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Application Number NDA 21-108

PHARMACOLOGY REVIEW(S)

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

KEY WORDS: tretinoin, photodamage, Renova
Reviewer Name: Amy Nostrandt
Division Name: Division of Dermatologic and Dental Drug Products
HFD# 540

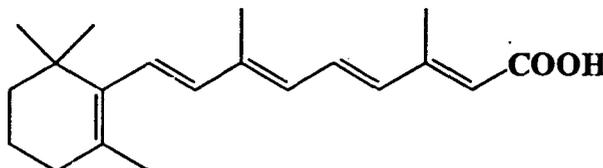
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Review Completion Date: 6/29/2000

Review number: 1
IND/NDA number: NDA 21-108
Serial number/date/type of submission: original submission, received 9/1/1999
Information to sponsor: Yes () No (X)
Sponsor (or agent): Johnson and Johnson Consumer Companies, Inc.
Manufacturer for drug substance: []

Drug:

Code Name: drug product - TEC-II 0.02%, drug substance - RWJ 8203-000, ORF 8203
Generic Name: tretinoin, all-*trans*-retinoic acid (tRA)
Trade Name: Renova
Chemical Name: tretinoin, all-*trans*-retinoic acid, (all-E)-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8 nonatetraenoic acid
CAS Registry Number: not provided
Molecular Formula/ Molecular Weight/Structure:



Molecular Weight: 300.44
Molecular Formula: C₂₀H₂₈O₂

Relevant INDs/NDAs/DMFs: IND — NDA 16-921, NDA 17-340, NDA 17-579, NDA 17-955, and NDA 19-963.

Drug Class: retinoid

Indication: adjunctive agent for use in the mitigation (palliation) of fine and coarse wrinkles, mottled hyperpigmentation, and tactile roughness of facial skin

Clinical formulation:

Component
tretinoin, USP
caprylic/capric triglyceride
steareth 20

%w/w
[]

stearic acid, NF	[]
cetyl alcohol, NF	
stearyl alcohol, NF	
benzyl alcohol, NF	
steareth 2	
xanthan gum, NF	
methylparaben, NF	
propylparaben, NF	
butylated hydroxytoluene, NF	
[]	
Total	100.00

Route of administration: topical to the skin

Proposed clinical protocol or Use: The drug is to be applied to the face once daily before retiring. The area is to be cleansed thoroughly before the product is applied.

Previous clinical experience:

Four 24-week phase 3 studies were conducted in Caucasian subjects. Additional studies have evaluated long-term use for up to 52-76 weeks. Reported adverse events included skin irritation, dryness, peeling, and erythema. Signs were generally mild or moderate in severity. One case of severe facial cellulitis was considered to be possibly related to the study drug. Dermal safety studies and a percutaneous absorption study were also performed.

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

Introduction and drug history:

The subject of the current submission is a 0.02% tretinoin cream formulation for the topical treatment of specific aspects of photoaging. The Applicant currently markets a 0.05% tretinoin cream for a similar set of signs attributed to photoaging. The product that is the subject of this NDA is purported to be less irritating than the currently marketed formulation.

Studies reviewed within this submission:

No new studies were submitted. Pertinent journal articles included in the submission and studies previously reviewed are summarized below.

The application states that a dermal irritation study and an ocular irritation study were performed with a formulation similar to the TEC-II formulation that is the subject of this NDA, but these studies were submitted and reviewed under NDA 19-963, for the marketed Renova (0.05% tretinoin) formulation. They are summarized in the special toxicology studies section.

Studies not reviewed within this submission:

Studies from the Applicant's previous submissions for other tretinoin formulations are summarized in the appropriate sections.

PHARMACOLOGY:

The following information is summarized in the overall pharmacology summaries, and in references 1 and 2.

Mechanism of Action: Tretinoin binds to cellular retinoic acid binding proteins (CRABP) in the cytoplasm of cells and to retinoic acid receptors in the nucleus (RAR). The latter are members of the steroid/thyroid hormone superfamily of nuclear receptors and are activated upon binding tretinoin. The ligand-receptor complex binds to retinoic acid response elements on DNA to control gene expression. RAR's are reported to be present on keratinocytes, fibroblasts, and melanocytes. The sponsor's summary states that the predominant RAR subtype in skin is RAR γ . The Applicant states that retinoic acid acts on all skin cell types. The Applicant claims phenotypic-dependent effects consistent with a different set of gene expression activities in pathologic skin cells as compared to normal skin cells, e.g. lightening of pigmented lesions but not normally pigmented skin. RAR's are reported to be highly conserved between various mammalian species. The sponsor states that RAR γ -RAR α heterodimers may form complexes with AP-1, activator protein-1, a dimer of the products of c-fos and c-jun that regulates transcription of genes involved in the control of growth and differentiation. The result is transrepression; neither factor can activate its respective response element-containing genes.

Drug Activity Related to Proposed Indication: The Applicant cites studies in the literature in which all-*trans*-retinoic acid (ATRA) enhanced formation of a zone of new dermal connective tissue, stimulated the synthesis of new collagen, and effaced grossly observed skin wrinkles in the hairless mouse. These effects are not seen in non-photodamaged skin. The Applicant states that one study demonstrated a tretinoin-induced increase in tropoelastin in the upper dermis. Other studies showed tretinoin to alter cytoskeletal protein expression in rhino mouse epidermis by altering the composition of epidermal and stratum corneum keratins, decreasing the expression of filaggrin and increasing the amount of a non-keratin glycoprotein. Additional studies showed that topically applied ATRA induced epidermal hyperplasia in mouse and guinea pig skin. It also stimulated epidermal DNA synthesis in hairless mouse skin.

The Applicant cites studies where tretinoin decreased MSH-induced and UVB-induced melanogenesis in S91 melanoma cells and in cultured melanocytes. In a study in Skh-II hairless lightly pigmented mice, concurrent treatment with 0.1% tretinoin prevented the UVB-induced increase in pigmentation. *Reviewer's comment: In a 1999 study in pigmented mice, tretinoin was shown to augment melanocyte activation and enhance melanogenesis, making melanocytes more sensitive to activation by ultraviolet radiation.*

Reference no. 3 is a journal article by Gendimenico GJ et al. (Evaluation of topical retinoids for cutaneous pharmacological activity in Yucatan microswine. Arch. Dermatol. Res. (1995) 287:675-679). The authors propose that microswine skin might be better as a model for topical retinoids than mouse or rabbit. Micropigs were treated topically with retinoids for five days/week for 5 weeks. The authors report that porcine skin was functionally responsive in terms of epidermal hyperplasia and transepidermal water loss. The potency of the compounds tested was similar to that seen in mice. One structural analog was tested that is known to be inactive on RAR's, and it had no characteristic retinoid effect on swine epidermis. The authors noted that there is at least one synthetic retinoid reported in the literature that was active in the mouse, but not in human skin. This was purported to be due to differences in skin metabolism.

The authors suggest that metabolic properties may also make porcine skin a more relevant model for human skin.

Ancillary Pharmacology Studies: One study cited indicated that topically applied ATRA lacked topical anti-inflammatory activity in the arachidonic acid-induced ear edema model of skin inflammation in the mouse. Another demonstrated that tretinoin was inactive against anaerobic and aerobic bacteria (gram + and gram -), yeast, fungi and protozoa in vitro at 50 µg/ml. Tretinoin was shown not to inhibit porcine pancreatic phospholipase A₂ at concentrations of 10-100 µM.

Summary of pharmacology: In addition to the above, the Applicant states, "Many of the same pharmacological activities observed in preclinical studies with tretinoin are also seen in clinical studies. These include effects on the epidermis, dermis and pigmentary system. This is to be expected since the RAR nuclear receptors are highly conserved between various mammalian species." *Reviewer's comment: The adverse effects of tretinoin are also mediated by these receptors, so conservation of these receptors between species indicates as well that many of the adverse effects seen in preclinical studies may be seen in clinical use.*

SAFETY PHARMACOLOGY:

The following information was provided in the overall summaries and in the summary provided as Reference no. 1:

Neurological effects: Tretinoin administered orally to mice at a dose of 10 mg/kg resulted in "borderline" CNS depression (decreased open field activity in one of three mice) for up to 24 hours after treatment. No effect was seen on pupil size. A slight increase in rectal temperature was noted at 4 hours after treatment.

Cardiovascular effects: Tretinoin administered intravenously to dogs at a dose of 5 mg/kg had no significantly different effects from vehicle on myocardial contractility, stroke volume and stroke work, heart rate, and myocardial perfusion. No arrhythmogenic activity was noted.

Pulmonary effects: Tretinoin administered intravenously to dogs at a dose of 5 mg/kg resulted in slight increases (7.7-13.3%) in airway resistance during infusion. Increases were seen in compliance during and after infusion, but the effect was of less magnitude than that seen in the vehicle controls.

Renal effects: not studied

Gastrointestinal effects: not studied

Abuse liability: not studied

Other: none

Summary: No effects were seen on the CNS, cardiovascular, or pulmonary systems that were believed to be biologically significant.

PHARMACOKINETICS/TOXICOKINETICS:

Summaries of studies from NDA 19-963 for Renova (0.05% tretinoin) cream and other tretinoin formulations are provided in the general summary and in Reference no. 1. Pharmacokinetic data also are supplied as Reference nos. 19 through 43. No nonclinical studies were performed using the TEC-II formulation that is the subject of this application.

Absorption:

Reference no. 20 (RWJ study report no. DM-92008) was a study in mice topically administered 0.03, 0.3, or 3 mg/kg ³H-tretinoin in SD 40 ethanol. Samples were collected at 1, 3, 5, 8 and 24 hours after the dose. The amount of drug absorbed was proportional to the dose. A plateau appeared at 1-3 hours post-dose and was stable for approximately 8 hours after application. Plasma radioactivity concentrations were 3.5, 26.9, and 97.5 ng-equivalents/ml at the low, mid-, and high doses, respectively at 5 hours (plateau phase). The report states that the duration of the plateau varied with dose: the curve at the low dose was on the decline at 24 hours, while the curve was still plateaued for the mid-dose and was still increasing at 24 hours for the high dose. The objective of the study was to determine the optimal sampling time for a dermal carcinogenicity study; it was decided that sampling would be optimal at 5 hours post-dose (mid-point of plateau phase).

In a 104-week dermal carcinogenicity study in mice at doses of 0.025, 0.5, and 1 mg/kg, samples were collected at 5 hours after ³H-tretinoin dose on day 1, and at scheduled single applications of ³H-tretinoin at 1, 6, 12, and 24 months. A dose-dependent increase in mean plasma radioactivity was seen which remained relatively constant throughout the study. The sponsor states that there was no significant difference between concentrations at different time points, but there appears to be a trend toward a peak at 1 month and decline of up to one half by 12 months (at 1 month – 2.8, 46, and 96 ng-equiv./mL). Interestingly, analysis by HPLC of plasma revealed tretinoin concentrations below the limit of quantitation. *Reviewer's comment: This information is from the overall summary in this application. Additional information was provided in carcinogenicity summaries, but the study report is not provided, nor is the entire set of data.*

Reference no. 27 is a journal article (J. Invest. Dermatol. 95:523-529, 1990) describing a study in hamsters. Doses of 17 µg/kg and 8.7 mg/kg ³H-tretinoin in acetone were applied to hamster skin. The results of the study indicated rapid percutaneous absorption and dose-dependent rates of elimination. The authors state that "prolonged absorption from the skin appears to contribute to high total bioavailability of topical retinoid." The data were reported to fit a two-compartment open model with a brief lag time and first order uptake and elimination. C_{max} of radioactivity was 2.3 ng-equiv/mL at 12 hours for the low dose and 900 ng-equiv/mL at 36 hours for the high dose. C_{max} of tretinoin by HPLC was 60 pg/g for the low dose (considered endogenous) and, after the high dose, was 75 ng/ml. The half-life of the first elimination phase was 1 hour. The authors state that C_{max} was 2% that after an equivalent oral dose, but the AUC topical was 60% of AUC oral (parent compound only). *Reviewer's comment: Clearly the use of C_{max} alone as an indication of relative bioavailability of topical formulations grossly underestimates exposure.* The authors saw secondary peaks that tended to rise toward the end of study as the concentration of total radioactivity declined; the authors stated that this suggested that the skin acts as a depot for tretinoin from which a slow systemic release of drug is possible.

