

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER: 20-922

PHARMACOLOGY REVIEW(S)

MAR 30 1999

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

NDA No.: 20-922

Date: 3/29/99

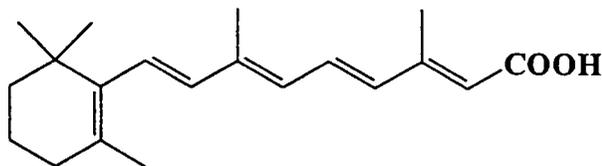
Name of Drug: Solagé, 2% 4-hydroxyanisole (4-methoxyphenol, mequinol, hydroquinone methyl ether, BMS-181158), and 0.01% All-Trans Retinoic Acid (ATRA, tretinoin, BMS-181159) solution

Structure:

4-hydroxyanisole
 $C_7H_8O_2$
MW=124.14



all-trans retinoic acid
 $C_{20}H_{28}O_2$
MW=300.44



Pharmacological Category: depigmenting agent

Sponsor: Bristol-Myers Squibb

Indication: treatment of solar lentigines resulting from chronic sun exposure

Route of Administration: topical

**APPEARS THIS WAY
ON ORIGINAL**

Formulation:

ingredient	% (w/v)	% (v/v)
4-hydroxyanisole	2.0	
tretinoin, USP	0.01	
ethyl alcohol, USP		77.8
PEG-8 (polyethylene glycol 400, NF)		<input type="text"/>
butylated hydroxytoluene, NF		
ascorbic acid, USP		
citric acid, USP		
ascorbyl palmitate, NF		
disodium EDTA, USP		
purified water, USP		

Related submissions: IND

INTRODUCTION

The sponsor faxed proposed label revisions on 3/26/99 following a teleconference on 3/25/99.

LABEL REVIEW:

The applicant proposed the following rewording to sections of the label related to nonclinical studies. Reviewer revisions are highlighted and justifications are in italics.

CONTRAINDICATIONS: **The combination of mequinol and tretinoin may cause fetal harm when administered to a pregnant woman. Due to the known effects of these active ingredients, SOLAGÉ Topical Solution should not be used in women of child-bearing potential.**

Reviewer's comment: The wording should remain as last proposed by the Division. The applicant provided an extensive review of 4-hydroxyanisole (mequinol) as an ingredient in cosmetic products which concluded that this material was unsafe for use in cosmetics. That article cited a developmental toxicology study in rats in which a 5% 4-hydroxyanisole cream caused increased pre-implantation embryonic loss and decreased pup body weights. In reproductive toxicology studies conducted by the sponsor, increased pre-implantation loss was seen in a rat segment I study at the high dose that was not statistically significant and statistically significant reduction in body weights in male pups. In the segment II rabbit study, increased pre-implantation loss was seen in 4-hydroxyanisole treated groups. Since the applicant's studies do show effects that corroborate published findings of this material, there does seem to be an effect of the mequinol component as well as the retinoid component.

In a dermal teratology study in New Zealand White rabbits, there were no statistically significant differences among treatment groups in fetal malformation data; however, marked hydrocephaly with visible doming of the head was observed in one mid-dose litter (12 and 0.06 mg/kg or 132 and 0.66 mg/m² of 4-hydroxyanisole and tretinoin, respectively) and two fetuses in one high dose litter (40 and 0.2 mg/kg or 440/2.2 mg/m² of mequinol and tretinoin, respectively) SOLAGÉ, and two high-dose tretinoin (0.2 mg/kg, 2.2 mg/m²) treated litters.

These malformations were considered to be treatment-related and due to the known effects of tretinoin. This was further supported by coincident appearance of other malformations associated with tretinoin, such as cleft palate and appendicular skeletal defects. No effects attributed to treatment were observed in rabbits in that study treated topically with 4-hydroxyanisole alone (dose 40 mg/kg, 440 mg/m²). A no-observed effect level (NOEL) for teratogenicity in rabbits was established at 4 and 0.02 mg/kg (44 and 0.22 mg/m² 4-hydroxyanisole and tretinoin, respectively) [redacted] for SOLAGE, which is approximately the maximum possible human daily dose, based on clinical application to 5% of total body surface area. Plasma tretinoin concentrations were not raised above endogenous levels, even at teratogenic doses. Plasma mequinol concentrations in rabbits at the NOEL at one hour after application were 124 ng/ml, or approximately twelve times the mean peak plasma concentrations of that substance seen in human subjects in a clinical pharmacokinetics study.

Reviewer's comment: It is appropriate that the label mention that plasma tretinoin concentrations were not measurable above background, but it should be made clear that teratogenic effects were seen at unmeasurable exposures. Alternatively, reference to tretinoin plasma concentrations can be omitted as it was in the Division's last version of the label.

In a repeated study in pregnant rabbits administered the same dose levels as the study described above, additional precautionary measures were taken to prevent ingestion. Although there is no evidence to confirm that ingestion occurred in the initial study, [redacted]

[redacted]. Precautionary measures additionally limited transdermal absorption to a six hour exposure period, or approximately one-fourth of the human clinical daily continuous exposure time. This study did not show any significant teratogenic effects at doses up to approximately 13 times the human dose on a mg/m² basis. However a concurrent tretinoin dose group (0.2 mg/kg/day) did include two litters with limb malformations [redacted]

Reviewer's comment: The applicant stated in the teleconference that it was remembered that a yellow tretinoin residue was left on the skin of the treated animals in the first segment II rabbit study (completed in 1993). The study report states that there was yellow-orange discoloration of the residual hair at the treatment site, but identifies this with irritation, and not with residual material. It seems more likely that this staining would be due to exudate at irritated skin sites. No mention is made in the study report that residual drug was left on the skin or that ingestion was suspected.

The applicant objected to the [redacted] exposure time being described as "not relevant to human clinical exposure," so revised wording is inserted. It is also important to note that clinical exposures will be continuous, but rabbit exposures were not.

The original wording regarding limb malformations should remain. Retinoid effects on limb development are well documented. A developmental toxicology study should not recognize only the worst possible effects of a drug, but should identify and acknowledge all of them.

[redacted]

Reviewer's comment: The applicant again wants to compare their study to the published study of Renova. This is not relevant or applicable. This drug is a combination drug in an unrelated vehicle with a different regimen for clinical use.

The entire paragraph should be deleted.

In a published study in albino rats (J. Am. Coll. Toxicology 4(5):31-63, 1985), topical application of 5% mequinol in a cream vehicle during gestation was embryotoxic and embryolethal. Embryonic loss prior to implantation was noted in that study where animals were treated throughout gestation. Coincidentally, mean pre-implantation embryonic loss was increased in the first rabbit study in all mequinol-treated groups relative to control and in the high dose mequinol/tretinoin and tretinoin only treated groups in the second study. In those studies, dosing began at gestation day 6, when implantation is purported to occur. Increased preimplantation loss was also noted at the high combination dose in a study of early embryonic effects in rats, as was decreased body weight in male pups; these findings are consistent with the published study.

Reviewer's comment: The sponsor chose to delete the above paragraph. It should remain and should include information from the segment I rat study (added last sentence).

SOLAGÉ was not teratogenic in Sprague-Dawley rats when given in topical doses of 80 and 0.4 mg/kg 4-hydroxyanisole and tretinoin, respectively (480 and 2.4 mg/m² or 11 times the maximum human dose). The maximum human dose is defined as [redacted] to 5% of the total body surface area.

Reviewer's comment: This or other wording to further clarify the dose definition should be added.

...

Carcinogenesis, Mutagenesis, Impairment of Fertility: Although a dermal carcinogenicity study in CD-1 mice indicated that SOLAGÉ applied topically at daily doses up to 80 and 0.4 mg/kg (240 and 1.2 mg/m²) of 4-hydroxyanisole and tretinoin, respectively, representing approximately 5 times the maximum possible systemic human exposure, was not carcinogenic, in a photocarcinogenicity study utilizing Crl:Skh-1(hr/hr BR) hairless albino mice, median time to onset of tumors decreased. Also, the number of tumors increased in all dose groups administered 1.4, 4.3, or 14 µl of SOLAGÉ/cm² of skin [redacted] (24 and 0.12, 72 and 0.36, or 240 and 1.2 mg/m² of mequinol and tretinoin, respectively) [redacted] 0.6, 1.9, or 6.5 times the daily human dose on a mg/m² basis following chronic topical dosing with intercurrent exposure to ultraviolet radiation for up to 40 weeks. Similar animal studies have shown an increased tumorigenic risk with the use of retinoids when followed by ultraviolet radiation. Although the significance of these studies to human use is not clear, patients using this product should be advised to avoid or minimize exposure to either sunlight or artificial ultraviolet irradiation sources.

Reviewer's comment: The original wording of the first sentence should be preserved. Expression of the dose in μl applied per cm^2 of skin is appropriate because the effects examined are at the level of the skin. Additional information should be added to express doses in mg/m^2 and to define the multiples of human topical dose.

4-Hydroxyanisole was non-mutagenic in the Ames/Salmonella assay using strains TA98, TA100, TA1535, and TA1537, all of which are insensitive to mutagenic effects of structurally related quinones. SOLAGÈ was non-genotoxic in an *in vivo* dermal micronucleus assay in rats, but exposure of bone marrow to drug was not demonstrated.

Reviewer's comment: Public information is available regarding the mutagenicity of hydroquinone and the insensitivity of these strains to detect this effect. The above rewording should be included.

The additional clause is added to the description of the micronucleus assay in rats, as effects on bone marrow components might not have been seen if the material did not reach the target tissue.

A dermal reproduction study with SOLAGÈ in Sprague-Dawley rats at a daily dose of 80 and 0.4 mg/kg (480 and 2.4 mg/m^2) of 4-hydroxyanisole and tretinoin, respectively, approximately 11 times the maximum possible human exposure, assuming 100% bioavailability following topical application to 5% of the total body surface area [redacted] showed no impairment of fertility.

[redacted] /S/

Amy C. Nostrandt, D.V.M., Ph.D. 3/29/99
Pharmacologist/Toxicologist

cc:

NDA 20-922

HFD-340

HFD-540

HFD-540/PHARM/Nostrandt

HFD-540/TLPHARM/Jacobs

HFD-540/MO/Cook

HFD-540/CHEM/Timmer

HFD-540/PMS/Cross

[redacted] /S/ 5-29-99
for Jacobs

Concurrence Only:

HFD-540/DD/WILKIN

[redacted] /S/ 3/30/99

HFD-540/TLPHARM/JACOBS

[redacted]

NOV 16 1998

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

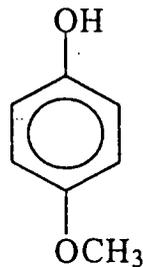
NDA No.: 20-922

Date Submitted: 12/30/97
Date CDER Received: 12/30/97
Date Assigned: 1/7/98
Date First Draft of Review Completed: 6/23/98
Date Review Completed: 11/16/98

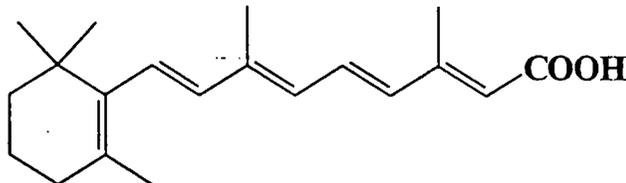
Name of Drug: 2% 4-hydroxyanisole (4-methoxyphenol, mequinol, hydroquinone methyl ether, BMS-181158), and 0.01% All-Trans Retinoic Acid (ATRA, tretinoin, BMS-181159) solution

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Sponsor: Bristol-Myers Squibb

Indication: treatment of solar lentigines, [redacted] resulting from chronic sun exposure

Route of Administration: topical

Formulation:

<u>ingredient</u>	<u>% (w/v)</u>	<u>% (v/v)</u>
4-hydroxyanisole	2.0	
tretinoin, USP	0.01	
ethyl alcohol, USP		77.8
PEG-8 (polyethylene glycol 400, NF)		
butylated hydroxytoluene, NF		
ascorbic acid, USP		
citric acid, USP		
ascorbyl palmitate, NF		
disodium EDTA, USP		
purified water, USP		

Related submissions: IND

Index of Nonclinical Studies:

Nonclinical Toxicology studies

1. Dermal study of fertility and early embryonic development in rats
2. Dermal study of embryo-fetal development in rats
3. Dermal study of pre- and postnatal development in the rat
4. Dermal carcinogenesis assay in CD-1 mouse

Nonclinical Pharmacokinetics studies

1. Toxicokinetics of BMS-181158 and BMS-181159 in rats during a 6-month dermal toxicity study.
2. Verification of exposure to BMS-181158 and BMS-181159 in rabbits during a segment II dermal teratology study.