In Reference no. 30 (Franz and Lehman J. Toxicol. – Cut. & Ocular Toxicol. 8:517-524, 1989-1990), a single topical application of 200 mg radiolabeled 0.05% Retin-A cream (100 µg tretinoin) was made to 100 cm² area of shaved skin in monkeys. Absorption of retinoic acid was 9.6% and 48.3% of the dose for normal and dermatitic skin, respectively. In human subjects, the authors report that absorption was 5.3% in normal skin and 7.2% in dermatitic skin.

Additional information was provided in the sponsor's summaries:

The sponsor states that studies of ³H-tretinoin in mice demonstrate rapid penetration through the epidermis into the dermis. The amount absorbed was dose-related.

After oral administration to mice, rats, dogs and hamsters, tretinoin was readily absorbed from the gastrointestinal tract. Oral bioavailability was approximately 40% from solution or capsule formulations. Plasma concentrations decreased over time with repeated oral administration to rats and monkeys due to enhanced clearance, suggesting that tretinoin induces its own metabolism and/or elimination. (*Reviewer's comment: Studies in the literature also indicate that tretinoin induces its own metabolism, possibly to active metabolites. It also upregulates synthesis of intracellular binding proteins, which could result in enhanced intracellular uptake, rather than elimination of active retinoid from the body.*) Oral administration of 10 mg/kg to mice resulted in a mean C_{max} of 4.5 µg/mL at 45 min post-dose, with a plateau phase of approximately 2 hours.

Intravenous administration of 10 mg/kg to male mice resulted in a serum tretinoin concentration of 11,000 ng/mL at 5 minutes, a plateau concentration of 5000 ng/ml between 1 and 2 hours after dosing, and a rapid elimination phase; levels were not measurable (<100 ng/ml) at 7 hours. The summary states that this is consistent with a capacity-limited saturable process.

Rats administered 1-5 mg/kg intravenously exhibited a plateau phase that ranged from 1.5-3 hours, depending on dose. It was followed by an exponential phase with a mean half-life of 0.78-0.93 hours. In a second study of 0.015-5 mg/kg, a similar profile was seen, but the terminal half-life was approximately 20 minutes.

Reference no. 39 is RW Johnson PRI report #DMR 1415, a report of a study of percutaneous absorption of ³H-tretinoin in human volunteers. A single topical dose of 50 µg in 100 mg cream formulations (3 formulations total) was applied, with or without 28 days of pretreatment with non-labeled cream. The mean peak C_{max} ranged from 12-21 pg-equivalents/mL for the six treatment groups. Cumulative elimination in the urine ranged from 0.94-1.47% of applied dose, and fecal elimination ranged from 0.44-0.66%. Percutaneous absorption was estimated to be 1.38-2.13% of the applied dose.

Reference no. 40 is RW Johnson PRI report #DM-91020-A, a report of a study of absorption of 0.05% tretinoin in TEC-II cream after a single topical dose with and without 28 days of pretreatment. ³H-tretinoin was included in the formulation, but plasma analysis was performed using a HPLC assay. Tretinoin concentrations ranged from below quantifiable levels (<2 ng/mL) to ———. After 28 days of pretreatment, mean plasma concentrations were not significantly different from baseline. No significant difference from baseline was reported after application of the labeled dose. The sponsor concluded that this is evidence of minimal absorption and that there is no significant potential for tretinoin from TEC-II to accumulate and perturb the endogenous pool of tretinoin or its metabolites.

Reviewer's comment: If the endogenous concentration is far below the limit of quantitation, then any measurable levels may be evidence of alteration to endogenous pools. There may also be a depot of material in the skin that can be slowly released over time at levels too low to quantify by HPLC. The sponsor has pointed out that nuclear retinoic acid receptors are in the same gene family as a number of hormone receptors; this may imply that relatively subtle alterations to normal homeostasis may have some effects.

In Reference no. 42 (Clewell et al. J. Am. Acad. Dermatol. 36:S77-S85, 1997), a report of a study supported by the sponsor and authored by one of their scientists, a PBPK model is described that estimates the internal exposure to retinoids after topical treatment with 0.05% tretinoin cream to be minimal in comparison to that for teratogenic oral doses. The model-derived dose surrogate AUC for tretinoin applied to the face arms and chest was 0.39 ng•hr/mL;

that for the monkey administered 5 mg/kg po is 5960 ng•hr/mL. The model does not appear to take into account active metabolites of tretinoin.

Distribution:

In the sponsor's summary, they describe the distribution of ³H-tretinoin after single oral dose to rats at 3.5 mg/kg and hamsters at 10.5 mg/kg as being to all sampled tissues. Concentrations were highest in liver and fat. In nonpregnant rats, 72 hours after dosing, ovaries retained the lowest percentage of total radioactivity. In pregnant hamsters, 96 hours after an oral dose of 10.5 mg/kg, plasma radioactivity concentrations (approximately 900 ng-equiv/g) were approximately equal to that in placenta and fetus. The sponsor states that studies have shown that plasma AUC is best pharmacokinetic correlate of embryotoxic and teratogenic potential across species and across different routes of administration in the same species (supported by reference no. 38, below). After a single intravenous dose of 10 mg/kg to mice, tretinoin was rapidly taken into liver, kidney and lungs; at 8 hours measurable levels (>0.1 mg/mL) were still present in brain (1.2 µg/mL), liver (0.3 µg/mL) and intestine (0.1 µg/mL), although plasma levels were no longer measurable. After oral administration of 10 mg/kg to mice, maximum tissue concentrations were achieved in 0.5-2 hours. Those concentrations exceeded plasma concentrations by 1.5-fold or greater in liver, lung, intestine, kidney, spleen, fat, heart, and brain. Levels plateaued for up to three hours, then declined with a half-life ranging from 25-68 minutes.

In Reference no. 22 (Tzimas G. et al. Developmental stage-associated differences in the transplacental distribution of 13-*cis*- and all-*trans*-retinoic acid as well as their glucuronides in rats and mice. Toxicol. Appl. Pharmacol. 133:91-101, 1995), the authors state that the rodent placenta develops from a choriovitelline to chorioallantoic type in late organogenesis. Two experiments were performed to evaluate relative transplacental passage of retinoids in the two placental types. In the first, 75 mg/kg/day 13-*cis*-retinoic acid was administered orally to pregnant rats on either gestation days (gd) 7-12 or 11-16. More efficient transplacental passage of the drug was found on gd 16 than on gd 12, as evidenced by higher ratios of embryo to maternal plasma concentrations and embryo to maternal AUC's. In the second experiment, 13-*cis*-retinoic acid or all-*trans*-retinoic acid was given as a single oral dose to pregnant mice on gd 11 or on gd 14, at two dose levels, 10 or 100 mg/kg. The embryo to maternal plasma concentration ratio for 13-*cis*-retinoic acid was significantly higher on gd 14 than on gd 11 at the low dose. All-*trans*-retinoic acid and its 4-oxo metabolite were transferred efficiently to mouse embryo at both times. The authors state that the study suggests that the transfer of 13-*cis*-retinoic acid is more efficient in late organogenesis due to the development of a chorioallantoic placenta. They also state that this would explain the higher placental transfer and teratogenic potency of 13-*cis*-retinoic acid in nonhuman primates and presumably in humans, both of which have a chorioallantoic placenta during susceptible developmental stages to 13-*cis*-retinoic acid teratogenicity.

Reference no. 24 is R.W. Johnson PRI research report no. DMR 1404, "Limited tissue distribution study of ³H-all-*trans*-retinoic acid (RWJ 8203) in male and female Long-Evans rats following a single oral dose." A single dose of 0.78 mg (females) or 0.88 mg (males) tretinoin was administered in soybean oil by gavage after an overnight fast. Plasma and tissue levels in lens, eye, bone marrow, fat, skin, liver, kidney testes, and ovaries were measured from three rats per sex at 4, 24, 48, and 72 hours after dosing. At four hours after the dose, as much as 25% of administered radioactivity was recovered, and approximately 2% was recovered at 72 hours. Tissue levels were highest in bone marrow, fat, skin and liver at all time points. At four hours,

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levels in fat and liver exceeded those in plasma by 2-3 fold, and levels in those tissues were near plasma levels at other time points.

Collins et al. in Reference no. 25 (Toxicol. Appl. Pharmacol. 127:132-144, 1994) demonstrated that transport of all-*trans*-retinoic acid across the placenta in rats was greater than that of 13-*cis*-retinoic acid.

Collins et al. in Reference no. 26 (Toxicol. Appl. Pharmacol. 130:9-18, 1995) determined maternal and embryonic AUC's in rats and rabbits after a dose of 6 mg/kg either as a single dose on gestation day 12 or as multiple doses on gestation days 7-12. Plasma samples were collected after the gd 12 dose. AUC's were lower by a factor of 9 after multiple dosing in the rat and by a factor of 2 in the rabbit. The effect was diminished in the embryo. The sponsor speculates that a relative increase in placental transfer was seen after multiple dosing, possibly due to an increased availability of binding sites (e.g. CRABP) in the embryo.

Reference no. 28 is an article by Howard et al. (Arch. Toxicol. 63:112-120, 1989) describing a comparative study of all-*trans*-retinoic acid, 13-*cis*-retinoic acid, all-*trans*-4-oxo-retinoic acid, 9-*cis*-retinal, and all-*trans*-retinyl acetate in pregnant hamsters. The authors state, "Maternal peak circulating concentrations of the parent retinoids, total radioactivity, plasma pharmacokinetic parameters or the total concentrations of residual radioactivity in fetal tissues could not be correlated with the differences in teratogenic potencies of these retinoids." Animals treated with 13-*cis*-retinoic acid had the greatest AUC, slowest clearance, and the longest elimination half-life.

In Reference no. 29 (Tzimas et al. Teratology 54:255-265, 1996), tretinoin was administered orally to pregnant cynomolgus monkeys at a dose of 5 mg/kg once daily from gd 16 to 26 and twice daily from gd 27 to 31. Plasma concentrations of tretinoin were lower at later time points, consistent with data from other species and inducible metabolism. Embryonic concentrations were approximately 40% of maternal plasma levels.

Reference no. 31 (Adamson et al. J. Natl. Cancer Inst. 84:1332-1335, 1992) describes a study of intravenous administration of all-*trans*-retinoic acid to rhesus monkeys at doses of 20, 50, and 100 mg/m² (sponsor's summary states 1, 2.5, and 5 mg/kg). The time course included three distinct phases: a brief initial exponential decline, followed by a relative plateau, the duration of which was dose-dependent, followed by a terminal exponential decay. The authors state that this pattern is consistent with a capacity-limited saturable elimination process. The half-life averaged 19 minutes, and the mean Km was 3.2 µM. AUC's were 15, 58, and 112 µM•hr at the low, mid-, and high doses, respectively. Dose dependent kinetics were hypothesized to occur in human patients treated orally with all-*trans*-retinoic acid for oncology indications.

Reference no. 32 (Sandberg et al. Drug Metab. Disp. 22:154-160, 1994) describes the pharmacokinetics after intravenous administration of 13-*cis*-, all-*trans*- (both at 0.25 or 0.0125 mg/kg), 13-*cis*-4-oxo- and all-*trans*-4-oxo- (both at 0.25 mg/kg) retinoic acid. Clearance and volume of distribution were dose-dependent at steady state, with greater values at the lower dose. Elimination half-lives for the *cis* compounds were not dose-dependent. The plasma-time concentration curves suggested that <30% of each compound was available in the central compartment for elimination in the post-distribution phase.

In Reference no. 38 (Tzimas et al. Toxicol. Appl. Pharmacol. 143:436-444, 1997), all-*trans*-retinoic acid was administered to rats on gd 9 at a dose of 5 mg/kg either by subcutaneous injection or orally. That dose was embryotoxic (lethality and skeletal defects) by the subcutaneous route, but not by the oral route. Cmax values seen in animals dosed by the two routes were similar Cmax, but plasma AUC's were three-fold higher after subcutaneous injection than after oral administration. The author's hypothesize that this was due to slower uptake and

limited detoxification by β -glucuronidation after subcutaneous injection. The formation of the glucuronide metabolite was much more extensive after oral than after subcutaneous administration. The authors concluded that AUC values were better correlates with embryotoxic outcome than was C_{max} .

In Reference no. 41 (Creech Kraft et al. Toxicol. Appl. Pharmacol. 100:162-176, 1989), placental transfer of all-*trans*-retinoic acid to the mouse embryo was greater than for the *cis* isomer. All-*trans*-retinoic acid and its 4-oxo metabolite both were described as extremely teratogenic in this study. At 8 hours after administration of all-*trans*-retinoic acid, embryo/maternal ratios of *trans* isomers were greater than 1. The authors propose a model of facilitated transport of all- *trans* forms to the developing embryo.

Reference 43 (Smith et al. Biochem. J. 132:821-827, 1973) documents that tretinoin is transported bound to serum albumin in the rat and not bound to retinol binding protein. The authors found 5-10% of radiolabel from the dose in carcasses and concluded that retinoic acid was not stored in tissues. The highest levels of label in this study were found in liver, kidneys, and intestine.

Metabolism:

Tretinoin is metabolized by oxidation by cytochrome P-450 enzymes to polar metabolites in liver and skin microsomes. It induces its own metabolism *in vivo* and *in vitro*. P-450 metabolism is inhibited by pretreatment with ketoconazole *in vitro* and *in vivo* in rats (Reference no. 35 – Van den Bossche et al. Skin Pharmacol. 1:176-185, 1988, and Reference no. 36 – van Wauwe et al. J. Pharmacol. Exper. Ther. 245:718-22, 1988)

The sponsor's summary describes the pathways involving shortening of the tetraene side chain, *cis-trans* isomerization, hydroxylation of the cyclohexene ring methyl groups, oxidation of the cyclohexene ring to a cyclohexone ring, epoxidation of the ring to a 5,6-epoxy metabolite followed by a rearrangement to 5,8-oxo-retinoic acid, and glucuronide or taurine conjugation. They state that in rodents the main metabolic pathways are isomerization to 13-*cis*-retinoic acid and oxidation to 4-oxo-tretinoin.

They describe studies in pregnant mice, rats, and rabbits administered tretinoin, where tretinoin, 13-*cis*-retinoic acid, 4-oxo-tretinoin, and retinol were found both in maternal plasma and in the embryo. The glucuronide was found in maternal plasma and embryo in the mouse, but only in maternal plasma in the rat and rabbit. Retinyl palmitate/oleate levels were measured in rat and rabbit; maternal plasma levels were detected in both species; levels were found in the embryo in the rabbit.

The sponsor states that endogenous tretinoin levels in embryos are 9-16 ng/ml in the mouse, 5-19 ng/ml in the rat, and approximately 10 ng/ml in the monkey.

Collins, et al. in Reference no. 25 (Toxicol. Appl. Pharmacol. 127:132-144, 1994) examined maternal and embryonic pharmacokinetics after equipotent teratogenic doses of all-*trans*-retinoic acid, 13-*cis*-retinoic acid, and retinyl palmitate to rats. The predominant maternal metabolites were the respective glucuronides, but these were not detected in the embryo.

Reference no. 33 (Creech Kraft et al. Drug Metab. Disp. 19:317-324, 1991) describes a study in cynomolgus monkeys. Oral doses of 2 and 10 mg/kg/day of 13-*cis*- and all-*trans*-retinoic acid were administered for ten days. Samples were taken for pharmacokinetics on day 1 and day 10. AUC was proportional to the administered dose. Elimination of all-*trans*-retinoic acid was more rapid than that of 13-*cis*-retinoic acid and increased with repeated dosing. The main metabolite was all-*trans*-retinoic acid glucuronide. All-*trans*-retinoic acid and its 4-oxo derivative were both teratogenic; the authors note that both of these compounds are found in human serum after dosing with 13-*cis*-retinoic acid. They suggest that the mouse may be

appropriate for study of all-*trans*-retinoic acid and the monkey a better model for studies of 13-*cis*-retinoic acid.

In Reference no. 34, Tzimas et al. (J Nutr. 126:2159-2171, 1996) describe a study in which 10 mg/kg retinyl palmitate was administered orally to rabbits from gd 7-12. The authors state that physiological concentrations (5-8.3 nmol/L) of all-*trans*- and 13-*cis*-retinoic acid were similar to endogenous levels in human plasma. Findings in the rabbit embryos included high amounts of retinol and esters, lower amounts of all-*trans*-4-oxo-retinoic acid, and levels of all-*trans*-retinoic acid that were 100% higher than endogenous levels. Embryonic AUC values suggested that embryotoxicity of this dosing regimen was mainly due to the action of all-*trans*-retinoic acid. One finding considered remarkable by the authors was the marginal increase of maternal plasma concentration of all-*trans*-retinoic acid RA over endogenous levels. They noted that this suggests a high risk of teratogenic effects even in the absence of high elevation of plasma all-*trans*-retinoic acid. ✓

Reference no. 37 (Adamson et al. Cancer Res. 53:472-476, 1993) describes a study of repeat intravenous dosing to rhesus monkeys of 50 mg/m² all-*trans*-retinoic acid by bolus injection for 8 days. One additional dose was given after 7 days without drug. The time course included a plateau phase, the duration of which decreased during the period of repeat dosing, followed by a terminal exponential decay phase. This was again consistent with a saturable process. The V_{max} of that process increased from 0.06 μmol/min on the first day to 0.17 μmol/min on day 8. This was considered consistent with induction of an enzymatic process. Cellular retinoic acid binding protein (CRABP) was measured in skin biopsy specimens and was shown to be rapidly induced. Levels were approximately three times baseline by day 3 and remained that high through the dosing period. They diminished following the 7-day drug free period.

Elimination:

The elimination kinetics have been described as dose-dependent and triphasic. The sponsor describes a rapid distributional phase, a plateau phase which is increased in duration with increased dose, and a third more rapid terminal elimination phase. As stated earlier, the plateau indicates the existence of a saturable elimination process. Elimination is via the urinary and biliary/fecal routes. The sponsor states in one of the summaries that the side chain terminal carboxy group is partly excreted as expired CO₂.

The sponsor describes additional information in their summaries:

In mice, the excretion of radioactivity after oral administration of ¹⁴C-tretinoin was monitored for 72 hours. Twenty percent was excreted in the urine as polar metabolites, 86% was excreted in the feces as parent compound, 13-*cis*-retinoic acid, and polar metabolites (the proportion of polar metabolites increased with time), and 12% in expired air.

In the rat, 0.1 mg/kg ³H-tretinoin was administered intravenously to bile duct-cannulated rats. At 18 hours, 18.4% of the radioactive dose had been excreted in the urine and 60% had been excreted in bile. It was concluded that tretinoin was well absorbed after oral administration and primarily excreted in bile.

In Reference no. 24 described under Distribution, cumulative excretion of radioactivity in rats over 0-72 hours was 14% in urine, 52% in feces, for a total of 66% excreted. The calculated elimination half-lives were 19.1 hours from female rat plasma and 21.7 hours from male rat plasma. The elimination half-life was longer from some tissues, e.g. 37.4 hours from the ovaries.

Other studies:

The sponsor's summary states that tretinoin is highly bound to plasma proteins in rats and humans (87% in rats and >95% in humans).

The sponsor's summary also discusses enzyme induction and inhibition:

The cytochrome P-450 mediated system present in rat skin microsomes can metabolize tretinoin and is inducible by tretinoin and by phenobarbital (Reference no. 21- Drug Metab. Dispos. 12:63-67, 1984). It can be inhibited by topical antifungals. Topical application of 1 mg of tretinoin induced a 3- to 4-fold increase in microsomal metabolism of tretinoin. The sponsor's summary also states that "mechanistic studies suggest that an increase in skin concentrations of the specific cellular retinoic acid binding protein (CRABP) may also contribute to the enhanced clearance of tretinoin on repeated dermal application."

In hamster liver microsomes, two polar metabolites of tretinoin, 4-oxo-tretinoin and 4-hydroxy-tretinoin are formed. Ketoconazole inhibits their formation in a competitive manner.

In *in vivo* rat studies, ketoconazole inhibited liver metabolism of intravenously administered tretinoin. The effect on plasma concentrations was described as significant.

TOXICOLOGY:

General Comments: Cross-referenced studies were previously submitted to support Retin-A and Renova formulations of tretinoin and are summarized here. The sponsor has cross-referenced NDA's 16-921 (for Retin-A® solution), 17-340, 17-522 and 19-049 (for Retin-A® creams), 17-579 and 17-955 (for Retin-A® gel) and 19-963 (for Renova® 0.05% tretinoin emollient cream). The information below is presented in the current submission in overall summaries and in the summary provided as reference no. 1.

Overall Toxicology Summary:**Acute Toxicity Studies:**

Single dose studies were performed by the oral route in mice, rats, and dogs, by the dermal route in rabbits, and by the intravenous route in mice and rats. LD₅₀ values determined in those studies are presented in the table below:

Species	Formulation	Oral LD ₅₀	IV LD ₅₀	Topical LD ₅₀
Mouse	pure drug substance	> 5 g/kg		
	0.025% tretinoin emollient cream	> 15 ml/kg (> 3.6 mg/kg)		
	0.1% gel	12.6 - 19.0 ml/kg	5.2 ml/kg	
	0.1% cream	> 44 ml/kg		
Rat	0.025% tretinoin emollient cream	> 15 ml/kg (> 3.6 mg/kg)		
	0.1% gel	8.7 - 20.9 ml/kg	8.7 ml/kg	
	0.1% cream	> 44.0 ml/kg		
	0.05% gel	20.2 ml/kg		
Dog	0.1% gel	> 10 ml/kg		
Rabbit	0.1% gel			> 9.4 ml/kg (> 0.5 mg/kg)

Study DS-5035: 5 CD-1 mice/sex were dosed by gavage with vehicle or 1, 3, or 5 g/kg tretinoin as a 0.75% solution in hydroxypropylmethylcellulose (HPMC). Deaths occurred at the high (1) and low (2, one was intubation error) doses. Clinical signs included alopecia, urine staining, and slight body weight loss that recovered in the second week after dosing.

Study DS-1701: Five mice/sex were dosed by gavage with 3.6 mg tretinoin/kg (15 ml/kg of 0.025% tretinoin cream; HED=0.3 mg/kg tretinoin). There were no deaths and no significant clinical findings.

Study DS-1702: Five Sprague-Dawley rats/sex dosed by gavage with 3.6 mg tretinoin/kg (15 ml/kg of TEC-1 0.025% cream; HED=0.6 mg/kg tretinoin). One vehicle control male died due to an error in intubation. There were no significant clinical findings.

Study DS-1704 was a single dose study in four NZW rabbits/sex dosed topically with 0.05 and 0.5 mg tretinoin/kg as 0.2 or 2 g/kg of a 0.025% tretinoin emollient cream (TEC-1) or vehicle. Application sites were abraded immediately prior to dosing and the test article was applied under an occlusive patch for 24 hours. There were no deaths. Local effects included erythema, edema, scaling, flaking, and epithelial thickening at the application site after treatment with either test article or placebo. Decreased erythrocyte count, hematocrit, hemoglobin and total protein were seen in males at 0.5 mg/kg (HED=167 mg/kg cream, 42 µg/kg tretinoin, 10 times the clinical dose).