INTRODUCTION

Most of the nonclinical studies submitted under this NDA were previously reviewed under IND They are discussed in the summary at the end of this review and cross-referenced as follows:

<u>Study</u>	<u>in review of submission #</u>
Nonclinical Toxicology studies	
Single-dose dermal toxicity study of rabbits	000
Single-dose oral toxicity study of rats	000
Two-month dermal rangefinding phototoxicity study in hairless mice	009/031
Three-month dermal rangefinding study in mice	009/025
Two-week dermal toxicity study in rats with ethanolic solutions	000
Six-month dermal study in rats, one-month interim report	000
Six-month dermal toxicity study of rats	004
Dermal teratology study in rabbits	010
Ames/Salmonella microbial mutagenicity assay	000

Micronucleus evaluation of a six-month dermal toxicity study in rats	007
Twelve-month photocarcinogenicity with ultraviolet radiation in hairless mice	038
Primary skin irritation study in rabbits	000
Primary skin irritation study in rabbits	038
UVA dermal phototoxicity study in guinea pigs	000
Dermal sensitization study in guinea pigs	000
Nonclinical Pharmacokinetics studies	
In vitro skin penetration	000
In vitro human skin permeation	000
Tissue distribution of radioactivity following intravenous administration of [³ H]TRA to male Long-Evans rats	046
Nonclinical Pharmacology studies	
Melanotic hyperpigmentation model: depigmentation of normal Yucatan pig skin	000
Melanocytic hyperpigmentation model: UVR-induced pigmentation	000
Studies on the mechanism(s) of action of induced depigmentation	000

It is unclear from the sponsor's multiples of human dose in the studies reviewed below exactly what the maximum human dose is expected to be. The proposed label states that safety factors are based on use over 5% of body surface area, but that patients in clinical studies were treated over an average of 1.7% of body surface area. Using the 5% value and assuming that 30 g of material would cover 100% of body surface area, then 1.5 g would be the maximum dose, administered bid for a total of 3.0 g per 60 kg human per day, or approximately 0.05 g (50 mg) of material per kg body weight. The resulting maximum systemic daily dose would be 1 mg 4-hydroxyanisole/0.005 mg tretinoin per kg or 37 mg 4-hydroxyanisole/0.185 mg tretinoin per m² total body surface area per day. The latter dose was used below to calculate systemic animal doses as multiples of the human dose. For topical application, 3.0 g of drug product applied to 5% of 1.62 m² (0.081 m², or 810 cm²) would yield 60 mg 4-hydroxyanisole/0.3 mg tretinoin per 810 cm², or 0.074 mg 4-hydroxyanisole/0.00037 mg tretinoin per cm² of skin (3.7 μl/cm²).

NONCLINICAL TOXICOLOGY STUDIES

- Study title:** Dermal study of fertility and early embryonic development in rats

Study number: 96672

Performing organization: [REDACTED]

Drug lot and batch: BMS-181158/BMS-181159 solution B95L008 (W1133-M-08-B) [REDACTED] 96017 and B95L009 (W1133-M-08-B) [REDACTED] 96009

Date of study: 12/17/96-2/14/97

GLP compliance: yes

Study design:

Dosing: once daily, topically to clipped areas of the skin of the back (approximately 10% of body surface area), unoccluded for 6 hours per day. Males were dosed for 4 weeks and females

for 2 weeks prior to mating; males and females were dosed throughout the cohabitation period for a maximum of 3 weeks; females were dosed through gestation day 7, then sacrificed on gestation day 15; males were dosed through the day before the scheduled sacrifice.

Dose groups: vehicle (4 ml/kg/day), 1, 2, 4 ml/kg/day (20/.1, 40/.2, 80/.4 mg/kg/day or 120/.6, 240/1.2, 480/2.4 mg/m²/day of 4-hydroxyanisole/tretinoin, respectively), or 3, 6.5, and 13 times the human dose on a mg/m² basis. The topical application to 10% total body surface area would be 0.120/0.0006, 0.240/0.0012, 0.480/0.0024 mg/cm²/day of 4-hydroxyanisole/tretinoin, respectively (6, 12, and 24 µl applied per cm² of skin). These represent 1.6, 3.2, and 6.5 times the human topical application rate.

Formulation: clinical formulation of 2% 4-hydroxyanisole/0.01% tretinoin

Test animals: CrI:CD[®](SD)BR rats, 25/sex/group, approximately 12 weeks old at the initiation of breeding. Animals were collared and exposed to the test article for 6 hours per day. Males were paired with females in the same treatment group on a 1:1 basis. Laparotomies were performed on females on gestation day 15.

Findings:

Deaths: One male in the control group died on study day 11.

Clinical signs: No systemic signs were observed. Body weight was decreased in high dose males on days 17-59 and was statistically significantly lower than controls on days 24-56. Body weight gains were decreased from study days 0-7, 10-24, 35-38, 42-45, and 49-52 in high dose males.

Dose-related dermal irritation was observed in all treated groups, consisting of slight to severe erythema and edema, eschar, fissuring (males only), desquamation, and exfoliation. Sporadic vocalization occurred in mid and high dose animals when dosed. No dermal effects were seen in vehicle control animals.

Reproductive parameters: In males, mating and fertility parameters, sperm evaluation, and gonadal weights were not affected by treatment. Histological examination of the testes revealed no treatment-related changes.

In females, estrous cycling, mating, fertility and intrauterine parameters were not affected by treatment. Intrauterine survival of F₁ embryos was comparable between treated and control groups. Pre-implantation loss was slightly increased at the high dose relative to control, but the difference was not significant, and the measure was within the range of historical controls for the contract laboratory. Postimplantation loss and mean numbers of viable embryos, corpora lutea and implantation sites were similar in treated and control groups.

Pre-coital intervals were not affected by treatment.

Organ weights: Testicular weights appeared to be decreased in high dose males; statistical significance was seen only for the absolute weight of the right testis, but not for the relative weight. This finding may be attributable to the final body weight being lower than control.

Mean ovarian weights (absolute and relative) were decreased in mid-dose females, and absolute ovarian weight in high dose females appeared lower than control, but the latter was not statistically significant. Since there was no dose-related trend, this was not considered to be an effect of treatment.

It was concluded that these above differences were not treatment related. Therefore, no treatment-related effects were observed in brain, pituitary gland, ovary, or testis/epididymis

weights. Retinoids have been associated with testicular degeneration, however, the finding of lower testicular weight was not associated with histopathological changes.

Pathological examination: There were no treatment-related findings on gross necropsy. Microscopic examination was performed on selected tissues from 10 males each in the control and high dose groups and revealed no treatment-related effects.

The parental systemic NOAEL was determined to be 40/0.2 mg/kg/day (0.240/0.0012 mg/cm² application site). The NOEL for dermal irritation was less than 20/0.1 mg/kg/day (0.120/0.0006 mg/cm² application site). The NOAEL for reproductive performance was considered to be greater than 80/0.4 mg/kg/day (0.480/0.0024 mg/cm² application site). 4-hydroxyanisole 2%/tretinoin 0.01% was not considered to be a reproductive toxicant in this study.

2. **Study title:** Dermal study of embryo-fetal development in rats

Study number: 96671

Performing organization: [REDACTED]

Drug lot and batch: BMS-181158/BMS-181159 solution B95L008 (W1133-M-08-B) 96017

Date of study: 12/31/96-1/24/97

GLP compliance: yes

Study design:

Dosing: once daily, topically to clipped areas of the skin of the back (approximately 10% of body surface area), unoccluded for 6 hours per day on days 6-15 of gestation.

Dose groups: vehicle (4 ml/kg/day), 1, 2, 4 ml/kg/day (20/.1, 40/.2, 80/.4 mg/kg/day or 120/.6, 240/1.2, 480/2.4 mg/m²/day of 4-hydroxyanisole/tretinoin, respectively), or 3, 6.5, and 13 times the human dose on a mg/m² basis. The topical application to 10% total body surface area would be 0.120/0.0006, 0.240/0.0012, 0.480/0.0024 mg/cm²/day of 4-hydroxyanisole/tretinoin, respectively (6, 12, and 24 µl applied per cm² of skin). These represent 1.6, 3.2, and 6.5 times the human topical application rate.

Formulation: clinical formulation of 2% 4-hydroxyanisole/0.01% tretinoin

Test animals: CrI:CD[®](SD)BR rats, 25 pregnant females/group, approximately 12 weeks old at the time of breeding. Animals were collared and exposed to the test article for 6 hours per day. Laparotomy was performed on gestation day 20 to examine reproductive organs and fetuses.

Findings:

Deaths: none

Clinical signs: The dams exhibited no systemic signs of toxicity. Treated animals did exhibit dose-related irritation consisting of very slight to slight erythema and desquamation in all treated groups. The incidence of the latter was increased in the high dose group. Local irritation was resolved in many animals by gestation day 20. Eschar was noted in one high dose animal on gestation days 19 and 20. Control animals were unaffected.

The mean body weight in the high dose group was reduced and was significantly different from control on gestation days 12-16. A significant decrease in body weight gain was observed in high dose animals on gestation days 6-12, and food consumption in that group was decreased

during the entire treatment period. Also in the high dose group, net body weight and net body weight gain were reduced, but gravid uterine weight was comparable to that of control animals. Reproductive/fetal parameters: Intrauterine growth and survival were concluded to be unaffected by treatment. One high dose female did have 6 dead fetuses and 2 late resorptions, but these were attributed to infection.

Mean fetal body weight was slightly decreased in the high dose group due to the one female with pathologic intrauterine abnormalities and a resulting low mean litter weight. However, there were no indications of growth retardation, and the mean fetal body weight in that group was not significantly different from control when that litter was excluded. The test article was not implicated. Interestingly, even with the exclusion of that litter, fetal body weights for male pups were significantly reduced at the high dose.

No significant differences were seen in numbers of viable or dead fetuses, postimplantation loss, pre-implantation loss, and numbers of corpora lutea and implantation sites. No external malformations or variations were seen. Visceral malformations were noted in one fetus each in control (ventricular septal defect) and high dose (situs inversus) groups. No visceral variations were noted. A skeletal malformation was found in one fetus in the low dose group (vertebral anomaly). Skeletal variations were similar in incidence and type in all groups. Those fetal malformations and variations observed were considered to be spontaneous and not related to treatment.

Pathological examination: At necropsy, one high dose female had clear fluid in the uterus and a second had greenish uterine fluid, 6 dead fetuses and 2 late resorptions. The latter was considered unusual and probably related to infection. Pathological findings in a few low and mid dose females did not appear to be related to treatment.

The systemic maternal NOAEL was 40/0.2 mg/kg/day (0.240/0.0012 mg/cm² application site), based on body weight changes, and the NOAEL for developmental toxicity was greater than 80/0.4 mg/kg/day (0.480/0.0024 mg/cm² application site). The test article was determined not to be a developmental toxicant in this study.

Reviewer's comment: Positive results were seen in the developmental toxicity study in rabbits (see reviews of serial # 010). Due to retinoid-related teratogenesis seen in that study, the sponsor has proposed Pregnancy category X for their labeling. In a subsequent update to the NDA, dated 5/5/98, the sponsor has indicated that the rabbit study is being repeated using additional precautions to prevent ingestion of test material. The report of that study is anticipated in the third quarter of 1998. In the original IND, the sponsor also provided a report from the published literature in which 4-hydroxyanisole alone, applied topically as either a 5% or 25% formulation was embryotoxic and embryolethal, though not teratogenic.

3. Study title: Dermal study of pre- and postnatal development in the rat
Study number: 96670
Performing organization:
Drug lot and batch: BMS-181158/BMS-181159 solution B95L008 (W1133-M-08-B) 96017
Date of study: 9/10/96-2/4/97
GLP compliance: yes

Study design:

Dosing: once daily, topically to clipped areas of the skin of the back (approximately 10% of body surface area), unoccluded (except during lactation) for 6 hours per day from day 6 of gestation through lactation day 20 (F₀ animals only).

Dose groups: vehicle (6 ml/kg/day), 0.6, 2.0, 6.0 ml/kg/day (12/0.06, 40/.2, 120/.6 mg/kg/day or 72/.36, 240/1.2, 720/3.6 mg/m²/day of 4-hydroxyanisole/tretinoin, respectively), or 2, 6.5, and 19 times the human dose on a mg/m² basis. The topical application to 10% total body surface area would be 0.072/0.00036, 0.240/0.0012, and 0.720/0.0036 mg/cm²/day of 4-hydroxyanisole/tretinoin, respectively (3.6, 12, and 36 µl applied per cm² of skin). These represent 1, 3.2, and 10 times the human topical application rate.

Formulation: clinical formulation of 2% 4-hydroxyanisole/0.01% tretinoin

Test animals: CrI:CD[®](SD)BR rats, 25 pregnant females/group, approximately 12 weeks old at the time of breeding. Animals were collared and exposed to the test article for 6 hours per day. Dams allowed to deliver naturally, and the litters were monitored. At postnatal day 4, litters were culled to 8 pups each (4/sex when possible). At 8-13 days of age, 25 male and 25 female F₁ pups per group were randomly selected for evaluation of physical and functional development and reproductive performance. Of those, 10/sex/group were selected for evaluation of sensory function and behavioral testing (motor activity, learning, memory). F₁ animals were mated; females underwent laparotomy on gestation day 20, and F₂ fetuses were evaluated. F₁ males were necropsied after F₁ females.