Repeated Dose Toxicity Studies:

Multidose studies by the oral or parenteral routes revealed that the target systems for tretinoin are skin, skeleton, hematopoietic system, liver, testes and nervous system. Common signs in rats and mice were reported to be cartilage and bone resorption, fracture, subcutaneous and internal hemorrhage, increased cerebrospinal fluid (CSF) pressure, fatty infiltration of the liver, and epithelial drying and scaling.

In subchronic and chronic studies in rats, mice, and dogs, the application states that toxicity similar to that seen in humans was observed. Signs included decreased body weight, hair thinning, dermatitis, mucocutaneous lesions, increased serum alkaline phosphatase, triglyceride levels, and transaminase activities, decreased albumin, hemoglobin, and erythrocyte counts, hepatic pathology, and testicular atrophy with interrupted spermatogenesis. The sponsor reports bone fractures, corneal opacities, epiphora, cardiovascular lesions, and erythrophagocytosis in lymph nodes after ingestion of relatively large doses of tretinoin for long periods of time. Testicular toxicity was characterized by coagulative necrosis and arrested spermatogenesis; Sertoli cells were the last to be affected. Lesions were also reported in the thymus.

Repeated dose dermal studies were performed in rats and rabbits. Local effects in both species were similar and were time- and dose-related in severity. Findings included erythema, hyperkeratosis, edema, epidermal hyperplasia with polymorphonuclear leukocyte (PMN) infiltration, multifocal hemorrhage and/or ulceration, microabscesses of the subcutis, decreased dermal elasticity, acanthosis, parakeratosis and increased density of subepithelial dermal connective tissue at higher concentrations or prolonged duration of treatment. Dermal irritation was reversible, but NOEL's were not identified. Systemic toxicity was seen in some dermal studies, consisting of slight reversible decreases in erythrocyte parameters, and leukocytosis and accelerated sedimentation rate in rabbits treated with a 0.1% gel formulation.

Studies conducted in support of Renova 0.05% tretinoin emollient cream:

Study DS89722 was a range-finding study in mice preliminary to a dermal carcinogenicity study. Five mice per sex were treated topically three times per week for three

months. Doses were 0, 0.03, 0.06, 0.14, 0.286, 0.714, 1.429 mg/kg/day tretinoin. Dose-related signs were seen at 0.06 mg/kg and above, including scaling, erythema, eschar, epithelial thickening, keratinization, and increased density of subepithelial dermal connective tissue.

Study DS91029 was submitted as Reference no. 17. A four-week dermal study of 0.05% TEC1 or TEC1A was conducted in two groups of six NXW rabbits per group (sites on 3 animals per group were abraded). Each animal was treated with the respective formulation article and its vehicle at separate sites, 0.5 ml/site/day (0.05 g/100 ml * 0.5 mL = 0.25 mg per rabbit; 0.25mg/1.8 kg = 0.14 mg/kg; HED=0.05 mg/kg) once daily, six days per week. The test article material was on the skin for approximately 22 hours per day. Animals were examined weekly and at 24, 46, and 72 hours after the last dose. No effects were seen on clinical observations or body weight gain. By week 1, treatment with both formulations resulted in severe erythema and well-defined to moderate edema. By week 3, skin reactions had progressed to eschar and severe erythema, with no change in edema. After the last dose, eschar and severe erythema were seen at all three time points. At 24 hours, edema was well-defined to moderate. By 72 hours, edema was only well-defined. While severe dermal reactions were observed for both formulations at intact and abraded sites, vehicle formulations produced moderate dermal irritation. The sponsor concluded that both active formulations were severe cumulative dermal irritants to rabbit skin under intact and abraded conditions. At necropsy, minimal to mild reddening was seen at vehicle sites, minimal to severe reddening was seen at test article sites, and scabs were seen at one TEC-1 vehicle site and at all active formulation sites. Histological examination revealed epidermal changes including sloughing of surface epithelium, marked hyperplasia/hypertrophy (acanthosis), parakeratosis, hyperkeratosis, multifocal hemorrhage and/or ulceration, and deeper foci of hemorrhage with necrosis of hair follicles. In the dermis, findings included moderate to severe inflammation that was described as subepidermal, chronic suppurative, and associated with epithelial lesions, microabscesses, moderate to marked fibrosis, and increased thickness. No effect was seen on the subcutis. Findings were similar findings between the two formulations and were less severe in vehicle-treated sites.

Studies conducted in support of Retin-A formulations (study numbers were not provided):

- Five rats/sex were treated topically for 6 weeks with doses of 0.02, 0.1, or 0.5 mg/kg/day. Findings included decreased body weight gain at 0.5 mg/kg, dose-related hyperkeratosis, leukocyte infiltration and necrotic inflammation.
- Fifteen rats/sex were treated orally in the diet for 12 weeks with 0, 0.4, 2, or 10 mg/kg/day. Findings included a dose-related decrease in erythrocyte parameters, increased serum alkaline phosphatase, increased liver weight, and basophilia of hepatic cytoplasm at 2 and 10 mg/kg. A NOEL was not identified.
- Three rats/sex were treated intraperitoneally for 11 or 18 days with doses of 8.7, 17.5, or 26.2 mg/kg/day tretinoin. Findings included dose-related decrease in body weight gain, a slight decrease in erythrocyte parameters, dose-related increase in fractures, enlarged Kupffer cells in the liver, and acanthosis of the skin. No NOEL was identified.
- Eight rabbits/group were treated topically for three weeks with 0, 0.1, 0.5, or 1 mL/kg/day of 0.1% tretinoin cream. Findings included dose-related erythema and hyperkeratosis, slight dose-related decrease in hematocrit and hemoglobin, and epidermal hyperplasia and hyperkeratosis in all treated groups.
- Five NZW rabbits/sex were treated topically for three weeks with 0, 0.1, 0.5 or 1 mL/kg/day of 0.1% tretinoin gel. Findings included dose-related erythema, hyperkeratosis, epidermal hyperplasia with PMN infiltration, decreased body weight, and increased erythrocyte sedimentation rate at the high dose.

- Five rabbits/sex were treated topically for 6 weeks with 0, 0.1, 0.5 or 1 mL/kg/day of 0.1% tretinoin gel. Deaths occurred in 1 mid- and 2 high dose animals (deaths in the two high dose animals were attributed to respiratory infection). Findings included erythema, hyperkeratosis, epidermal hyperplasia, decreased elasticity of the skin, decreased body weights, decreased erythrocyte parameters, and leukocytosis, all dose-related in severity.

CARCINOGENICITY:

Study DS-92116-E, a dermal carcinogenicity study in mice, was described in the sponsor's overall summaries in this submission. Crl:CD-1(ICR)BR mice, 50/sex/group, were treated topically for 91 weeks. Dosing was three times per week with 0, vehicle (unidentified), 0.025, 0.5, or 1.0 mg/kg tretinoin as 1.0 mL/animal to a 3x5 cm clipped area in the scapular region (concentrations were 0.0008, 0.017, and 0.035%, respectively). Clinical signs included alopecia, scaling, edema, erythema, eschar, and ulceration in all groups, but more frequently in tretinoin-treated groups. Flaccid skin tone was seen mostly in males at the mid- and high doses. Histological examination revealed lymphocytic/plasmacytic hyperplasia in lymph nodes near treated skin sites at 0.5 mg/kg and higher. In the skin, acanthosis was seen in all drug-treated groups. Pustular dermatitis was seen mostly at the mid- and high doses. Other findings noted in the previous reviews included lymphocytic/plasmacytic hyperplasia of lymph nodes (including those not adjacent to the treatment area) and spleen, myeloid hyperplasia of bone marrow, and papillary necrosis of the kidney.

One squamous cell carcinoma was seen in each of the in mid- and high dose groups (The sponsor states that this was within reported spontaneous incidence, but higher than the laboratory's historical range.) at the treatment site. Three and two papillomas were found in females in the mid- and high dose groups, respectively, in the treatment area were believed by the sponsor to be due to irritation. (*Reviewer's comment: No skin tumors were seen at treatment sites in the control or low dose groups.*) One distant carcinoma was seen in one animal in each of the low and high dose groups; both were believed to be unrelated to treatment. There appeared to be an increase in liver tumors in treated males; the previous reviewer observed a dose-related trend when statistical analysis was performed of total liver tumors that were found to be fatal or possibly fatal. The low dose was considered to be the MTD; other doses were considered to exceed the MTD due to skin irritation. The sponsor states that lethality was similar across all groups, but a previous review of in-life data notes that deaths appeared to be earlier in mid- and high dose groups.

In satellite pharmacokinetic groups, plasma ³H-tretinoin concentrations were determined on day 1, at months 1, 6, 12, and weeks 62, 64, and 65 to verify drug absorption. Plasma was collected at 5 hours post-dose, a time previously determined to be the approximate midpoint of a maximal plateau phase (see pharmacokinetic section, Reference no. 20), to estimate C_{max}. A dose-dependent increase was seen in the absorption of tretinoin. On day 1, plasma concentrations were 2.71, 40.4, and 70.8 ng-equivalents/mL in the low, mid-, and high dose groups, respectively. No significant change was reported during the course of the study. *Reviewer's comment: The lack of change over time is unusual. Usually tretinoin concentrations become lower over time due to metabolic induction. It is also important to note that at 0.5 and 1 mg/kg or 10 and 20 times the human dose after BSA normalization, plasma concentrations are significantly greater than background (<5 ng/mL) and that the low dose is half the human dose normalized for total body surface area.*

The sponsor concluded that the study demonstrated no definitive evidence of carcinogenicity.

A report of a "Topical retinoic acid and experimental photocarcinogenesis in hairless mice" (research report #77-50 (6)) is provided as Reference no. 16. Groups of 30 —hairless albino mice per sex were treated with vehicle (methanol), vehicle and solar simulated radiation (180 J/m²/day, UVR), 0.001% tretinoin in methanol and UVR, or 0.01% tretinoin in methanol and UVR. Animals were topically treated with tretinoin or vehicle beginning two weeks prior to UVR exposure, so that treatment with tretinoin was for 30 weeks, with UVR exposure for the last 28 weeks of that period. Exposure was intercurrent, with two hours of solar simulation exposure in the morning and test article application in the afternoon. Animals were examined weekly by two independent blinded examiners and were necropsied at 18 months. An increased photocarcinogenic response was seen at both tretinoin concentrations. The time to onset of tumors was shortened (about 20 weeks with tretinoin and UVR vs. about 40 weeks with vehicle and UVR) and the number of tumors was increased. No tumors were seen in the vehicle-treated group that was not exposed to UVR.

The sponsor cites literature studies (12) in which tretinoin enhanced photocarcinogenesis (6), inhibited photocarcinogenesis (4) or had no effect (2). None of those studies were submitted.

Reviewer's comment: It is important to note that not all studies published in the literature were performed with concurrent or intercurrent exposure to the test article and solar simulated radiation. The design of those studies showing inhibition of photocarcinogenesis generally involved a period of exposure to UV radiation, followed by a later period of tretinoin treatment without continued UV exposure. Appropriately performed studies, in which exposure to tretinoin and solar simulated radiation are concurrent or intercurrent, show enhancement of photocarcinogenesis.