Findings:

Deaths: No F₀ dams died spontaneously during the study. During the first week of lactation, all dams and offspring in the high dose group were euthanized due to extreme irritation at the application site.

Clinical signs: In F₀ animals, dose related irritation was noted in treated groups, consisting of very slight to severe erythema (first noted on study day 8), very slight to moderate edema, including fissuring (especially at the high dose), desquamation (first noted on study day 13), eschar, focal eschar and exfoliation (first noted on study day 14) at the treatment sites.

Vocalization was observed on application of the test material in mid and high dose groups. High dose animals exhibited significant decreases in body weight on gestation day 20 and lactation day 1, in mean body weight gain during gestation, and in food consumption during gestation days 9-12. Increased food consumption in first few days of lactation was observed in those animals before they were sacrificed for humane reasons.

In F₁ animals, drug related changes were only observed at the maternally toxic high dose. In that group, there was increased pup mortality, decreased pup body weight, and an increased incidence of clinical signs; signs seen in high dose pups included small size, hypoactivity, cool to the touch, and pale in appearance.

As adults F₁ males from treated groups exhibited body weights that appeared to lag behind those of controls in a dose-dependent manner, but that finding was not statistically significant. There was no apparent effect on male body weight gain at any dose. No significant effect was seen on body weight, body weight gain, gestation body weight or gestation body weight gain in females.

Reproductive parameters: Six dams each in the mid- and high dose groups failed to deliver by post-mating day 25, as compared to two each in the control and low dose groups; all but one of the controls were found to be nongravid. Four high dose females had total litter loss between lactation days 1 and 5. Reduced F₁ pup survival and a higher rate of missing or cannibalized pups was seen in high dose litters after postnatal day (PND) 1. High dose F₁ pups had reduced body weights, and there was an increased incidence of F₁ pup clinical and necropsy findings.

Developmental parameters were included in F₁ pup observations. Balanopreputial separation and vaginal patency were unaffected by treatment, although females in treated groups appeared to lag behind controls in timing of the latter measure, but not in a dose-related manner. Auditory startle testing on or about PND 21 and 60 revealed no treatment-related effects. Motor activity (total and ambulatory) measurements were made on or about PND 13, 17, 21, and 60. On PND 13 there was apparently less activity in treated groups, but the variability was so great in controls at this time point that there was no significant difference. It is likely that this is too early an age for motor activity to be a sensitive measure. Variability was too high at PND 17 and 21 for meaningful interpretation as well. At PND 60, variability was less and there was no effect of treatment on total or ambulatory counts. Testing in the water maze was initiated between PND 20-23 and between PND 57-62 and evaluated swimming ability, learning and memory. No effect of treatment was seen.

Estrous cycling in F₁ females and reproductive performance in F₁ animals was unaffected by treatment. Gravid uterine weights and F₂ fetuses were also unaffected. There did appear to be an increase in early resorptions (and therefore post-implantation loss) in treated groups compared to control (not statistically significant).

Pathological examination: On gross necropsy, the only treatment-related finding in F₀ dams was reddening, thickening and scabbing of skin at treated sites.

In F₁ pups found dead or euthanized, gross findings in the high dose group included absence of milk in the stomach, renal papilla not developed or not fully developed and/or distended ureters or urinary bladder. One external malformation (anury) was noted in one animal in one litter. At the low dose, one pup was found to have the renal papilla not fully developed.

In F₁ euthanized surplus pups, high dose animals were again noted with absence of milk in the stomach, and one litter had pups in which the renal papilla was not developed or not fully developed and/or ureters or urinary bladder were distended. Also at the high dose there was one pup in one litter with a hemorrhagic ring around iris.

In F₁ adults, no significant findings were seen that could be attributed to treatment. One low dose female had an enlarged spleen (also seen in a control female) and one mid-dose female had clear fluid in one uterine horn.

In F₂ pups, two low dose fetuses in two different litters were seen with external malformations (one with omphalocele and a second with craniorachischisis and a curly tail). These were considered to be within the range of historical controls. No external malformations were seen in the mid-dose group.

Based on this study, the maternal, neonatal, and developmental NOAELs were determined to be 40/0.2 mg/kg/day, or 12 µl/cm² (3.2 times the human application rate).

4. Study title: Dermal carcinogenesis assay in CD-1 mouse

Study number: 93720

Performing organization:

Drug lot and batch: # B93K009

Date of study: 1/25/94 to 1/29/96

GLP compliance: yes

Study design:

Dosing: dermal, unoccluded, qd for 104 weeks to clipped sites on the back (approximately 6 cm²).

Dose groups: untreated control, vehicle (100 µl), 10, 30, 100 µl/mouse/day (8/0.04, 24/0.12, 80/0.4 mg/kg/day 4-hydroxyanisole/tretinoin, respectively, or 24/0.12, 72/0.36, 240/1.2 mg/m²/day). These doses represent 0.65, 2, and 6.5 times the maximum human dose on a mg/m² basis. The topical application to a 6 cm² site would be 1.7, 5, and 17 µl/cm², or 0.034/0.00017, 0.10/0.0005, and 0.340/0.0017 mg/cm² of skin/day of 4-hydroxyanisole/tretinoin, respectively. These represent 0.5, 1.4, and 4.6 times the human topical application rate.

Formulation: clinical formulation of 2% 4-hydroxyanisole/0.01% tretinoin

Test animals: Crl:CD-1[®](ICR)BR VAF/Plus mice, 6-7 weeks of age at start of study, 50/sex/group.

Findings:

Deaths: The sponsor's trend analysis revealed dose-related statistically significant decreases in survival in mid- and high-dose males and in high-dose females (*Reviewer's comment: The results of the statistical reviewer's analysis appear to be in agreement*). Those groups also exhibited the greatest degree of dermal irritation. Death, in general, was most often attributed to generalized age-related amyloidosis. The numbers of animals surviving to study termination are shown in the following table:

Group:	untreated control	vehicle control	10 µl/day	30 µl/day	100 µl/day
males	19/50 (38%)	22/50 (44%)	16/50 (32%)	8/49 (16%)	6/50 (12%)
females	17/49 (35%)	21/50 (42%)	21/50 (42%)	20/50 (40%)	10/48 (21%)

Clinical signs: Dose-related dermal irritation was seen, initially greater in males than in females, although in the end it was concluded that there were no gender differences. Effects on skin at the treatment site included very slight to severe erythema, very slight to moderate edema, desquamation, scabbing, and slight eschar in high dose. In mid- and high-dose animals the application site was also described as exhibiting leathery skin, exfoliation, thickening, sores, ulceration, and atonia. Vehicle control animals exhibited a low incidence of irritation. Signs in that group consisted of scabs, sores, alopecia, staining of fur, and/or swelling. The sponsor reported a low incidence of non-dermal palpable masses and/or visible skin growths in both treated and control groups, none of which were considered to be treatment-related. Macroscopic skin masses were observed at the treatment site in one vehicle control male (negative for

neoplasia on microscopic examination) and one high dose female (benign keratoacanthoma). No systemic signs were reported, but there seemed to be an increased incidence of ocular opacities in treated animals, particularly toward the end of the treatment period. The report states that there were no consistent or biologically significant changes in body weight. However, in males there appeared to be dose-related decreases in body weight, particularly after day 100. These changes were slight, but most pronounced in the mid- and high-dose groups. This effect may have been secondary to irritation. In females, body weight in the vehicle group was lower than in other groups for most of study, and body weight in the high dose group was actually highest at some intervals.

Reviewer's comment: The statistical reviewer's analysis revealed dose-related statistically significant decreases in body weight in males relative to control after 40 weeks of treatment.

Clinical chemistry and hematology: (samples collected on day of sacrifice)

Hematological changes consisted of increased % eosinophils in mid- and high-dose males, and decreased % monocytes and increased % eosinophils in high-dose females. These do not appear to be biologically significant.

Pathological examination: Test-article related findings on gross necropsy were related to dermal irritation of treated skin. In males, there was an increased incidence at the dosing site of desquamation, scabs and exfoliation in treated groups, as well as eschar, ulceration, erythema and edema in those that died before the scheduled termination. Findings in treated females were similar, with the addition of eschar and ulceration in animals at the terminal sacrifice. Splenic enlargement was seen in both treated males and females. There also seemed to be an increased incidence of ocular opacities in treated animals, but no dose-related ocular lesions were reported on histopathological examination. ✓

Histologic examination revealed treatment-related changes in treated skin in vehicle and treated groups at all doses. These changes consisted of acanthosis and hyperkeratosis of the epidermis, chronic inflammation of the dermis with inflammatory cell infiltration (and increased collagen and fibrous tissue in more extensive cases), epidermal inflammatory exudate, and focal epidermal necrosis. These findings were dose-related in incidence and severity. Low or single incidences of amyloidosis, foci of dense mast cell infiltration, or focal vesicular degeneration of the epidermis were seen in treated groups. Some similar changes were seen at adjacent untreated sites at lower incidence and severity and were considered to be related to migration of the test article.

Other changes present were considered to be secondary to cutaneous inflammation, including bone marrow hyperplasia, increased extramedullary hematopoiesis in spleen, and lymphocytic/plasmacytic hyperplasia in lymph nodes. The most common incidental finding in all groups was systemic amyloidosis involving kidneys, gastrointestinal tract, liver, heart, adrenals, ovaries most frequently. That finding was considered to be age- and not treatment-related.

Tumor findings:

No treatment-related neoplasms were noted either systemically or at the application sites. Incidental tumors involving the lymphoreticular system, lung, and/or liver were found at single or ✓

low incidence, or were comparable among groups. They were considered not unusual for mice of this age and strain.

The only primary skin tumor was one benign keratoacanthoma in dorsal skin of one high dose female. The histopathology report states that this is a rare spontaneous tumor in this strain of mouse. The sponsor cites a paper by Maita, et al. (Toxicologic Pathology 16:340-349, 1988), that summarizes spontaneous tumor incidence in control animals of this species and strain from 11 2-year carcinogenicity studies. Those authors reported one incidence each in males and females, out of 891 and 890 animals, respectively. Although this tumor was seen in a high dose animal at the treated site, it did appear at a low incidence and has been reported to occur as a spontaneous tumor at low incidence. It therefore seems likely that the lesion was not related to treatment. A trichoepithelioma was reported in a mid-dose female.

The statistical reviewer's analysis revealed no tumor differences or trends that were statistically significant in the overall study. Among males, there were no statistically significant trends or pairwise differences among dose groups. Among females, there was a statistically significant evidence of trend in stromal sarcoma of the cervix, due to two occurrences, both in the high dose group. (*Reviewer's comment: In Charles River's historical database of spontaneous neoplastic lesions in this strain of mouse, dated March, 1995, endometrial stromal sarcoma of the uterus/cervix is documented as up to 14% incidence with a mean incidence of 2.45% in 24-month studies.*)

After executive CAC review of the study results, a survival-adjusted analysis was requested, as was combination of certain tissues and lesions (i.e. combination of stromal polyps and stromal sarcomas across uterus and cervix). The only difference in the results of that analysis from those of the original analysis was a slightly significant test of trend ($p=0.0049$) for the pooled stromal polyps and sarcomas of the cervix, but there was no significant difference when these lesions were pooled across uterus and cervix. As previously discussed, this does not appear to be treatment-related when considered in light of historical incidence of these lesions.

NONCLINICAL PHARMACOKINETICS STUDIES

1. **Study title:** Toxicokinetics of BMS-181158 and BMS-181159 in rats during a 6-month dermal toxicity study.

Study number: 92005 (pharmacokinetics report MAP930740039)

Performing organization: Bristol-Myers Squibb

Drug lot and batch: not specified

Date of study: report date 11/24/93

GLP compliance: yes

Study design:

Dosing: topically to the skin, daily for 6 months

Dose groups: vehicle, 4.0/0.02, 12.0/0.06, 40.0/0.20, and 40.0/0 mg/kg/day of 4-hydroxyanisole/tretinoin (24/0.12, 72/0.36, 240/1.2, 240/0 mg/m²/day, respectively; the doses for groups 2-4 are 0.65, 2, and 6.5 times the human dose, respectively).