The sponsor notes that hairless albino mice may not be an ideal model. The current label for Renova (tretinoin) 0.05% does state that the relevance of this study to humans is unknown. The sponsor also states that no evidence of photocarcinogenesis has been seen in pigmented mice, but provides no data to support that statement. *Reviewer's comment: In a recent study, Halliday et al. (J. Invest. Dermatol. 114:923-927, 2000) demonstrated that dark pigmentation provided some protection, but was not enough to overcome the augmentation of photocarcinogenesis by retinoic acid.*

Summary Conclusions and Recommendations

In a dermal carcinogenicity study in mice, topical application of tretinoin three days per week for 91 weeks at doses up to 1 mg/kg (HED=0.08 mg/kg, 20 times the systemic human dose) in a 0.035% formulation (1.75 times the 0.02% concentration of the current drug product) was considered by the sponsor to be not carcinogenic. The incidence of skin tumors in females and fatal liver tumors in males make the results seem more equivocal than negative. Information regarding those tumors has been included in the label for Renova (0.05% tretinoin) cream and should be in this one as well.

In a photo co-carcinogenicity study in hairless mice, topical application of 0.001 or 0.01% tretinoin enhanced UV-induced carcinogenesis.

It is recommended that these studies be reported in the label as previously recommended for Renova (tretinoin) 0.05%. Proposed wording is presented in the label review below.

IMMUNOTOXICOLOGY:

No immunotoxicology studies were submitted or referenced.

REPRODUCTIVE TOXICOLOGY:**Summary:**

The sponsor has provided as reference no. 6 a summary and study report for "A segment II developmental toxicity study in female Wistar rats administered all-*trans* retinoic acid (RWJ-8203-000) dermally," conducted by _____ study number 66036, sponsor study no. DS90042). That segment II dermal study was conducted in female Wistar rats using a 0.1% tretinoin solution in an ethanolic vehicle. Doses were 0 (vehicle), 1.0, 2.5, and 5.0 mg/kg/day administered on gd 6-16 to groups of 25 mated female rats. (*Reviewer's comment: Previous reviews indicate that the animals were not restrained, and the sponsor later communicated that restraint was unnecessary as the animals were not inclined to ingest the test article in an ethanolic vehicle.*) Originally, additional groups received 10 and 20 mg/kg/day, but severe irritation after 5-6 days of treatment necessitated termination of those groups. Dose-related dermal irritation, decreased food consumption, decreased body weights, and decreased thymus weights were seen at 2.5 mg/kg/day and above. Maternal liver weights (absolute; relative liver weights were slightly greater than control.) and fetal body weights were decreased at 5.0 mg/kg/day. There was a significant increase in supernumerary ribs at 2.5 and 5.0 mg/kg. Although not statistically significant, there appeared to be a trend toward increased incidence of enlarged cardiac atria in treated groups. (*Reviewer's comment: Fetal anomalies were expressed and analyzed as mean number of affected fetuses per litter rather than the preferred litter incidence. It is possible that significant differences could have been missed in this way.*) The NOEL was considered to be 1.0 mg/kg (HED=0.17 mg/kg or 42 times the estimated clinical dose).

The sponsor also provided as reference no. 7 a summary and study report for "A segment II developmental toxicity study in female Wistar rats administered all-*trans* retinoic acid (RWJ-8203-000) orally," conducted by _____ study number 66037, sponsor study no. DS90045). That study of tretinoin suspended in 0.5% hydroxypropylmethyl cellulose and 1% ethanol was performed in Wistar rats. Doses were 0 (vehicle), 1.0, 2.5, 5.0 and 10.0 mg/kg/day administered on gd 6-15 to groups of 25 mated female rats. The sponsor's summary states that there were no remarkable findings on dam necropsy. No other treatment-related maternal effects were reported. Although not statistically significant, fetal weights in the two highest dose groups appeared to be lower than controls, and there appeared to be a slight increase in early resorptions in the 10 mg/kg/day group. Cleft palate was observed in three of 23 litters at 10 mg/kg. There was a dose-related increase in supernumerary ribs in all treated groups that was statistically significant at 5.0 and 10 mg/kg. Statistically significant increases in the incidence of incomplete ossification of the cranial bones were reported at 5.0 and 10.0 mg/kg. There was also a trend toward increased incidence of incomplete ossification of other bones in those two groups. (*Reviewer's comment: Fetal anomalies were expressed and analyzed as mean number of affected fetuses per litter rather than the preferred litter incidence. It is possible that significant differences could have been missed in this way. The original review of this study noted an apparent increased incidence of enlarged cardiac atria in the 5.0 mg/kg group, but this is not apparent from the data expressed in this manner. The litter incidence of this anomaly is not provided.*) All of these effects were reported to have occurred in the absence of any maternal toxicity. The NOEL was considered to be 1.0 mg/kg/day (HED=0.17 mg/kg or 42 times the estimated clinical dose).

Reference no. 8 consists of a summary and partial report of a study of developmental toxicology of topically applied Renova in rabbits (study no. DS-93024, performed by _____). The sponsor's conclusion was that the drug was not teratogenic at 0.05 mg/kg/day (HED=0.017 mg/kg, 4 times proposed human dose), the lowest dose tested. (Reviewer's comment: *As can be seen from the synopsis of the study that follows, that dose was not a no effect level. There was a low incidence of retinoid-associated effects.*) A dermal segment II study was conducted in NZW rabbits using 0.005% and 0.05% tretinoin emollient cream. Dose groups consisted of 20 mated females and were treated with 0 (untreated), 0 (vehicle), 0.05, or 0.5 mg/kg/day on gd 7-19 (Reviewer's comment: *The report states that the female rabbits were mated on four consecutive days and that the last day of mating was considered to be day 0 of gestation. It seems that the actual day of gestation could differ by as much as 3-4 days from the presumed day of gestation. Since there are critical developmental periods for structures affected by retinoids, it seems possible that a delayed start of treatment could miss some of those critical periods, and effects would not be seen even if systemic exposure was sufficient to cause those effects.*). Elizabethan collars were worn from gd 0-22. Animals were restrained in stocks for a 6-hour treatment period each day, followed by washing of the treatment site and replacement of the collar. Blood samples were collected prior to the first dose, 6 and 24 hours after dosing on gd 19 and again on gd 29 prior to sacrifice. There was increased mortality in the two treated groups (Reviewer's comment: *In a previous study conducted with tretinoin microsphere gel, the design of which was used in the current study, increased mortality was attributed to the collars and stocks; see summary of reference 13 below.*) and a significant increase in abortions in the 0.5 mg/kg group. The litters of does that died or aborted generally consisted of resorbed conceptuses (three deaths and seven abortions in the high dose group and three of the deaths and one abortion in the low dose group; 50% and 20% of the dams in each respective group). Grade 2 erythema and desquamation were seen in vehicle control animals and grade 4 erythema and grade 3 desquamation were seen in tretinoin-treated animals. Grade 1 fissuring was noted in vehicle and tretinoin-treated groups, and eschar, edema, and atonia were noted at 0.5 mg/kg. (Reviewer's comment: *The choice of doses was based upon the results of a two-week dose range finding study in timed-pregnant rabbits in which 0.5 mg/kg/day resulted in only moderate erythema and very slight edema. It is unclear why the same dose and regimen in this study for the same duration resulted in substantially more severe irritation.*) Dose-dependent body weight and gain reductions were seen in tretinoin-treated animals. Food consumption was significantly reduced in high dose animals. Necropsy findings included evidence of systemic infection in two high dose animals that the report stated was probably secondary to skin irritation (Reviewer's comment: *This seems unlikely; there was fairly extensive thoracic and abdominal organ involvement that may have been a confounding factor in evaluation of maternal mortality.*) Pharmacokinetic data was not included in this submission. Based on an earlier review of this study, tretinoin concentrations in 0.05 mg/kg animals were above but near the LOQ (_____ on gd 19; in 0.5 mg/kg animals, tretinoin was not detectable, but its metabolites 13-cis retinoic acid and 13-cis-4-oxo retinoic acid were detectable. On gd 29, tretinoin was detectable in high dose animals. Only 8-17 (a previous review stated 8-14, apparently omitting the untreated control group) litters were evaluated in the vehicle and tretinoin-treated groups (less than the 16-20 recommended by ICH). Embryo-fetal death was increased in drug-treated groups. Resorptions were significantly increased and fetal body weights were decreased at 0.5 mg/kg (Reviewer's comment: *The significant increase in resorptions in the high dose group was based on analysis of surviving does. Since resorptions were prevalent in does that died or aborted, it seems likely that resorptions would have also been significantly increased in the low dose group, had the four animals that died or aborted*

and had litters consisting of late resorptions been included in the analysis). Cleft palate was found in association with open eyelids and fused sternal centers and an alteration in frontal bone ossification in one 0.05 mg/kg litter and a second litter in that group had enlarged eyebulges, broad and wavy ribs, wavy scapular alae, and a variation in skull ossification. A fetus in a third litter had malformed cervical vertebrae and a variation in sternal ossification. Litter incidences of fetal alterations in this group were not increased, even though fetal incidences of retinoid-associated alterations were. Microphthalmia and a variation in skull ossification were seen in one 0.5 mg/kg litter. The litter and fetal incidences of fused nasal bones (up to 6 of 8 litters) and irregularly shaped scapular alae (2 of 8 litters) were significantly increased in the high dose group. In this study, tretinoin was concluded to be embryotoxic, but not teratogenic. No NOAEL was identified.

References 9 and 10 are protocols for segment I studies in male and female rats. Draft study reports were submitted on 12/16/1999 to this application and final reports were submitted to NDA 19-963 on 3/28/2000. In both male and female rats, a parental NOAEL was not identified, but the adverse effects at the lowest dose (0.1 mg/kg, HED=0.017 mg/kg) were limited to skin irritation. The two highest doses were associated with some degree of body weight loss or reduced body weight gains, but the effects in females appeared to resolve during the study. In males, effects were seen on epididymal weights at 0.5 mg/kg/day (HED=0.08 mg/kg/day, 20 times the clinical dose), and there appeared to be a reduction in sperm count and motility at that dose. In females, there appeared to be an increase in nonviable embryos at doses of 0.25 mg/kg (HED=0.04 mg/kg/day, 10 times the clinical dose) and above, a finding that is consistent with systemic retinoid exposure in early gestation. Based on these findings, the reproductive NOAEL's were 0.25 mg/kg (HED=0.04 mg/kg/day, or 10 times the maximum human topical dose) in males and 0.1 mg/kg (HED=0.017 mg/kg, or 4 times the maximum human topical dose) in females. The reviews of those studies are reproduced below:

1. Study title: Fertility study of Renova ® administered topically to male rats (Segment 1 evaluation)

Study No: 98-014T

Site and testing facility: _____

GLP compliance: yes

QA- Reports Yes (X) No ():

Lot and batch numbers: lots 26A604 and 28H815

Protocol reviewed by Division Yes (X) No ():

Methods:

- Species/strain: Crl:CD _____ (Sprague-Dawley)
- Doses employed: Groups 1-5 were treated with 0 (untreated), 0 (vehicle), 0.1, 0.25, and 0.5 mg/kg/day of tretinoin, respectively (0, 1, 0.2, 0.5, and 1 ml/kg of the respective formulations)
- Route of Administration: topical to clipped skin of the back
- Study Design: Test article was administered as a single daily application to males only beginning 70 days before cohabitation, continuing through the cohabitation period (maximum 21 days), and through the day before sacrifice in Groups 2-5. Dosages were adjusted daily for body weight and were given at approximately the same time each day. The test article was applied under a semi-occluded bandage for six hours per day; the bandage was then removed and the site washed with soap and water. Elizabethan collars were worn during the exposure period. During the cohabitation period, females were removed from the cages during dose exposure.

- Number of animals/sex/dosing group: 25/sex/group; only males were treated with the test article.
- Parameters and endpoints evaluated: Male rats were observed twice daily for viability and weekly for general appearance during the acclimation period. During the treatment period, clinical observations and grading for signs of skin irritation were performed daily before dosing and on the day of sacrifice. Flaking, edema, and erythema were scored on a scale of 0-3, and the presence of scabs, fissuring and ulceration were noted. Body weights were recorded weekly during the acclimation phase, daily during the dosing period, and on the day of sacrifice. Feed consumption was measured daily up to the cohabitation period. Clinical observations and body weights were recorded for untreated female rats at least weekly prior to gestation and on gestation days (gd) 0, 7 and 13. Feed consumption for females was recorded on gd 0, 7, and 13.

Surviving male rats were sacrificed by CO₂ asphyxiation and subjected to gross necropsy. Right and left testes, left epididymis (whole and cauda), right epididymis, seminal vesicles (with and without fluid), and prostate were weighed. Sperm concentration and motility were evaluated in samples taken from the left cauda epididymis. The remainder of the left epididymis, the right epididymis, prostate, seminal vesicles, and testes were retained in appropriate fixative for possible future examination. Gross lesions were retained in formalin.

Female rats were sacrificed by CO₂ asphyxiation on gd 13. Gross necropsy was performed, and ovaries and gross lesions were preserved and retained. The number of corpora lutea were recorded for each ovary. The uterus was examined for pregnancy, number and distribution of implantations, and viable and nonviable embryos. Placentae were examined for abnormalities.

- Statistical evaluations: Proportion data, such as clinical observations were analyzed for homogeneity of variance. Continuous data, such as body weights and feed consumption were analyzed using Bartlett's test for homogeneity of variance and ANOVA, when appropriate, followed by Dunnett's test to compare groups. If Bartlett's test was significant, ($p \leq 0.05$), the Kruskal-Wallis test was used followed by Dunn's method of multiple comparisons or Fisher's exact test. Count data at Cesarean-sectioning was evaluated using the Kruskal-Wallis test. Sperm motility (expressed as percentages) were subjected to arcsine transformation and analyzed by parametric methods.

Results:

- Mortality: One male in the high dose group was found dead on day 12 of the study. No clear cause of death could be determined, and the death was not attributed to the test article.
- Clinical signs: Vocalization was significantly increased in rats at the mid- and high doses and was noted in four rats in the low dose tretinoin group. This was considered to be related to administration of the test article and increased skin irritation. There was a dose-related incidence of urine stained abdominal fur that was significantly different from untreated control in the mid- and high dose groups.
- Skin observations:

In the vehicle control group, the incidence of grades 1 and 2 erythema, edema, and flaking, ulceration, and scabs were significantly increased relative to untreated control.

In the high dose male that died, grade 3 flaking, grade 2 erythema, grade 2 edema, and a scab were noted prior to death.

Skin reactions were dose-dependent in incidence and severity. In all three tretinoin-treated groups, the incidences of grades 1-3 erythema and flaking, grades 1 and 2 edema, ulceration, and scabbing were significantly different from untreated controls. In addition, grade 3 edema was significantly different from untreated controls in the high dose group, and fissuring was significantly different from control in the mid- and high dose groups. In the low dose group, grade 3 edema and fissuring were not seen.

- **Body weight:** Vehicle-treated animals had decreased body weight and body weight gains relative to untreated controls. Average body weights were reduced for the entire predosing period after the first week and at termination in the mid- and high dose groups. Body weight gains were significantly different from untreated control for most weeks of the pre-dosing period and for the total study interval. Body weight and gain were not affected in the low dose group (*Reviewer's comment: It seems unusual that vehicle-treated animals had reduced body weights while low dose animals had unaffected body weights relative to untreated controls.*)

- **Food consumption:** Absolute feed consumption (g/day) was decreased after day 43 of the study, but relative feed consumption (g/kg/day) was increased in the mid- and high dose groups throughout the pre-mating period after the first week, relative to untreated controls. In females, relative feed consumption was increased in animals mated to high dose animals during the first week of pregnancy, relative to the untreated control group. Since females were not treated, it seems unlikely that this difference was related to treatment of the corresponding males.

- **Toxicokinetics:** not performed

- **Fertility in Males**

- **In-life observations:** Fertility index, numbers of days to inseminate, number of rats that mated, and number of rats with confirmed mating dates during the first two weeks of cohabitation were unaffected at all doses. *Reviewer's comment: The number of pregnant females reported in the table of fertility parameters in males does not match the number of pregnant females reported in tables of observations in females. However, the difference does not appear to change the above conclusions.*

- **Terminal and Necroscopic evaluations:** The terminal body weights of rats in the mid- and high dose groups were significantly lower than those of untreated controls. The absolute weights of the left and right epididymis at the high dose were significantly reduced relative to untreated controls. Relative weights of left and right epididymides, left cauda epididymis, and both testes were significantly increased relative to untreated controls in the mid- and high dose groups. (*Reviewer's comment: The applicant did not consider the reduction in absolute epididymis weights to be a sign of toxicity, since the relative weights were increased. It is important to note that relative weights of the testes were increased, reflecting decreased terminal body weight, without a decrease in absolute weights. Relative organ: brain weights were not recorded.*) No gross lesions at necropsy were considered to be related to the test article.

Sperm parameters (number motile and nonmotile, percent motile, total sperm count and density) were not significantly affected by treatment, although there was a downward trend in sperm count and density in treated groups. The mean number motile and total counts were slightly lower in the high dose group than in controls, but the high variability appears to have precluded the difference from being significant. No histological evaluation of the male reproductive tract was performed.

- Conclusions: A NOAEL was not identified, but the adverse effects at the lowest dose (0.1 mg/kg, HED=0.017 mg/kg) were limited to skin irritation. The two highest doses were associated with reduced body weight and body weight gains. The report states that the reproductive NOAEL was greater than 0.5 mg/kg/day (HED=0.08 mg/kg/day), the highest dose tested. However, effects were seen on epididymal weights in the high dose group, which may indicate that the reproductive NOAEL was actually 0.25 mg/kg (HED=0.04 mg/kg/day) in this study. The applicant should perform histological evaluation of the preserved samples from the male reproductive organs.

2. Study title: Fertility study of Renova® administered topically to female rats (Segment 1 evaluation)

Study No: and number: 98-015T

Site and testing facility: _____

GLP compliance: yes

QA- Reports Yes (X) No ()

Lot and batch numbers: lots 26A604 and 28H815

Protocol reviewed by Division Yes (X) No ():

Methods:

- Species/strain: Cri:CDu _____ (Sprague-Dawley)
- Doses employed: Groups 1-5 were treated with 0 (untreated), 0 (vehicle), 0.1, 0.25, and 0.5 mg/kg/day of tretinoin (in the 0.05% cream), respectively (0, 1, 0.2, 0.5, and 1 ml/kg of the respective formulations)
- Route of Administration: topical to clipped skin of the back; test site was approximately 5 cm by 22 cm.
- Study Design: Test article was administered as a single daily application beginning 15 days before cohabitation, continuing through the cohabitation period (maximum 21 days), and through gestation day (gd) 7. Dosages were adjusted daily for body weight and were given at approximately the same time each day. The test article was applied under a semi-occluded bandage for six hours per day; the bandage was then removed and the site washed with soap and water. Elizabethan collars were worn during the exposure period. During the cohabitation period, females were separated from breeder males during dose exposure.
- Number of animals/sex/dosing group: 25/sex/group; only females were treated with the test article.
- Parameters and endpoints evaluated: Female rats were observed twice daily for viability and weekly for general appearance during the acclimation and pre-dosing period. Clinical observations, examination for abortions, premature deliveries and deaths, and grading for signs of skin irritation were performed daily before dosing, daily in the post-dosing period, and on the day of sacrifice. Flaking, edema, and erythema were scored on a scale of 0-3, and the presence of scabs, fissuring or ulceration was noted. Body weights were recorded weekly during the acclimation phase, daily during the dosing and post-dosing periods, and on the day of sacrifice. Feed consumption was measured daily during the dosing period.

Surviving female rats were sacrificed by CO₂ asphyxiation on gd 13 and subjected to gross necropsy. Ovaries and tissues with gross lesions were preserved in formalin. The number of corpora lutea were recorded for each ovary. The uterus was examined for pregnancy, number and distribution of implantations, and viable and nonviable embryos. Placentae were examined for abnormalities.

- Statistical evaluations: Proportion data, such as clinical observations were analyzed for homogeneity of variance. Continuous data, such as body weights and feed consumption were analyzed using Bartlett's test for homogeneity of variance and ANOVA, when appropriate, followed by Dunnett's test to compare groups. If Bartlett's test was significant, ($p \leq 0.05$), the Kruskal-Wallis test was used followed by Dunn's method of multiple comparisons or Fisher's exact test. Count data obtained at the time of Caesarian section were analyzed using the Kruskal-Wallis test.

Results:

- Mortality: There were no unscheduled deaths.
- Clinical signs: Vocalization was associated with administration of the test article to irritated skin in the high dose group and to one animal in the mid-dose group.
- Skin observations: The incidence of grade 1 erythema and flaking were increased in the vehicle group relative to untreated controls. The incidence and severity of all skin reactions were dose-dependent. The incidences of grade 1-2 erythema, grade 1 edema, and grade 1-2 flaking were greater than that in the untreated control group in all tretinoin-treated groups during the prehabitation and gestation periods. The incidence of grade 2 edema was greater than control in the mid-dose group during the gestation period and in the high dose group during prehabitation and gestation periods. Grade 3 flaking was significantly greater than control in the high dose group during the prehabitation and gestation periods. Ulcerations and scabs were seen predominantly in the mid- and high dose groups (significant only in the high dose group during gestation), while fissuring was limited to one animal in the high dose group.
- Body weight: Rats in all groups had body weight loss during the first week of the prehabitation period. There were statistically significant body weight losses in the mid- and high dose groups relative to untreated controls during this time. Weight loss in the vehicle and low dose groups was greater than in untreated controls, but was not statistically significant.

All groups had body weight gain in the second week. Body weight gains in all treated groups were slightly greater than the untreated control group (*Reviewer's comment: This would appear to indicate that the treated animals were recovering from the initial stress of treatment and were experiencing compensatory weight gain.*) For the two weeks as a whole, there were no significant differences for any group compared to the untreated control group.

During gestation, body weight gains in all treated groups were slightly greater than in the untreated control group. In the high dose group, body weights were reduced on gd 0-8, but body weight gain in the post-treatment period, gd 8-13, was significantly increased relative to untreated control. Average daily body weights in all treated groups did not differ from those of the untreated control group during this period.

- Food consumption: Absolute (g/day) food consumption was significantly reduced during the first week of the study relative to untreated control in the high dose group; relative food consumption was also reduced, but not significantly. Absolute and relative food consumption values were increased relative to untreated control during the second week of the study in that same group. (*Reviewer's comment: This again would appear to indicate that the treated animals were recovering from the initial stress of treatment.*) Over the entire prehabitation period, absolute and relative food consumption was comparable to the untreated control group in all treated groups.

During the gestation period, absolute and relative food consumption values were increased in all treated groups. This was statistically significant on gd 8-13 in the

vehicle, mid-, and high dose groups. Relative food consumption was significantly increased relative to untreated control for all tretinoin-treated groups for gd 1-13.

- Toxicokinetics: not performed

- Fertility and Early Embryonic Development in Females

- In-life observations: There were no significant differences among the five groups in average numbers of estrous stages per 14 days, number of days in cohabitation, or percentage of pregnant rats. All female rats had a confirmed mating date. There were 22, 22, 24, 23, and 21 pregnant rats in the untreated, vehicle, 0.1, 0.25, and 0.5 mg/kg/day groups, respectively.

- Terminal and Necroscopic evaluations: There were no treatment-related gross lesions other than the observed skin lesions.

The litter averages for corpora lutea, implantations, viable embryos, nonviable embryos, dams with any nonviable embryos, and the percentage of nonviable embryos per litter were not significantly different. However, there was a trend toward an increase in nonviable embryos and the percentage of nonviable embryos per litter in the mid- and high dose groups. (*Reviewer's comment: The sample size may have been too small to detect a difference statistically. An increase in intrauterine deaths would be consistent with known effects of systemic retinoid exposure.*) One mid-dose animal had a litter consisting of one nonviable embryo; the report stated that this was within the range of historical controls at the laboratory conducting the study.