Formulation: 2% 4-hydroxyanisole/0.01% tretinoin in an ethanolic vehicle

Test animals: Harlan Sprague-Dawley albino rats, 16/sex/group. The skin at the application site was clipped in all animals and abraded in 8/sex/group. Blood was collected for pharmacokinetic evaluation from 5/sex/group (2-3 abraded, 2-3 not) at 1, 3, 6, and 24 hours after a daily dose during weeks 4 and 21. Concentrations of 4-hydroxyanisole were measured using [redacted] with a range of quantitation of [redacted]

Findings:

Serum concentrations and AUC for 4-hydroxyanisole increased in a dose-related manner. Those values were also higher in group 4, treated with the high dose of the combination, than in group 5, treated with the high dose of 4-hydroxyanisole alone. Values were higher at week 21 than at week 4. No differences were noted in 4-hydroxyanisole concentrations between animals with abraded and non-abraded skin. Some samples from vehicle-treated animals contained low concentrations of 4-hydroxyanisole; this was considered to be due to contamination during sample processing and not biologically relevant. This does appear to reflect on the quality of sample handling and processing and ultimately on the quality of the resulting data. Tretinoin concentrations were reported to be mostly less than [redacted] (LOQ). Those samples with higher concentrations were reported to be mostly near the naturally occurring level of 1.3 ng/ml. However, serum concentrations in some animals at various time points reached as high as 5.8 ng/ml at week 4 and as high as 24.3 ng/ml at week 21.

Mean serum concentrations of 4-hydroxyanisole (4-HA) in ng/ml:

Dose 4-HA/tretinoin (mg/kg/day)	Week 4 - hours after dose				AUC _{0-24 hr} (ng·hr/ml)	Week 21 - hours after dose				AUC _{0-24 hr} (ng·hr/ml)
	1	3	6	24		1	3	6	24	
4.0/0.02	23.9	10.0	3.3	<LOQ	95.5	105	16.4	39.4	1.1	623
12.0/0.06	96.9	26.7	13.8	<LOQ	357	140	34.6	29.6	7.1	675
40.0/0.20	348	136	54.1	14.8	1571	327	208	72.0	57.4	2312
40.0/0	70.4	81.4	60.2	14.4	1078	149	175	77.6	38.8	1845

2. **Study title:** Verification of exposure to BMS-181158 and BMS-181159 in rabbits during a segment II dermal teratology study.
- Study number:** 92714 (pharmacokinetics report MAP930740056)
received 5/26/98 in an amendment to the original NDA
- Performing organization:** Bristol-Myers Squibb
- Drug lot and batch:** 2% 4-hydroxyanisole solution W1133-M-09-A, lot no. B92H007
2% 4-hydroxyanisole/0.01% tretinoin solution W1133-M-08-A, lot no. B92H006
0.01% tretinoin solution W1133-M-10-A, lot no. B92H008
- Date of study:** report date 12/9/93
- GLP compliance:** yes

Study design:

Dosing: topically to clipped and/or abraded areas of skin, daily on gestation days 6-18

Dose groups: vehicle, 40 mg/kg/day (480 mg/m²/day) 4-hydroxyanisole, 4/0.02, 12/0.06, 40/0.2 mg/kg/day (48/0.24, 144/0.72, 480/2.4 mg/m²/day) 4-hydroxyanisole/tretinoin, and 0.2 mg/kg/day tretinoin (2.4 mg/m²/day); doses in groups 3-5 are 1.3, 4, and 13 times the maximum human dose, respectively.

Reviewer's comment: *Retinoid-associated malformations were seen in litters from the mid and high dose combination groups and from the tretinoin only group.* ✓

Formulation: 2% 4-hydroxyanisole and/or 0.01% tretinoin solution

Test animals: pregnant female rabbits

Blood samples were taken for pharmacokinetic evaluation at one hour after dosing on gestation day 18 from 3 dams with abraded skin and 2 with intact skin from each dose group. Plasma drug concentrations were determined by [redacted] methods. The ranges of the standard curves were [redacted] for 4-hydroxyanisole and [redacted] for tretinoin.

Findings:

Plasma concentrations of 4-hydroxyanisole increased in a dose-related manner in groups 3-5, which were treated with increasing doses of the combination product. Within each group, mean 4-hydroxyanisole concentrations were higher in animals with intact skin (124, 375, and 793 ng/ml in groups 3-5, respectively) than in animals with abraded skin (80, 192, and 598 ng/ml in groups 3-5, respectively). However, since only one time point was sampled, it is possible that earlier and higher peak concentrations were seen in animals with abraded application sites.

Mean plasma concentrations of 4-hydroxyanisole in group 2 animals, treated with 40 mg/kg/day of 4-hydroxyanisole alone (203 and 259 ng/ml in animals with abraded and intact skin, respectively) were 3-fold lower than in group 5, treated with 40 mg/kg/day 4-hydroxyanisole plus 0.2 mg/kg/day tretinoin (598 and 793 ng/ml in animals with abraded and intact skin, respectively). This may indicate that animals treated with the combination product had higher systemic exposure to 4-hydroxyanisole than did those treated with that product alone. While comparison is difficult with only one time point, this finding is consistent with other animal and with human pharmacokinetic studies.

Serum tretinoin concentrations were below the LOQ of [redacted] in all groups (*Reviewer's comment: Tretinoin has been shown to be teratogenic in other studies while associated plasma concentrations were not distinguishable from background values*). Low concentrations of 4-hydroxyanisole were found in all samples from group 6, treated with tretinoin alone. This was stated to be due to contamination during collection, processing, or analysis of samples. Again this appears to reflect on the standard of laboratory procedures and on the quality of the data. ✓

SUMMARY

In a single dose oral study in Sprague-Dawley rats, the minimum lethal dose was greater than 5.0 ml/kg (100 mg 4-hydroxyanisole/0.5 mg tretinoin per kg, 600/3.0 mg/m²). Signs seen at that dose included decreased locomotor activity and ataxia, which were considered to be due to

the high alcohol content of the test material. Most animals were reported to have appeared normal by 4 hours after treatment.

A single topical dose of 2.0 ml/kg (40 mg 4-hydroxyanisole/0.2 mg tretinoin per kg) to New Zealand White rabbits under occlusion resulted in transient skin irritation consisting of well-defined erythema and very slight edema. Very slight erythema and edema persisted during the 14-day observation period. A lesser degree of irritation was noted with the vehicle.

In a 14-day dermal study in Sprague-Dawley rats, doses of 2 ml/kg/day of 2% 4-hydroxyanisole/0.01% tretinoin (40 mg 4-hydroxyanisole/0.2 mg tretinoin per kg) or 4% 4-hydroxyanisole/0.01% tretinoin (80 mg 4-hydroxyanisole/0.2 mg tretinoin per kg) resulted in irritation and focal eschar. Severity was slightly increased with dose. Vehicle treatment in females resulted in slight transient irritation. Histological examination of treated groups revealed dermal and epidermal inflammation, acanthosis, hydropic degeneration, and incipient fibrosis.

A 3-month dermal range-finding study was conducted in CD-1 mice at doses of 0 (vehicle), 10, 25, 50, 100, and 150 μ l/day of 2% 4-hydroxyanisole/0.01% tretinoin (8/0.04, 20/0.1, 40/0.2, 80/0.4, and 120/0.6 mg/kg/day). Dermal irritation, consisting primarily of erythema, was seen in all treated groups, as well as some edema, eschar, desquamation, and pinpoint scabbing. Spleen weights were increased in high dose animals. Dose-related acanthosis was seen histologically. Pharmacokinetic evaluation revealed dose-related systemic exposure to both 4-hydroxyanisole and tretinoin.

In a 6-month dermal study in Sprague-Dawley rats, treatments included 4/0.02, 12/0.06, and 40/0.2 mg/kg/day 4-hydroxyanisole/tretinoin and 40 mg/kg 4-hydroxyanisole alone. An interim evaluation at one month revealed dose related scab formation, acanthosis, and dermal inflammation at all combination dose levels. Corneal opacities were reported in drug-treated animals beginning in the first month, but were not considered to be treatment related. Such lesions are known to be side effects of tretinoin, but can be due to other factors in laboratory animals. Animals treated with the high dose combination had decreased mean hemoglobin, hematocrit, and RBC count. At 6 months, animals in the high dose combination group had a slight increase in neutrophil count and females in the 4-hydroxyanisole only group had slightly decreased serum potassium. Females in the high dose combination group had slightly increased liver weights. Time and dose-related irritation was evident at the treatment site. Histopathologic evaluation of the treatment sites revealed dose-related incidence and severity of acanthosis, dermal inflammation, scabbing, dermal hemorrhage or edema, erosion, ulceration. In animals treated with 4-hydroxyanisole alone, minimal scabbing, acanthosis, or chronic-active inflammation were observed. Pharmacokinetic evaluation revealed dose-related exposure to 4-hydroxyanisole, with higher plasma concentrations of that drug seen after administration of the combination than after 4-hydroxyanisole alone. Plasma 4-hydroxyanisole concentrations and AUC's were higher at week 21 than at week 4.

In a two-month range-finding phototoxicity study in hairless mice, doses of 0 (vehicle), 10, 30, and 100 μ l/day of 2% 4-hydroxyanisole/0.01% tretinoin (8/0.04, 24/0.12, and 80/0.4 mg/kg/day) were applied 5 days per week followed by exposure to UVB radiation at 0.272 MED (272 RBU). Additional control groups were exposed to UVB radiation alone at 0.272 MED and 0.382 MED (272 and 382 RBU, respectively). Dryness and flaking were seen in all three drug

treated groups, and wrinkling was seen particularly in the mid- and high dose groups. No such effect was seen in controls. Enlarged lymph nodes were found in all treated groups and in one untreated high UV control animal and were considered to be related to UV exposure, although the frequency of this observation was increased in drug-treated groups. Histologically, treatment-related acanthosis, inflammation, hyperkeratosis, and parakeratosis were noted. Minimal acanthosis was seen in all three control groups.

Reproductive toxicology studies were performed using topical dosing of the clinical formulation and included a study of fertility and early embryonic development in Sprague-Dawley rats, developmental toxicity studies in Sprague-Dawley rats and in New Zealand White rabbits, and peri/postnatal developmental toxicology in Sprague-Dawley rats. The segment I and II studies in rats at doses up to 80 mg 4-hydroxyanisole/0.4 mg tretinoin per kg were negative for reproductive/developmental effects, with the possible exception of reduced body weight of male pups from dams treated with the highest dose in the segment II study. The developmental toxicology study in rabbits revealed retinoid-associated malformations in mid- and high-dose combination groups (12/0.06 and 40/0.2 mg 4-hydroxyanisole/tretinoin per kg, respectively) and in the tretinoin treated group (0.2 mg/kg). Plasma samples collected on gestational day 18 indicated dose-related exposure to 4-hydroxyanisole, but plasma tretinoin concentrations were below the limit of quantitation. Higher plasma concentrations of 4-hydroxyanisole were seen in animals treated with the combination than in those treated with 4-hydroxyanisole alone. Maternal effects were limited to irritation at the treatment site. In the peri/postnatal developmental toxicology study in rats at doses up to 120 mg 4-hydroxyanisole/0.6 mg tretinoin per kg, the animals in the high dose group were terminated early due to severe irritation. Treatment related effects were seen only at the maternally toxic high dose and included increased pup mortality, lower pup weights, and incomplete development of the kidney. The NOAEL in this study was 40 mg 4-hydroxyanisole/0.2 mg tretinoin per kg. In the original IND, the sponsor also provided a report from the published literature in which 4-hydroxyanisole alone, applied topically to pregnant rats as either a 5% or 25% formulation was embryotoxic and embryolethal, though not teratogenic.

A 2-year dermal carcinogenicity study in CD-1 mice at doses up to 80 mg 4-hydroxyanisole/0.4 mg tretinoin per kg was negative for treatment-related effects on tumor development. In a 1-year photo co-carcinogenicity study in hairless mice, there was a significant enhancement of UV-induced skin carcinogenesis and a significantly shorter time to tumor onset in all treated groups.

Genotoxicity testing of the drug product was problematic due to the high ethanol content of the vehicle. Low tolerance to the formulation by bacteria and mammalian cells in vitro was demonstrated, and it was agreed by the previous reviewer that further attempts at genotoxicity testing were not necessary. Separate testing of the drug substances in a non-ethanolic vehicle was not pursued. 4-hydroxyanisole alone in an aqueous solution or in DMSO was tested in the Ames/Salmonella assay, and the drug product was tested by micronucleus evaluation of bone marrow from Sprague-Dawley rats treated topically for 6 months. The results of both tests were negative. However, the strains of Salmonella typhimurium used in the Ames assay (TA98, TA100, TA1535 and TA1537) were not sensitive to oxidizing mutagens (ref. Casarett and

Doull's Toxicology, 5th ed.; ICH guidelines). Since it is conceivable that 4-hydroxyanisole may be a mutagen by such a mechanism, testing should have included an appropriate additional strain (*S. typhimurium* TA102, *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA [pKM101] are suggested in the ICH guidelines as a fifth test strain). Given the negative results of the micronucleus evaluation and the dermal carcinogenicity assay, it is unlikely that additional testing would be necessary for this drug.