- Conclusions: A maternal NOAEL was not identified, but the adverse effects at the lowest dose (0.1 mg/kg, HED=0.017 mg/kg) were limited to skin irritation. Alterations to body weight, weight gains, and food consumption in treated groups were followed by compensatory change early in the study. The reproductive NOAEL was reported to be 0.5 mg/kg/day (HED=0.08 mg/kg/day), the highest dose tested. However, if the increase in nonviable embryos at 0.25 mg/kg and above represents and effect of treatment, then the reproductive NOAEL would be 0.1 mg/kg (HED=0.017 mg/kg, or 4 times the maximum human topical dose).

Reference no. 11 is a journal article: Hendrickx AG and Hummler H. Teratogenicity of all-*trans* retinoic acid during early embryonic development in the cynomolgus monkey (*Macaca fascicularis*). *Teratology* 45:65-74, 1992. Sixteen pregnant females were administered all-*trans* retinoic acid per os daily from gd 10-20 and twice daily from gd 21-24. Dose groups were 5 mg/kg (n=9), 10 mg/kg (n=6), and 20 mg/kg (n=1). (*Reviewer's comment: The HED's for these doses are 1.7, 3.3, and 6.7 mg/kg, respectively, or ≥400 times the proposed human dose.*) Signs of hypervitaminosis A were reported in one low dose dam, three mid dose animals and in the high dose animal. Maternal weight loss was reported at 10 and 20 mg/kg. Increased embryoletality was seen in 22%, 50%, and 100% of animals in the three dose groups, respectively. Fetal alterations consisted of growth retardation in one of the seven fetuses in the 5 mg/kg group, and craniofacial malformations in all three viable fetuses in the 10 mg/kg group. Skeletal variations were reported in both 5 and 10 mg/kg groups; the total incidence was 70%, which was significantly higher than in historical controls. A developmental NOEL was not identified. The authors note agreement of the current study with other published studies of developmental toxicity of tretinoin in other primate species.

Reference 12 is a review of structure-activity and dose-response relationships of retinoids in neural and behavioral teratology studies (Adams J. *Neurotoxicology and Teratology* 15:193-202, 1993). Of retinol, all-*trans*-retinoic acid, and 13-*cis* retinoic acid, all-*trans*-retinoic acid

was determined to be the most potent in producing CNS and behavioral effects. It was concluded that gestation days 8-10 were the most sensitive time points in the rat for induction of gross CNS malformations, and gd 11-13 were the most sensitive time points in the rat for induction of postnatal effects (functional behavioral and neurochemical endpoints) in offspring with normal morphology.

A report of a segment II GLP study of 0.1% tretinoin microsponge gel conducted in CrI:CD ~~_____~~ (Sprague-Dawley) rats was provided as Reference no. 13 (sponsor study no. B0251S). The study drug product was applied topically at doses of 0 (vehicle), 0.2, 0.5, and 1.0 mg/kg/day tretinoin on gd 6-15. There were 25 mated females per group, plus 6 satellite animals per group for blood collection. Test material was applied to the clipped backs of the animals under occlusion for 24 hours; the site was then washed and the next dose was applied. Maternal effects included erythema, desquamation, edema and/or fissuring (the latter was in one mid-dose rat only). Food consumption and weight gain were decreased in all treated groups gd 12-16. Body weights were lower than controls in the mid- and high dose groups on gd 16 and in the high dose group only on gd 15. Litter observations were comparable between treated and control groups. No morphological alterations were considered biologically important; vertebral and rib malformations were reported in one 0.5 mg/kg litter and a bifid centrum in a thoracic vertebra was reported in one to two litters in each of the treated groups. Incomplete ossification in vertebrae, ribs and sternal centers were reported in treated and control groups. One 0.5 mg/kg fetus had dilation of the lateral ventricles of the brain, and one 1.0 mg/kg fetus had a dilated renal pelvis. Results of pharmacokinetic sampling may not have been submitted or reviewed.

Reference 14 is a report of a segment II GLP study of 0.1% tretinoin microsponge gel was conducted in artificially inseminated NZW rabbits (sponsor study no. B0252S). Topically applied doses were 0, 0.2, 0.5, and 1.0 mg/kg/day applied as increasing volumes of the 0.1% formulation on gd 7-19. After 24 hours, sites were wiped with a wet paper towel and dried before scoring for dermal irritation and application of the next dose. Dams wore Elizabethan collars on gd 2-20. There were 18 (original review stated 15) mated females per group and blood samples were taken from six animals in each group. Maternal effects included erythema, atonia, and desquamation in all groups, including control. Edema was noted in the skin of treated animals. Severity of signs of skin irritation was dose-dependent. No drug-related effects were seen on maternal body weight, gain, or feed consumption (Decreased body weight gain was reported in the original review in dams at 1.0 mg/kg, but this was apparently due to one animal that had a gastric trichobezoar.). Measurable concentrations of tretinoin or 13-cis tretinoin were found in the 0.5 and 1.0 mg/kg dose groups at 24 hours after the first and 13th (final) doses; no drug was detected 24 hours after the 12th dose; the sponsor believed that its presence was due to sporadic ingestion (*Reviewer's comment: As reported in the original review, actual values were dose-related and could also be reasonably explained by slow dermal absorption, biotransformation and cellular uptake. A pharmacokinetic study of radiolabeled tretinoin presumably applied topically revealed time and dose dependent plasma concentrations of the drug that were similar to the values obtained in this study.*). There were 11-14 evaluable litters per group (less than the 16-20 recommended by ICH). There appeared to be an increase in resorptions and fewer live fetuses per litter in the 0.5 mg/kg group. There was a dose-related trend in the mean percent of fetuses with any alteration, but there were no statistically significant increases in any treated group. However, when retinoid-associated malformations (domed head, hydrocephaly, cleft palate, flexed paws in association with dilation of brain ventricles, limb or rib malformations, dilation or hemorrhage of the lateral and/or third ventricles of the brain) were examined, the incidence of one or more was significantly increased in the 0.5 and 1.0 mg/kg groups. The presence of more than one retinoid-associated alteration in the same fetuses was

considered further evidence of a drug-related effect. Brain ventricle dilation was seen in one low dose fetus; this was within the range of historical controls. Flexed forepaws also occurred in the low dose fetus with dilation of brain ventricles, which may be evidence of a treatment effect. The developmental NOEL was considered at the time to be 0.2 mg/kg/day (HED=0.07 mg/kg, or 17 times the proposed human dose).

Reference 15 is a report of a second rabbit study (study no. B0289S) to replace the above study using 20 naturally mated animals per group. (*Reviewer's comment: If the presumed day of gestation was determined as for the study in reference 8, the actual day of gestation could be as much as 3-4 days later than the presumed day of gestation. Since there are critical developmental periods for structures affected by retinoids, it seems possible that a delayed start of treatment could miss some of those critical periods, and effects would not be seen even if systemic exposure was sufficient to cause those effects.*) Doses were 0 (untreated), 0 (vehicle), 0.5, or 1.0 mg/kg/day on gd 7-19, administered as increasing volumes of the 0.1% gel formulation (*Reviewer's comment: Administration of doses as increasing volumes can result in underdosing of higher dose animals when excessive volume falls off of the animals or the dose layer is thick enough to preclude optimal skin contact with drug substance.*). Animals were restrained in stocks for a 6 hour exposure period, after which the material was washed off and the Elizabethan collars were placed on the animals until the next dosing. Collars were worn through gd 22. A fifth group was treated with 1.0 mg/kg tretinoin for 24 hours per day and wore collars through gd 22. Blood samples were taken prior to the first dose on gd 7 and 2, 4, and 24 hours after the final dose on gd 19, and again on gd 29 at sacrifice and Caesarean section. Dam mortality was attributed to restraint in the stocks. Grade 1 and 2 erythema and desquamation were reported in all drug and vehicle treated groups (*Reviewer's comment: Decreased topical exposure to the drug relative to that in the study in reference 8 is evidenced by relatively lower incidence and severity of dermal irritation.*). No effects were seen on body weight or food consumption. Pretreatment tretinoin concentrations ranged from below the LOQ to — and were only measurable in 15/80 does. Variability in post-dose blood levels was high. The numbers of animals sampled per group at any time point ranged from 1-17. Retinoid concentrations after the 13th dose on gd 19 demonstrated a possible dose-related exposure to tretinoin, *cis*-retinoic acid, and *cis*-oxo-retinoic acid at points where those levels were determined. However, due to variability in the numbers of animals sampled and the omission of determination at certain time points in some groups, little conclusion about tretinoin concentration time course can be determined. The values were not significantly different from those seen in the previous study (as reported in the original reviews of the studies presented here as references 8 and 15). No drug-related effects on litter parameters or fetal alterations were noted. There appeared to be increased resorptions and resorptions per litter in the 0.5 mg/kg group. Fetal malformations were seen in the 1.0 mg/kg (24 hour) group: microphthalmia in one litter and a second litter with a fetus with no tail, malformations of the thoracic, lumbar and caudal vertebrae and cervical vertebral ossification variation. Although the litter incidences of these malformations were within the range of historical controls, they both may be retinoid-associated and were coincidentally both in the high-dose/24-hour exposure group. Yet, the developmental NOEL was considered to be 1.0 mg/kg (HED=0.33 mg/kg, or 83 times the proposed clinical dose).

In the general summary and in Reference no. 1, the sponsor describes a dermal rat study of 0.1 or 1 ml/kg/day of 0.05% tretinoin gel (0.05 or 0.5 mg/kg/day). The sponsor reports no maternal or fetal toxicity and no teratogenicity. At the high dose, there was an increase in anatomical variations, particularly incomplete ossification of bones of the skull.

An oral rat developmental toxicity study (no study number provided) is described. Doses were 1 or 2.5 mg/kg. Increased embryoletality was seen at 2.5 mg/kg, described as "only a modest increase in intrauterine death." The sponsor's summary in Reference no. 1 describes a treatment-related increase in anatomical variations (increased numbers of thoracic ribs or sternbrae).

Another study described in Reference no. 1 was a dermal developmental toxicology study in rabbits of three 0.1% tretinoin formulations. Doses were 0.15 and 0.6 mg/kg/day tretinoin in an ointment, 0.05 and 0.2 mg/kg in a cream, and 0.4 and 0.6 mg/kg in alcohol solution. Mean fetal weights appeared to be decreased, and there were increased resorptions and intrauterine deaths (increased embryoletality) in high dose cream and solution groups.

Another study described in Reference no. 1 was a topical study in rabbits of 0.1 or 1 ml/kg of 0.05% tretinoin gel (0.05 or 0.5 mg/kg/day tretinoin). Dose-related irritation was seen in the dams. An increase in resorptions was seen at the high dose. A treatment-related increase in incomplete ossification of the parietal bones was seen, consistent with findings previously reported in rats.

Also in the general summary, the sponsor states that tretinoin administered to monkeys resulted in a dose-dependent increase in embryoletality at 5-40 mg/kg (HED=1.7-13 mg/kg, 425-3000 times human dose). They also state that rats, at oral doses greater than or equal to 5 mg/kg (HED=0.8 mg/kg, 200 times human dose), effects on the central nervous system, postnatal death, growth reduction, and behavioral dysfunction were seen.

In the pharmacokinetic section of the general summary, the sponsor describes a topical study in pregnant hamsters of 3 daily doses of 10.5 mg/kg. The sponsor reports no developmental effects, but "gross cutaneous toxicity". A single oral dose of 10.5 mg/kg resulted in developmental effects in 50% of dosed hamsters. The sponsor cites this as evidence of lack of teratogenicity for topically applied tretinoin, "indicating that the threshold concentrations required for developmental effects are not achieved from this route of administration."

Reviewer's comment: The timing of the above dosing is not specified, and there is a critical and narrow window for the characteristic developmental effects of tretinoin. Also, it is not possible to have achieved steady-state plasma or tissue concentrations of the drug after only three topical exposures. The study does not prove lack of teratogenicity of topically applied tretinoin, as it was not performed adequately. It would be critical to have achieved steady state exposures at the critical developmental period.

Segment III studies described in the general summary include an oral study in rats at 10 mg/kg/day. Increased sensitivity was noted at gestation days 11-13; dosing at that time resulted in increased fetal mortality, and decreased body, cerebrum and cerebellum weights. In live fetuses, difficulty was seen in breathing and nursing and was associated with abnormal development of regions of the medulla. Cerebellar weight reductions resulted in effects on motor activity, but not learning behavior. Animals treated on gd 8-10 were most susceptible to malformations, such as exencephaly, and eye and skeletal defects.

Labeling Recommendations:

Addition of segment 1 study data should be added to the label, including the apparent effects on embryo viability and sperm counts. Recommended wording is provided in the label review below.

**APPEARS THIS WAY
ON ORIGINAL**

GENETIC TOXICOLOGY:**Summary:**

An *in vitro* Ames assay is described in the general summaries and in Reference no. 1. *Salmonella typhimurium* strains _____ and _____, and *E. coli* strain _____ were tested at 5-250 µg/plate, with and without metabolic activation. Tretinoin was negative for genotoxicity in this assay.

Reference no. 18 is a study report for study DS-9006, the *in vivo* micronucleus assay in CD-1 mice. The animals were given a single intraperitoneal dose of tretinoin in DMSO at 42, 105, or 210 mg/kg. Bone marrow was harvested at 6, 24, and 48 hours. The positive control was triethylenemelamine (TEM) in distilled water at a dose of 1 mg/kg. No increase in polychromatic erythrocytes (PCE) was seen in tretinoin-treated groups, while a significant increase was seen in the positive control group. The study was considered negative for genotoxicity.

SPECIAL TOXICOLOGY STUDIES:**General Comments:**

The only studies stated by the sponsor to have been performed with the proposed TEC-II formulation were a dermal irritation study (study #DS-7065) and an ocular irritation study (study #DS-7067), both in rabbits, submitted as Reference numbers 4 and 5. There were slight changes in the formulation from that used in these nonclinical studies and in clinical trials to the current proposed to-be-marketed formulation. Specifically, the test formulation contained less steareth-20 (listed as _____ in the to-be-marketed formulation), less stearic acid _____, less benzyl alcohol _____ and more steareth-2 (listed as _____). Additionally, the formulation tested contained _____ and did not contain the fragrance _____.

Both of these studies were submitted previously to and reviewed under NDA 19-963.

1. Study Title: Primary dermal irritation study of _____ (0.05% cream formulation #1) in rabbits

Study No: DS-7065

- route, form, volume, and infusion rate: 24 hour topical exposure to formulation containing 0.05% tretinoin

Six female NZW rabbits were treated. Sites were clipped free of fur. Test material and vehicle (0.5 ml/site) were each placed on abraded and non-abraded sites on each rabbit under a 1"x1" gauze pad and covered with occlusive wrap for 24 hours. Animals were sacrificed and discarded on day 12.

- Irritation scoring: Sites were scored for irritation at 24, 48, and 72 hours using a modified Draize score. Scoring continued daily until signs were resolved.

Results:

- Irritation scoring: At treated sites, moderate to severe erythema without eschar and very slight to moderate edema were seen at 24 hours. Well-defined, moderate erythema and very slight edema were present at 72 hours. No edema was present by day 4. Erythema was present through day 8 for intact sites and day 10 for abraded sites. The tretinoin cream formulation was considered to be a moderate dermal irritant in rabbits, as was the vehicle, but to a lesser degree. The degree of irritation was greater at abraded sites. The report states that the

degree of irritation was comparable to that of other tretinoin cream formulations when tested in rabbits.

- Clinical signs: none
- Body weights: no effects

2. Study Title: Ocular irritation study of ~~TEC-II~~ 0.05% cream formulation #1) in rabbits

Study No: DS-7067

- route, form, volume, and infusion rate: A single dose of 0.1 ml of 0.05% formulation or vehicle was instilled into the right eye of one of two groups of 9 female rabbits. The left eye served as an untreated control. Both eyes of 3 rabbits/group were irrigated with 30 mL of tepid water 20 seconds after dosing.

- Irritation scoring: Eyes were scored for irritation at 1, 24, 48, and 72 hours using the Draize method.

- Ophthalmoscopy: Examination with ~~fluorescein~~ staining was performed at 24 hours and later timepoints to aid in evaluation. The procedure was discontinued when stain retention was no longer observed.

Results:

- Irritation scoring: At 1 hour conjunctival irritation consisting of injection (slight) and diffuse (moderate) redness, chemosis, and discharge were seen in non-irrigated eyes treated with tretinoin at 1 hour. In irrigated eyes, only conjunctival redness was seen. Effects were decreased by 24 hours to slight conjunctival redness in two of the nine test article-treated eyes (both non-irrigated). At 48 hours, conjunctival redness persisted in one of those animals. No signs of irritation were seen at 72 hours in any animals. No corneal damage (ulcerations or opacities) was observed. Findings in vehicle control eyes were similar, but signs of irritation were less pronounced.

The 0.05% tretinoin emollient cream formulation was considered to be a minimal ocular irritant when not immediately irrigated and "practically nonirritating" when immediately irrigated. The report states that the findings are consistent with the degree of irritation produced by other ~~TEC-II~~ cream formulations.

Summary:

A formulation similar to the TEC-II formulation was found to be a moderate skin irritant and a minimal ocular irritant in the rabbit. Both dermal and ocular irritation in these tests were reversible. The sponsor states that these effects are consistent with the pharmacologic activity of tretinoin and with previously known information on tretinoin and tretinoin formulations.

Reviewer's comment: The sponsor states that the TEC-II formulation was developed to be less irritating than the marketed formulation.

In the general summaries and in Reference no. 1, the sponsor describes special toxicology studies performed with previous tretinoin formulations. Those formulations were also mild to moderate dermal irritants in rabbits (severe irritant in study DS-1699), minimal to non-irritants in rabbit eye, non-sensitizing in the guinea pig (*Reviewer's comment: Summary tables indicate that the placebo cream in two ~~formulations~~ was a weak to strong sensitizer, while the active formulation was a non-sensitizer.*), and negative for phototoxicity in the guinea pig. Additional dermal irritation studies were performed in guinea pigs and mice with gel formulations or tretinoin in acetone; irritation was not seen in the guinea pig, but was dose-related in mice.

OVERALL SUMMARY AND EVALUATION:

Introduction: The drug product that is the subject of this application is similar to the sponsor's currently marketed product for a similar indication, but contains a lower concentration of tretinoin in a different cream formulation.

Safety Evaluation: There is a history of clinical use of Renova (0.05% tretinoin). The current 0.02% formulation should be at least as safe as the marketed product. The most notable adverse effects of topical tretinoin formulations in nonclinical studies have been local effects characteristic of retinoids at the site of application. Some systemic effects have been reported after topical application in animal studies. These have included adverse effects on red blood cell parameters and on fertility and prenatal development. Tretinoin has also been shown to enhance photocarcinogenicity in appropriately designed studies.

Clinical Relevance of Safety Issues: Due to presumed differences in skin permeability characteristics between rabbits, rodents, and humans, it is unknown what the significance of these findings might be.

Other Clinically Relevant Issues: none

Conclusions: The application is approvable with the labeling revisions presented below.

Communication Review:**- Labeling Review (NDA):**

The label should be identical to that for Renova 0.05% tretinoin emollient cream, with the exception that dose multiples should be recalculated to compare animal exposures to clinical doses of tretinoin in the lower concentration cream, (0.02%).

Recommendations for changes to sections pertaining to pharmacology and toxicology are as follows. Additional changes may be made as necessary, pending the final revisions of the label for Renova 0.05% tretinoin emollient cream.

1. Under CLINICAL PHARMACOLOGY:

The effect of tretinoin on skin with ~~photodamage~~ photodamage has not been evaluated in animal studies. When hairless albino mice were treated topically with tretinoin shortly after

a period of UVB irradiation, new collagen formation was demonstrated only in photodamaged skin.

The transdermal absorption of tretinoin from various topical formulations ranged from 1% to 31% of applied dose, depending on whether it was applied to healthy skin or dermatitic skin.

2. Under **PRECAUTIONS:**

Carcinogenesis, Mutagenesis, Impairment of Fertility: In a 91-week dermal study in CD-1 mice administered 0.017% and 0.035% formulations,

cutaneous squamous cell carcinomas and papillomas in the treatment area were observed in some female mice. A dose related incidence of liver tumors in male mice was observed at those same doses. The biological significance of these findings is not clear because they occurred at doses that exceeded the dermal maximally tolerated dose (MTD) of tretinoin and because they were within the background natural occurrence rate for these tumors in this strain of mice. There was no evidence of carcinogenic potential when 0.025 mg/kg/day of tretinoin was administered topically to mice (0.5 times the human dose, adjusted for total body surface area).

For purposes of comparisons of the animal exposure to human exposure, the is defined as 1 gram of 0.02% RENOVA applied daily to a 50 kg person (0.004 mg/kg tretinoin).

Studies in hairless albino mice suggest that concurrent exposure to tretinoin may enhance the tumorigenic potential of carcinogenic doses of UVB and UVA light from a solar simulator.

There are other reports in New Zealand White rabbits administered doses of greater than 0.2 mg/kg/day (17 times the human _____ dose adjusted for total body surface area) of an increased incidence of domed head and hydrocephaly, typical of retinoid-induced fetal malformations in this species.

In contrast, several well-controlled animal studies have shown that dermally applied tretinoin may be fetotoxic, but not overtly teratogenic, in rats and rabbits at doses of 1.0 and 0.5 mg/kg/day, respectively (42 times the human _____ dose adjusted for total body surface area _____)

With widespread use of any drug, a small number of birth defect reports associated temporally with the administration of the drug would be expected by chance alone. Thirty human cases of temporally-associated congenital malformations have been reported during two decades of clinical use of another formulation of topical tretinoin (Retin-A). Although no definite pattern of teratogenicity and no causal association has been established from these cases, 5 of the reports describe the rare birth defect category holoprosencephaly (defects associated with incomplete midline development of the forebrain). The significance of these spontaneous reports in terms of risk to the fetus is not known. L

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Non-teratogenic effects:

Dermal tretinoin has been shown to be fetotoxic in rabbits when administered 0.5 mg/kg/day (42 times the _____ human dose normalized for total body surface area) _____ Oral tretinoin has been shown to be fetotoxic, resulting in skeletal variations and increased intrauterine death, in rats when administered 2.5 mg/kg/day (104 times the _____ human dose adjusted for total body surface area) _____

Nursing Mothers: It is not known whether this drug is excreted in human milk. _____

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Pediatric Use: Safety and effectiveness in patients less than 18 years of age have not been established.

Geriatric Use: _____

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

RECOMMENDATIONS:

Internal comments: From a nonclinical standpoint, the application is approvable with the above label revisions.

External Recommendations/Draft letter Content for Sponsor: none

Future development or NDA issues: none

/S/

[_____] 7/5/00

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

cc:

NDA 21-108

HFD-340

HFD-540

HFD-540/PHARM/Nostrandt

HFD-540/TLPHARM/Jacobs

HFD-540/MO/Luke

HFD-540/CHEM/Timmer

HFD-540/PMS/Cintron

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Draft date (# of drafts): 6/29/00 (2)

Concurrence Only:

HFD-540/DD/WILKIN

/S/] 7/16/00

HFD-540/TLPHARM/JACOBS

/S/ 7/5/00
acting TL in D.F.S. ✓

**APPEARS THIS WAY
ON ORIGINAL**