Although several prototype formulations caused mild to moderate skin irritation, none were classified as a primary skin irritant in primary skin irritation studies in New Zealand White rabbits. The 2% 4-hydroxyanisole/0.01% tretinoin formulation was not phototoxic in hairless albino guinea pigs following UVA exposure. The formulation was also considered to be non-sensitizing in a modified assay in hairless guinea pigs. Due to the high concentration of ethanol in the formulation, it would be considered to be a probable ocular irritant.

Pharmacokinetic studies included one in which [³H]-tretinoin was administered intravenously to male Long-Evans rats. The results of that study suggested extensive distribution of the test article and excretion primarily by biliary route. In an in vitro study of percutaneous absorption, 50-60% of 4-hydroxyanisole penetrated mouse skin over a 10 hour period, with less than 5% being retained in the epidermis and dermis. Concomitant application of a single dose of tretinoin did not enhance 4-hydroxyanisole penetration in this system. Similar results were seen in in vitro skin permeation studies of 4-hydroxyanisole using human cadaver skin. In vitro permeation of human skin by 0.01% tretinoin with or without 2% 4-hydroxyanisole was between 5-7% of the topically applied dose. Metabolism of 4-hydroxyanisole was discussed in literature references submitted with the original IND. In rabbits administered 4-hydroxyanisole orally, urinary metabolites consisted primarily of a glucuronide and a sulfate conjugate. A small amount of the drug was demethylated to yield hydroquinone in the rabbit, although this was not detected in the rat. In a study in human melanoma patients administered 4-hydroxyanisole by intraarterial infusion, urinary concentrations of unchanged 4-hydroxyanisole, 3,4-dihydroxyanisole, 3-hydroxy-4-methoxyanisole and 4-hydroxy-3-methoxyanisole were detected. These were excreted as glucuronide and sulfate conjugates.

Pharmacology studies in Yucatan minipig were conducted to evaluate potential efficacy and to determine optimal relative concentrations for the combination product. These studies demonstrated synergy between the two drug substances. The proposed combination drug product produced slight to moderate depigmentation with minimal irritation and did not kill melanocytes. (However, in a literature reference submitted to the original IND, six months of treatment of black guinea pigs with either 0.5% or 1.0% 4-hydroxyanisole resulted in degeneration of melanocytes.) Depigmentation of normal dark skin was demonstrated and was reversible. The combination was also shown to reverse UV light-induced hyperpigmentation in light skinned Yucatan minipigs. ✓

COMMENTS

The chemistry review of the referenced DMF for 4-hydroxyanisole revealed a lack of identification and quantification of impurities in that drug substance. It is possible that unqualified impurities may exist in large enough amounts to necessitate additional testing prior ✓

to approval. If it can be demonstrated that the impurities were present in the test material used in long-term nonclinical studies, additional testing would not be necessary.

The proposed label calls for twice daily dosing. Nonclinical studies of this drug were performed using once daily dosing. The longer duration of topical exposure in the clinical setting may allow for greater systemic exposure than would be seen if the same dose were applied once daily for a limited period of time. This should be taken into consideration when making cross-species extrapolation from nonclinical studies in which dosing was performed once daily. Additionally, twice daily clinical dosing would introduce the possibility of concurrent exposure to sunlight. Other tretinoin topical preparations are applied at night only. Since photo cocarcinogenicity studies of tretinoin indicate that tretinoin may enhance sunlight-induced carcinogenesis when exposure to the drug and sunlight are concurrent, and since the dosing instructions for the current product are not consistent with existing labels for tretinoin-containing products, the implications for risk of enhancement of sunlight-induced carcinogenesis are uncertain. There may be reduced concern due to the fact that the patient population is one that is likely to be avoiding sun exposure.

REGULATORY CONCLUSIONS

From a pharmacology/toxicology standpoint this drug product is approvable, pending satisfactory limitation of impurities in the 4-hydroxyanisole component and with the minor labeling changes described below.

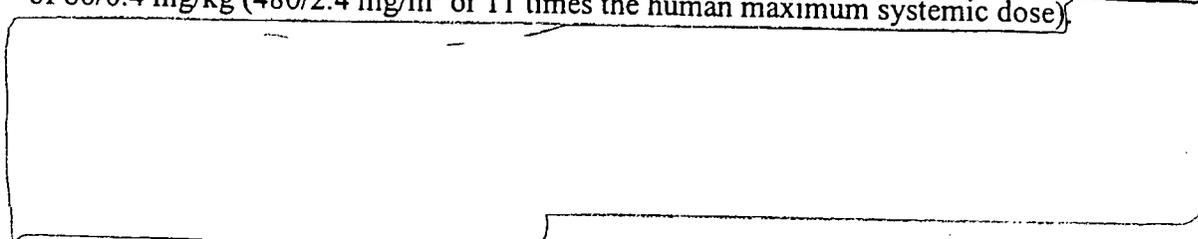
RECOMMENDATIONS

The following modifications should be made to the label:

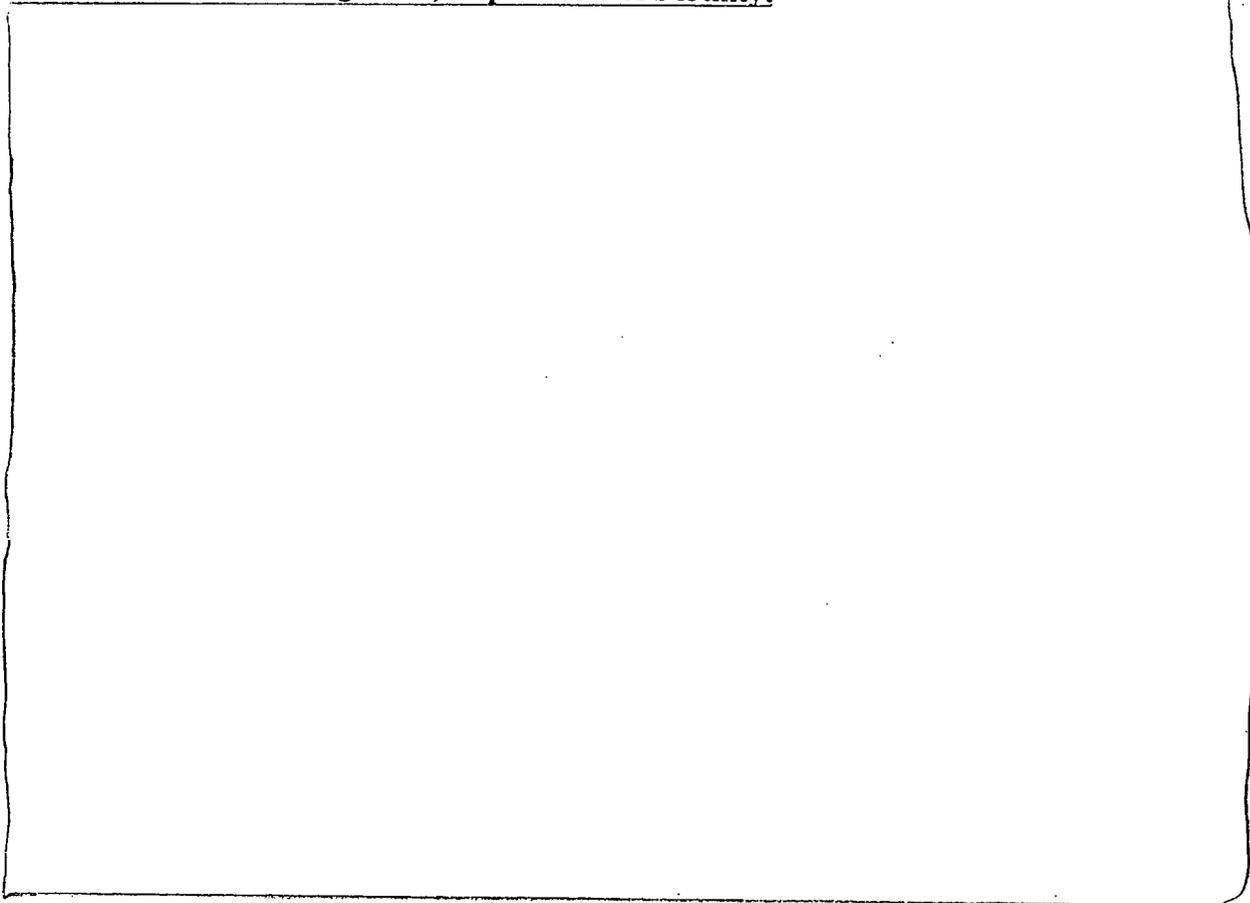
- **CONTRAINDICATIONS:** [REDACTED]

In a dermal teratology study in New Zealand White rabbits, there were no statistically significant differences among treatment groups in fetal malformation data; however, marked hydrocephaly with visible doming of the head was observed in one mid-dose litter (12/0.06 [4-hydroxyanisole/tretinoin] mg/kg, 132/0.66 mg/m²) and two fetuses in one high dose litter (40/0.2 [4-hydroxyanisole/tretinoin] mg/kg, 440/2.2 mg/m²) TRADENAME, and two high-dose tretinoin (0.2 mg/kg, 2.2 mg/m²) treated [REDACTED] litters. These [REDACTED] malformations were considered to be treatment-related and due to the known effects of tretinoin. This was further supported by coincident appearance of other malformations associated with tretinoin, such as cleft palate and appendicular skeletal defects. [REDACTED] No effects attributed to treatment were observed in rabbits treated with 4-hydroxyanisole alone (dose 40 mg/kg, 440 mg/m²). A no-observed effect level for teratogenicity in rabbits was established at 4/0.02 mg/kg (44/0.22 mg/m²) for TRADENAME, which is approximately the maximum possible human [REDACTED]

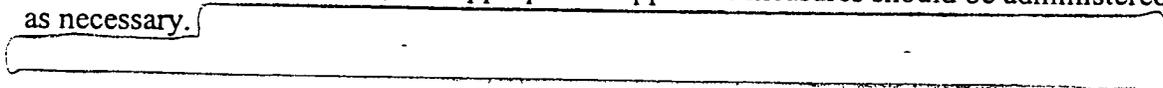
TRADENAME was not teratogenic in Sprague-Dawley rats when given in topical doses of 80/0.4 mg/kg (480/2.4 mg/m² or 11 times the human maximum systemic dose).



- **Carcinogenesis, Mutagenesis, Impairment of Fertility:**



- **OVERDOSAGE:** If TRADENAME is applied excessively, no more rapid or better results will be obtained and marked redness, peeling, or discomfort may occur. Oral ingestion of the drug may lead to the same adverse effects as those associated with excessive oral intake of vitamin A (hypervitaminosis A). If oral ingestion occurs, the patient should be monitored, and appropriate supportive measures should be administered as necessary.



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NDA 20-922

Review of Pharmacology and Toxicology Data

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[Redacted] /S/

Amy C. Nostrandt, D.V.M., Ph.D.
Pharmacologist/Toxicologist

11/16/98

cc:

NDA 20-922

HFD-340

HFD-540

HFD-540/PHARM/Nostrandt

HFD-540/TLPHARM/Jacobs

HFD-540/MO/Cook

HFD-540/CHEM/Timmer

HFD-540/PMS/Cross

Concurrence Only:

HFD-540/DD/WILKIN

HFD-540/TLPHARM/JACOBS

[Redacted] /S/

11/16/98

As above, It is not known whether tretinoin can act as a promoter on already UV-initiated skin. If that is the case, then the presence of actinic lentiginos increases the probability that the skin to be treated already has UV-initiated cells. If tretinoin is a promoter in the dark, then application at night, sunscreens, and sun avoidance will be insufficient protection. Labeling is deferred.

[Redacted] /S/

11/18/98

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

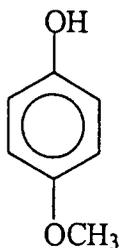
NDA No.: 20-922 (BZ)

Date Submitted: 2/5/99
 Date CDER Received: 2/8/99
 Date Assigned: 2/10/99
 Date Review Completed: 2/11/99

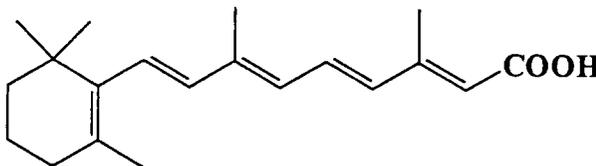
Name of Drug: 2% 4-hydroxyanisole (4-methoxyphenol, mequinol, hydroquinone methyl ether, BMS-181158), and 0.01% All-Trans Retinoic Acid (ATRA, tretinoín, BMS-181159) solution

Structure:

4-hydroxyanisole
 $C_7H_8O_2$
 MW=124.14



all-trans retinoic acid
 $C_{20}H_{28}O_2$
 MW=300.44



Pharmacological Category: depigmenting agent

Sponsor: Bristol-Myers Squibb

Indication: treatment of solar lentigines resulting from chronic sun exposure

Route of Administration: topical

**APPEARS THIS WAY
 ON ORIGINAL**

Formulation:

ingredient	% (w/v)	% (v/v)
4-hydroxyanisole	2.0	
tretinoin, USP	0.01	
ethyl alcohol, USP		77.8
PEG-8 (polyethylene glycol 400, NF)		
butylated hydroxytoluene, NF		
ascorbic acid, USP		
citric acid, USP		
ascorbyl palmitate, NF		
disodium EDTA, USP		
purified water, USP		

Related submissions: IND [redacted]

INTRODUCTION

The current submission includes new information regarding an impurity found in the 4-hydroxyanisole supplied by the new manufacturer, using a different synthesis from that used by the supplier of that material that was used in nonclinical and clinical studies. The material from [redacted] contains [redacted] and updated specifications are for this impurity to be up to [redacted] of the drug substance.

A one-half page summary is provided to support the safety of this impurity. The sponsor states that [redacted] is considered GRAS in foods. This is not relevant information to dermal exposure to this material, and provides no dose-response information. The sponsor also cites a literature review that documented single dose toxicity (oral LD₅₀ in rats = 3.6 g/kg; dermal LD₅₀ in rabbits = 5.0-g/kg). It may be of concern that, even though at a large dose, there may have been transcutaneous absorption of a single dose of [redacted] in rabbits that was sufficient to be lethal.

The sponsor states that human dermal safety trials revealed no irritation or sensitization at a concentration of 4% in an unspecified vehicle. They also state that the material has been found to be inactive for depigmentation in man.

The sponsor was requested by the project manager to provide the literature reference used as the basis of the provided summary (Food Chem. Toxicol. 16:715-6, 1978). It was provided by fax on 2/10/99 and consisted of a one page summary of literature citations. A copy will be officially submitted to the NDA. The following additional information was present:

No effects were seen in oral studies where [redacted] was included as up to 10% in the diet of rabbits for 1-2 months or was fed at up to 6 g/day for 1 month to three dogs. In rats fed [redacted] as 2-10% of the diet for 5 weeks, growth retardation was seen.

In dermal studies, slight to moderate irritation was seen in guinea pigs. The dermal LD₅₀ in rabbits is actually described as >5 g/kg; higher doses may not have been examined. In a 30-day topical study in rabbits (treated 5 days/week) at 1 or 10% [redacted] in 5 ml suntan lotion, the only effects were gross pitting of the kidney surface and epidermal atrophy, ulceration and marked irritation with sloughing. When exposure was limited to three hours/day for 30 days, skin effects were limited to mild to moderate erythema and sloughing.

In a study of toxicity of [redacted] was less toxic than [redacted] but did produce "slight cardiac disturbances and definite degenerative changes in liver and kidney." The article notes that the latter information is from an abstract that did not identify either the species or route of administration used.

COMMENTS

It is assumed that [redacted] was not present in the material used in previous nonclinical studies and was therefore not qualified in those studies. According to the new specifications provided in this submission, the only other potential impurity that may comprise more than [redacted] of the drug substance is [redacted] which is allowed at up to [redacted] of the drug substance.

CONCLUSIONS

Since the sponsor's specifications for [redacted] allow it to comprise up to [redacted] of the drug substance, data to support the safety of this impurity as it relates to the proposed use of their drug should be provided, i.e. dermal safety of chronic use. The same applies for any other impurities identified in the 4-hydroxyanisole from the new synthesis \geq [redacted] of the drug substance that were not present in the material used in the nonclinical testing. The provided information does include dermal toxicology testing of [redacted] for up to 30 days in rabbits and indicates potential for local effects as well as nephrotoxicity, hepatotoxicity, and cardiotoxicity. NOEL's were not established. In addition to full study reports of the data being unavailable, the referenced studies are quite old and predate GLP's. Under the circumstances it would seem prudent to perform at least one bridging study to demonstrate equivalent safety of the product containing [redacted] to the product used in long-term nonclinical studies.

RECOMMENDATIONS

Based on the provided nonclinical information, the drug product is approvable provided that:

1. The previously recommended label changes are made.
2. The applicant agrees to a phase 4 commitment to perform a 4-week bridging study in rabbits to demonstrate equivalency of the new 4-hydroxyanisole to that used in previous animal studies.

Alternatively, if analysis of reserve test article from long-term nonclinical studies reveals similar levels of the same impurity(ies) to that found in the new material, then those studies may be considered sufficient to qualify the impurity.

**APPEARS THIS WAY
ON ORIGINAL**

[redacted] /S/ 2/11/99
Amy C. Nostrandt, D.V.M., Ph.D.
Pharmacologist/Toxicologist

cc:

NDA

HFD-340

HFD-540

HFD-540/PHARM/Nostrandt

HFD-540/TLPHARM/Jacobs

HFD-540/MO/Cook

HFD-540/CHEM/Timmer

HFD-540/PMS/Cross

[Redacted]

Concurrence Only:

HFD-540/DD/WILKIN **TS** 2/29/99

HFD-540/TLPHARM/JACOBS **151** 2/11/99

**APPEARS THIS WAY
ON ORIGINAL**

FEB 17 1999

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

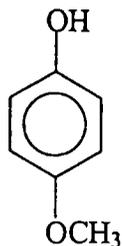
NDA No.: 20-922 (BL, #015)

Date Submitted: 2/5/99
Date CDER Received: 2/8/99
Date Assigned: 2/16/99
Date Review Completed: 2/17/99

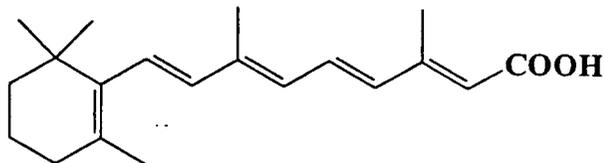
Name of Drug: 2% 4-hydroxyanisole (4-methoxyphenol, mequinol, hydroquinone methyl ether, BMS-181158), and 0.01% All-Trans Retinoic Acid (ATRA, tretinoin, BMS-181159) solution

Structure:

4-hydroxyanisole
 $C_7H_8O_2$
MW=124.14



all-trans retinoic acid
 $C_{20}H_{28}O_2$
MW=300.44



Pharmacological Category: depigmenting agent

Sponsor: Bristol-Myers Squibb

Indication: treatment of solar lentigines resulting from chronic sun exposure

Route of Administration: topical

APPEARS THIS WAY
ON ORIGINAL

Formulation:

ingredient	% (w/v)	% (v/v)
4-hydroxyanisole	2.0	
tretinoin, USP	0.01	
ethyl alcohol, USP		77.8
PEG-8 (polyethylene glycol 400, NF)		[redacted]
butylated hydroxytoluene, NF	[redacted]	
ascorbic acid, USP	[redacted]	
citric acid, USP	[redacted]	
ascorbyl palmitate, NF	[redacted]	
disodium EDTA, USP	[redacted]	
purified water, USP	[redacted]	

Related submissions: IND [redacted]

INTRODUCTION

The following incorporates changes to the pharmacology and toxicology sections of the label relative to previous recommendations, as decided in team labeling meetings on 2/16/99.

The major difference between the revised label and that included in this submission is the inclusion of some data from the repeated developmental toxicology study in rabbits and alteration of the pregnancy category from X [redacted] with [redacted]

[redacted] While balanced inclusion of data from the second study might be reasonable, the discussion of that study provided by the sponsor neglects to mention that maternal toxicity was not seen, although it is stated that this study is similar to a published study of Renova, in which maternal toxicity was documented at the high dose. The label also fails to mention teratogenicity seen in the tretinoin only dose group in the repeat study. An additional concern is that no pharmacokinetic evaluation was performed in the second study to confirm the sponsor's assumption that terata seen in the original study were the result of ingestion of the test material. It is important to note that plasma tretinoin levels in the original study were below the level of quantitation, even in dose groups where retinoid-associated malformations were seen.

Based on the above and the previous review of the repeated developmental toxicology study in rabbits, it is recommended that the pregnancy category remain X, and the label revisions remain as determined in the labeling meeting of 2/16/99. The repeated study was not included, as the length of explanation required to describe differences between the two studies and the flaws in the design of the second study would be excessive, and the discussion would not provide the physician with useful information.

RECOMMENDATIONS

1. Under **CLINICAL PHARMACOLOGY**, the paragraph describing [redacted] was removed.

2. Under **CONTRAINDICATIONS**:

[redacted] The combination of 4-hydroxyanisole and tretinoin may cause fetal harm when administered to a pregnant woman.

In a dermal teratology study in New Zealand White rabbits, there were no statistically significant differences among treatment groups in fetal malformation data; however, marked hydrocephaly with visible doming of the head was observed in one mid-dose litter [redacted] 12 and 0.06 mg/kg or 132 and 0.66 mg/m² of 4-hydroxyanisole and tretinoin, respectively) and two fetuses in one high dose litter [redacted] 40 and 0.2 mg/kg or 440/2.2 mg/m² of 4-hydroxyanisole and tretinoin, respectively) TRADENAME, and two high-dose tretinoin (0.2 mg/kg, 2.2 mg/m²) treated litters. These malformations were considered to be treatment-related and due to the known effects of tretinoin. This was further supported by coincident appearance of other malformations associated with tretinoin, such as cleft palate and appendicular skeletal defects. No effects attributed to treatment were observed in rabbits in that study treated topically with 4-hydroxyanisole alone (dose 40 mg/kg, 440 mg/m²). A no-observed effect level (NOEL) for teratogenicity in rabbits was established at 4 and 0.02 mg/kg (44 and 0.22 mg/m² 4-hydroxyanisole and tretinoin, respectively) for TRADENAME, which is approximately the maximum possible human systemic exposure, based on clinical application to 5% of total body surface area. Plasma [redacted] concentrations in rabbits at the NOEL at one hour after application were 124 ng/ml, or approximately twelve times the mean peak plasma concentrations of that substance seen in human subjects in a clinical pharmacokinetics study.

[redacted]

In a published study in albino rats (J. Am. Coll. Toxicology 4(5):31-63, 1985), topical application of 5% 4-hydroxyanisole in a cream vehicle during gestation was embryotoxic and embryolethal.

TRADENAME was not teratogenic in Sprague-Dawley rats when given in topical doses of 80 and 0.4 mg/kg 4-hydroxyanisole and tretinoin, respectively (480 and 2.4 mg/m² or 11 times the human maximum systemic dose) [redacted]

[redacted]

.....

[redacted]

3. Under Carcinogenesis, Mutagenesis, Impairment of Fertility:

Although a dermal carcinogenicity study in CD-1 mice indicated that TRADENAME applied topically at daily doses up to 80 and 0.4 mg/kg (240 and 1.2 mg/m²) of 4-hydroxyanisole and tretinoin, respectively, representing approximately 5 times the maximum possible systemic human exposure was not carcinogenic, in a photocarcinogenicity study utilizing Crl:Skh-

1(hr/hr BR) hairless albino mice, median time to onset of tumors decreased. Also, the number of tumors increased in all dose groups administered 1.4, 4.3, or 14 µl applied per cm² of skin.

Similar animal studies have shown an increased tumorigenic risk with the use of retinoids when followed by ultraviolet radiation. Although the significance of these studies to human use is not clear, patients using this product should be advised to avoid exposure to either sunlight or artificial ultraviolet irradiation sources.

4-Hydroxyanisole was non-mutagenic in the Ames/Salmonella assay using strains TA98, TA100, TA1535, and TA1537, all of which are insensitive to mutagenic effects of TRADENAME was non-genotoxic in an *in vivo* dermal micronucleus assay in rats.

A dermal reproduction study with TRADENAME in Sprague-Dawley rats at a daily dose of 80 and 0.4 mg/kg (480 and 2.4 mg/m²) of 4-hydroxyanisole and tretinoin, respectively, approximately 11 times the maximum possible human exposure, showed no impairment of fertility.

4. Under OVERDOSAGE:

If TRADENAME is applied excessively.....If oral ingestion occurs, the patient should be monitored, and appropriate supportive measures should be administered as necessary. An oral dose of 5.0 ml/kg (30 ml/m²)

Clinical signs observed were related to the high alcohol content (77%) of the drug formulation.

/S/ Amy C. Nostrandt, D.V.M., Ph.D. 2/17/99
Pharmacologist/Toxicologist

- cc:
- NDA
- HFD-340
- HFD-540
- HFD-540/PHARM/Nostrandt
- HFD-540/TLPHARM/Jacobs
- HFD-540/MO/Cook
- HFD-540/CHEM/Timmer
- HFD-540/PMS/Cross

Concurrence Only:
HFD-540/DD/WILKIN /S/ 3/17/99
HFD-540/TLPHARM/JACOBS /S/ 1/2/99

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

MAR 18 1999

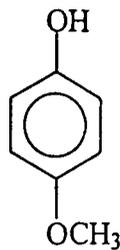
NDA No.: 20-922 (BL, #015)

Date: 3/12/99

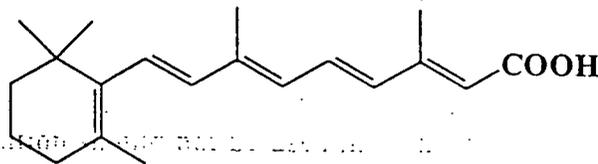
Name of Drug: 2% 4-hydroxyanisole (4-methoxyphenol, mequinol, hydroquinone methyl ether, BMS-181158), and 0.01% All-Trans Retinoic Acid (ATRA, tretinoin, BMS-181159) solution

Structure:

4-hydroxyanisole
 $C_7H_8O_2$
MW=124.14



all-trans retinoic acid
 $C_{20}H_{28}O_2$
MW=300.44



Pharmacological Category: depigmenting agent

Sponsor: Bristol-Myers Squibb -

Indication: treatment of solar lentigines resulting from chronic sun exposure

Route of Administration: topical

APPEARS THIS WAY
ON ORIGINAL

Formulation:

ingredient	% (w/v)	% (v/v)
4-hydroxyanisole	2.0	
tretinoin, USP	0.01	
ethyl alcohol, USP		77.8
PEG-8 (polyethylene glycol 400, NF)		
butylated hydroxytoluene, NF		
ascorbic acid, USP		
citric acid, USP		
ascorbyl palmitate, NF		
disodium EDTA, USP		
purified water, USP		

Related submissions: IND

INTRODUCTION

A discussion with the sponsor regarding the proposed pregnancy category and the repeated rabbit developmental toxicology studies took place on 3/11/99. The applicant originally proposed a pregnancy category X for their product based on the occurrence of retinoid-associated malformations in a rabbit developmental toxicology study employing topical exposure. They have since repeated that study, taking precautionary measures to prevent ingestion and to limit transdermal exposure in a manner that is inconsistent with the proposed clinical use. The formulation is an ethanolic solution, and it is unlikely that the animals in the original study were inclined to ingest it six hours after application, when collars were removed and the animals were returned to the cage. It is even less likely that it was possible to wipe it off onto the cage and ingest it.

The applicant has not produced pharmacokinetic evidence that the material was ingested; had it been ingested, then 4-hydroxyanisole would have been ingested in a proportional manner to tretinoin and should have been present in the plasma in unusually high concentrations. Plasma 4-hydroxyanisole concentrations were not more than 50% higher than in rats receiving the same doses; this much of a difference is not unexpected, given that xenobiotics are often found to be more readily absorbed across the skin of the rabbit than in the rat. Tretinoin might also have been detectable had the material been ingested. The applicant stated that the single blood sampling timepoint was prior to ingestion (at one hour after application, prior to collar removal). However, the sample was taken on gestation day 18, at the end of the dosing period. It was previously shown in rats that, even with dermal application, plasma concentrations of 4-hydroxyanisole are still present at the same dose as the high dose in the rabbit study at 24 hours. Plasma levels were also shown to increase over time after dermal exposure in rats at all doses. If the material were ingested, then an increase in plasma levels would be expected over a 12 day treatment period, and the 4-hydroxyanisole concentrations at the sampling timepoint should be higher in animals that had ingested the material than in animals that did not by more than an order of magnitude. Furthermore, even if it were assumed that the animals did ingest the material, the plasma levels of 4-hydroxyanisole at doses of the combination product in which retinoid-associated teratogenicity was seen were only one order of magnitude greater than the C_{max} values reported in human pharmacokinetic study subjects.

The applicant also noted that no effects were seen in the group in the first study treated with 4-hydroxyanisole alone. This is in contrast to a report of a study submitted by the applicant which documents increased preimplantation loss and reduced fetal body weights in dams treated with a 5% cream containing 4-hydroxyanisole. Coincidentally, increased preimplantation loss was seen in 4-hydroxyanisole treated groups relative to controls in the first study and in the high dose combination and tretinoin only groups in the second study. If the first dose on gestational day 6 was truly after implantation occurred, then this is not likely to be a treatment-related effect. However, if implantation occurred after application of the first dose, this may be a drug-related effect.

Regarding the assignment of a pregnancy category designation, the category X designation originally proposed by the applicant seemed appropriate, based on the original data and on the lack of any evidence of ingestion of test material in the initial rabbit study. It is important to note that the incidence of holoprosencephaly in human fetuses of patients treated with topical tretinoin in the first trimester of pregnancy is approximately 92-fold that of the population background incidence (Rosa F., Teratology 49:418-9, 1994). It is unknown what embryotoxic contribution 4-hydroxyanisole may make in clinical patients, and it is unclear whether or not tretinoin will have fetal effects in these patients under the conditions of use. Tretinoin effects may be possible, since tretinoin-related teratogenicity does occur at plasma concentrations that are indistinguishable from background. The combination may enhance developmental effects, and/or the vehicle may allow for greater absorption of one or both components, relative to marketed products.

Nonclinical studies were positive for developmental toxicity and support a pregnancy category X designation. In summary, the reasons for maintaining the pregnancy category as an X are as follows:

- 1) Retinoid-related teratogenicity was seen at doses resulting in plasma C_{max} values of 4-hydroxyanisole that were only one order of magnitude greater than those seen in human pharmacokinetics study subjects. The safety factor, based on plasma levels at the NOEL is barely one order of magnitude.
- 2) A study in the literature referenced in a comprehensive review submitted by the applicant indicated that 5% 4-hydroxyanisole was lethal to pre-implantation rabbit embryos. This effect was suggested as well in the original segment II rabbit study.

RECOMMENDATIONS

From a pharmacology/toxicology standpoint, the positive developmental toxicology data and the small safety factor for species extrapolation support a pregnancy category X designation. It is important that the label warnings against administration during pregnancy be strong, particularly in light of the evidence that the human exposure data for 4-hydroxyanisole is within one order of magnitude of that in animals at doses of the combination product in which retinoid-associated teratogenicity was seen, regardless of the route of administration.

Description of the repeated rabbit developmental toxicology study in the label should make it clear that ingestion in the first study was not confirmed and that transdermal absorption in the second study was artificially limited in a manner that is not relevant to the clinical use of this drug. Addition of a description of the repeated rabbit study and referenced information regarding 4-hydroxyanisole should be inserted as follows:

In a dermal teratology study in New Zealand White rabbits, there were no statistically significant differences among treatment groups in fetal malformation data; however, marked hydrocephaly with visible doming of the head was observed in one mid-dose litter (12 and 0.06 mg/kg or 132 and 0.66 mg/m² of 4-hydroxyanisole and tretinoin, respectively) and two fetuses in one high dose litter (40 and 0.2 mg/kg or 440/2.2 mg/m² of 4-hydroxyanisole and tretinoin, respectively) TRADENAME, and two high-dose tretinoin (0.2 mg/kg, 2.2 mg/m²) treated litters. These malformations were considered to be treatment-related and due to the known effects of tretinoin. This was further supported by coincident appearance of other malformations associated with tretinoin, such as cleft palate and appendicular skeletal defects. No effects attributed to treatment were observed in rabbits in that study treated topically with 4-hydroxyanisole alone (dose 40 mg/kg, 440 mg/m²). A no-observed effect level (NOEL) for teratogenicity in rabbits was established at 4 and 0.02 mg/kg (44 and 0.22 mg/m² 4-hydroxyanisole and tretinoin, respectively) for TRADENAME, which is approximately the maximum possible human [redacted] exposure, based on clinical application to 5% of total body surface area. Plasma [redacted] concentrations in rabbits at the NOEL at one hour after application were 124 ng/ml, or approximately twelve times the mean peak plasma concentrations of that substance seen in human subjects in a clinical pharmacokinetics study.

In a repeated study in pregnant rabbits administered the same dose levels as the study described above, additional precautionary measures were taken to prevent ingestion, although there is no evidence to confirm that ingestion occurred in the initial study. Precautionary measures additionally limited transdermal absorption to a six-hour exposure period [redacted]. [redacted] This study did not show any significant teratogenic effects at doses up to approximately 13 times the human dose on a mg/m² basis. However, a

concurrent tretinoin dose group (0.2 mg/kg/day) did include two litters with limb malformations;

In a published study in albino rats (J. Am. Coll. Toxicology 4(5):31-63, 1985), topical application of 5% 4-hydroxyanisole in a cream vehicle during gestation was embryotoxic and embryolethal. Embryonic loss prior to implantation was noted in that study where animals were treated throughout gestation. Coincidentally, mean preimplantation embryonic loss was increased in the first rabbit study in all 4-hydroxyanisole treated groups relative to control, and in the high dose 4-hydroxyanisole/tretinoin and tretinoin only treated groups in the second study. In those studies, dosing began at gestation day 6, when implantation is purported to occur.

No adequate or well-controlled trials have been conducted with SOLAGE in pregnant women.

TRADENAME was not teratogenic in Sprague-Dawley rats when given in topical doses of 80 and 0.4 mg/kg 4-hydroxyanisole and tretinoin, respectively (480 and 2.4 mg/m² or 11 times the human [redacted] dose).

[redacted]

.....

[redacted]

[redacted] /S/

Amy C. Nostrandt, D.V.M., Ph.D.
Pharmacologist/Toxicologist

3/15/99

- cc:
 - NDA
 - HFD-340
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- [redacted]

Concurrence Only:

HFD-540/DD/WILKIN /S/ 3/16/99

HFD-540/TLPHARM/JACOBS /S/ 3/15/99

NOV 10 1998

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

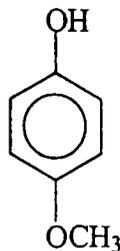
NDA No.: 20-922 (BZ)

Date Submitted: 9/17/98
Date CDER Received: 9/28/98
Date Assigned: 10/5/98
Date Review Completed: 11/6/98

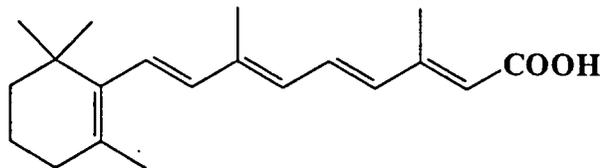
Name of Drug: 2% 4-hydroxyanisole (4-methoxyphenol, mequinol, hydroquinone methyl ether, BMS-181158), and 0.01% All-Trans Retinoic Acid (ATRA, tretinoin, BMS-181159) solution

Structure:

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all-trans retinoic acid
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MW=300.44



Pharmacological Category: depigmenting agent

Sponsor: Bristol-Myers Squibb

Indication: treatment of solar lentigines resulting from chronic sun exposure

Route of Administration: topical

Formulation:

ingredient	% (w/v)	% (v/v)
4-hydroxyanisole	2.0	
tretinoin, USP	0.01	
ethyl alcohol, USP		77.8
PEG-8 (polyethylene glycol 400, NF)		
butylated hydroxytoluene, NF		
ascorbic acid, USP		
citric acid, USP		
ascorbyl palmitate, NF		
disodium EDTA, USP		
purified water, USP		

Related submissions: IND [redacted]

INTRODUCTION

The current submission contains a final report of a dermal developmental toxicology study in rabbits. A similar study had been conducted previously, but retinoid-associated malformations were seen in the mid- and high dose groups treated with the 4-hydroxyanisole/tretinoin combination and in the group treated with tretinoin only (see reviews of serial # 010). On the basis of those study results, the sponsor originally proposed Pregnancy category X for their labeling. The rabbit developmental toxicology study was repeated with additional precautions taken to prevent ingestion of the test article. The sponsor has requested modification of the proposed labeling to reflect the results of the new study and specifically a change of pregnancy category from X [redacted]

In the original IND, the sponsor also provided a report from the published literature in which 4-hydroxyanisole alone, applied topically as either a 5% or 25% formulation was embryotoxic and embryolethal, though not teratogenic.

NONCLINICAL TOXICOLOGY STUDIES

1. Study title: Dermal study of embryo-fetal development in rabbits

Study number: 98612

Performing organization: [redacted]

Drug lot and batch: BMS-181158/BMS-181159 solution, lot # B97B003-2; 0.01% BMS-181159 solution, lot # B95L007-1

Date of study: 3/2-27/98 and 3/16/98-4/10/98

GLP compliance: yes

Study design:

Dosing: once daily, topically to clipped areas of the skin of the back (12 x 14 cm, approximately 10% of total body surface area), unoccluded for 6 hours per day on days 6-18 of gestation. (Reviewer's comment: The label specifies dosing twice daily for human patients.)

Dose groups: untreated control, vehicle (2 ml/kg/day), 0.2, 0.6, 2.0 ml/kg/day of 2% 4-hydroxyanisole/0.01% tretinoin solution (4/0.02, 12/0.06, 40/.2 mg/kg/day or 48/0.24, 144/0.72, 480/2.4 mg/m²/day of 4-hydroxyanisole/tretinoin, respectively), or 1.3, 3.9, and 13 times the human dose on a mg/m² basis. Another group received 0.2 mg/kg/day (2.4 mg/m²/day) tretinoin.

The doses of the 4-hydroxyanisole/tretinoin combination product represent 0.043/0.0002, 0.13/0.0006, and 0.43/0.002 mg 4-hydroxyanisole/tretinoin per cm² area of application, respectively, or 0.6, 1.8, and 5 times the local application dose in humans.

Formulation: clinical formulation of 2% 4-hydroxyanisole/0.01% tretinoin

Test animals: New Zealand White [Hra:(NZW)SPF] rabbits, 20 pregnant females/group, conducted as two consecutive replicate experiments with groups of 10 animals each. Some animals (1-2/group) were removed from the first replicate for reasons unrelated to treatment and were replaced with additional animals in the second replicate. (*Reviewer's comment: This appears to be two pooled experiments, where each individually consisted of too few animals per treatment group to assess the potential for developmental toxicity, according to ICH recommendations.*) Animals were restrained in stocks during exposure to the test article for 6 hours per day. The application sites were then washed with warm water and soap and dried. The rabbits were collared with Elizabethan collars and returned to clean cages. Caesarean sections were performed on gestation day 29 to examine reproductive organs and fetuses. (*Reviewer's comment: The treatment methodology in the previous study was similar, with the exceptions that the animals were collared only during the 6-hour exposure period and that half of the dams in each group were treated at abraded skin sites. The only other difference between the two studies was the inclusion of the washing step after the exposure period in the current study.*)

Findings:

Deaths: One dam in the vehicle control group and one dam in the 0.2 mg/kg tretinoin group died due to causes unrelated to treatment.

Clinical signs: Significant dose-related irritation of the skin was seen. Erythema was observed in the vehicle control group, 4-hydroxyanisole/tretinoin combination groups, and the tretinoin group and was dose-related in severity. Dose-related edema was seen in all 4-hydroxyanisole/tretinoin combination groups and in the tretinoin group. Dose-related desquamation and fissuring were seen in the middle and high dose 4-hydroxyanisole/tretinoin combination groups and the tretinoin group. A low incidence of desquamation was noted in the vehicle control group, but was not statistically significant. Atonia and hyperreactivity were seen in the high dose combination and tretinoin treated groups. The maximum grades for all indices of irritation were described as moderate.

Other clinical observations included abnormal feces, red substance in the cage pan (usually associated with abortion), head tilt, vocalization, and red substance in the urine. The incidence of these findings was not dose-related.

Small weight losses or lack of weight gains were seen in dams during the pre-dose period, during which the animals were acclimated to the stocks and Elizabethan collars. All groups, including controls, lost weight during gestation days 6-12. All groups gained weight during the remainder of the dose period, but the weight change over the entire dose period averaged a net loss for all groups. After dosing was discontinued, animals in each group gained an average of 400-470 g. Food consumption similarly decreased in all dose groups, including controls, during the dosing period and increased afterwards.

A true maternally toxic dose did not appear to be achieved, presumably due to the short exposure time (6 hours).

Reproductive/fetal parameters: Abortions occurred in all groups in a manner unrelated to dose: 3, 2, 3, 4, 3, and 1 dam in each of the untreated, vehicle, low, mid, and high dose combination, and tretinoin groups, respectively. Premature delivery on gestation day 29

occurred in one animal in each of the untreated and vehicle control groups. No information from these litters or from litters from animals found dead or euthanized in extremis was included in fetal and litter evaluations, even though many were late in term.

There were no significant differences in the numbers of corpora lutea or implantation sites, litter size, live fetuses, early or late resorptions, fetal body weights, sex ratios, or post-implantation loss. There were no dead fetuses in any group. However, the mean percent resorbed conceptuses per litter in the tretinoin group was higher than control, but was not statistically significant due to a very large standard deviation. Pre-implantation loss appeared slightly higher in the high dose combination and tretinoin-treated groups.

Fetal evaluations were made from 16, 15, 17, 16, 17, and 17 litters in the control, vehicle, low, middle, and high dose combination and tretinoin groups, respectively. The sponsor's statistical testing of the litter incidence of total alterations demonstrated that their incidence was similar across litters. Similar testing was not performed for the incidences of external, soft tissue, or skeletal malformations or variations. The vast majority of alterations were skeletal. The following alterations were noted that may be of concern:

- Two fetuses in two litters in the group receiving tretinoin only had limb malformations consisting of absence of the pollex and the associated skeletal malformations of the metacarpals and phalanges.
- One litter in the low dose combination group included a fetus with a cardiac malformation described as large heart and large vessels.
- Ten fetuses in six litters in the mid-dose combination group exhibited a variation in skull ossification described as displaced nasal midline suture. Statistically, there was a significant increase in the fetal incidence, but not the litter incidence of this variation. The incidence of this finding was 1, 2, 1, 2, and 1 litter(s) in the untreated, vehicle, low dose combination, high dose combination, and tretinoin treated groups, respectively.
- In the high dose combination group, three fetuses in two litters were noted with irregularly shaped scapular alae. One of those fetuses had additional alterations. Again, these represented a statistically significant increase in fetal incidence, but not in litter incidence of this variation. One fetus in one untreated control litter also had this alteration.
- Four litters in the high dose combination group were seen to include skull alterations of irregular ossification or fusion of frontal bones. The litter incidence was not statistically significantly higher than the incidences in other groups.
- Irregular ossification of the nasal bones of the skull, including displace midline suture occurred in 3, 2, 4, 6, 4, and 5 litters in the untreated, vehicle, low, middle, and high dose combination and tretinoin-treated groups, respectively. No statistical differences were noted, but the incidences do seem higher in drug-treated groups.
- A skull alteration described as a small eyesocket was noted in 1 litter in the tretinoin-treated group.
- Alterations to thoracic vertebrae appeared to be of significantly higher incidence in the high dose combination group.

Pathological examination: At necropsy, none of the findings in the dams were dose-dependent. Findings included clear gelatinous material in the stomach (reported to be associated with abortion), light green caseous material adhered to the endometrium in the uterine horn, gas

distention in the gastrointestinal tract, red brown or brown perivaginal substance (reported to be associated with unscheduled death or abortion), and green brown, red brown, or brown perianal substance.

COMMENTS

The developmental toxicology study in rabbits previously submitted to the original NDA was performed using adequate precautions to prevent ingestion: the rabbits wore an Elizabethan collar during the six-hour exposure period, after which the application sites were wiped with gauze. The only differences in performance of the current study were that animals were restrained in stocks for the 6-hour exposure period, no animals had abraded application sites, and the application sites were washed with soap and water at the end of the treatment period. In light of this, it is important to note that the current study does not negate the previous study. Systemic drug exposures in the current study may have been lower than in the previous study due to the short exposure period followed by removal of all residual test material and potentially some portion of the surface epithelium with associated test material due to the mechanical action of washing the site. Because precautions were taken in both studies to avoid ingestion of the test article, it seems unlikely that differences between results of the two studies were due solely to ingestion of the material in the first study.

It is important to note that the exposure period in both studies was once daily for six hours only. Clinical dosing is described in the label as twice daily, at least eight hours apart. It would appear that the duration of topical exposure in both rabbit teratology studies was considerably less than that experienced by clinical patients, i.e. drug was available at the skin surface for absorption for only 6 hours per day, while in humans it will likely be present at the skin surface for upwards of 24 hours per day. Similar studies have been accepted for previous tretinoin products, but the labeled dosing for those drugs was once daily, at night. The sponsor included with the submission a published report of a similar study using Renova[®] (tretinoin) in rabbits. The results of that study were negative, but doses were considered maternally toxic based on persistent weight loss and decreased feed consumption. In the current study, weight loss, if present, was slight and not persistent, and it occurred similarly in all dose groups, including controls. Feed consumption was similarly affected. The doses used in the current study are claimed to be maternally toxic based on local skin reactions alone; this seems insufficient in light of the severity of the local irritation (moderate) and the lack of other persistent maternal effects.

There is no data available for pharmacokinetic comparison between these rabbits and human patients. It has previously been demonstrated that deviations from baseline serum tretinoin concentrations are undetectable even at overtly teratogenic doses in rabbits, presumably due to cellular uptake of tretinoin and rapid metabolism of tretinoin to pharmacologically and toxicologically active substances, such as isotretinoin. Since this drug product is a combination of tretinoin with a substance that is not endogenous, pharmacokinetic data might have at least allowed some estimate of relative exposure based on 4-hydroxyanisole concentrations. The different ADME characteristics of the two drugs would prevent direct extrapolation, but could have provided some general estimate. Pharmacokinetic sampling was performed in the earlier rabbit developmental toxicology study: Mean peak plasma concentrations of 4-hydroxyanisole were of 192 ng/ml or higher in groups treated with combination drug in which retinoid associated

malformations were seen, and 80-124 ng/ml at the NOEL. As expected, mean tretinoin concentrations were considered indistinguishable from background. In a pharmacokinetic study in humans, the average peak serum concentration of 4-hydroxyanisole was approximately 10 ng/ml, with an AUC_{0-12h} of 33 ng·hr/ml.

For a study of this type, in which the endpoints examined are dependent on systemic exposure, ingestion of topically applied material does not necessarily invalidate the study. As long as there is some measure of comparative systemic exposure that can be compared between clinical patients treated in accordance with the proposed label and animals in which a toxicologic effect was seen, the data are still useful. In the case of the original rabbit developmental toxicology study, peak 4-hydroxyanisole concentrations were 80-124 ng/ml at the NOEL (4/0.02 mg 4-hydroxyanisole/tretinoin per kg/day, or 48/0.24 mg/m², or 1.3 times the maximum human dose). In the human pharmacokinetic study, peak 4-hydroxyanisole concentrations averaged approximately 10 ng/ml, or approximately 8 to 12-fold lower.

CONCLUSIONS

In short, the current study does not conclusively relieve concern regarding the risk of developmental toxicity as a result of topical treatment with 4-hydroxyanisole/tretinoin. The study was designed to minimize exposure to experimental animals while the clinical regimen would seem to maximize exposure to patients. The experimental exposure time in the current study is too short to extrapolate to twice daily dosing in clinical patients. In the previous study in which developmental toxicity was seen, the NOEL was 1.3 times the maximum human dose. Limb malformations seen in the current study in the tretinoin group are suggestive of sufficient systemic exposure to produce developmental toxicity.

RECOMMENDATIONS

The pregnancy category for this drug should remain X, based on the evidence for teratogenesis in the first study, on the comparison of human and animal exposure data that is available, and on the extended duration of topical exposure in clinical patients as a result of twice daily dosing. Additionally, literature submitted with the original IND indicated that topically applied 5% 4-hydroxyanisole was embryolethal and embryotoxic.

The sponsor's original proposed label should be modified as follows:

CONTRAINDICATIONS: [redacted] may cause fetal harm when administered to a pregnant woman.

In a dermal teratology study in New Zealand White rabbits, there were no statistically significant differences among treatment groups in fetal malformation data; however, marked hydrocephaly with visible doming of the head was observed in one mid-dose litter (12/0.06 [4-hydroxyanisole/tretinoin] mg/kg, 132/0.66 mg/m²) and two fetuses in one high dose litter (40/0.2 [4-hydroxyanisole/tretinoin] mg/kg, 440/2.2 mg/m²) TRADENAME, and two high-dose tretinoin (0.2 mg/kg, 2.2 mg/m²) treated [redacted] litters. These [redacted] malformations were considered to be treatment-related and due

to the known effects of tretinoin. This was further supported by coincident appearance of other malformations associated with tretinoin, such as cleft palate and appendicular skeletal defects. [redacted] No effects attributed to treatment were observed in rabbits treated with [redacted] alone (dose 40 mg/kg, 440 mg/m²). A no-observed effect level for teratogenicity in rabbits was established at 4/0.02 mg/kg (44/0.22 mg/m²) for TRADENAME, which is approximately the maximum possible human [redacted]

[redacted]

TRADENAME was not teratogenic in Sprague-Dawley rats when given in topical doses of 80/0.4 mg/kg (480/2.4 mg/m² or 11 times the human maximum [redacted] dose).

[redacted]

[redacted] /S/ 11/10/98
Amy C. Nostrand, D.V.M., Ph.D.
Pharmacologist/Toxicologist

- cc:
 - NDA
 - HFD-340
 - HFD-540
 - HFD-540/PHARM/Nostrandt
 - HFD-540/TLPHARM/Jacobs
 - HFD-540/MO/Cook
 - HFD-540/CHEM/Timmer
 - HFD-540/PMS/Cross
- [redacted]

Concurrence Only:
HFD-540/DD/WILKIN [redacted] /S/ 11/11/98
HFD-540/TLPHARM/JACOBS [redacted] /S/ 11/11/98