All-*Trans*-Retinyl Palmitate [CASRN 79-81-2]

Prepared October 2000

SUMMARY

Retinyl palmitate was selected by the Center for Food Safety and Applied Nutrition for photo-toxicity and photocarcinogenicity testing based on the increasingly widespread use of this compound in cosmetic retail products for use on sun-exposed skin, the biochemical and histological cutaneous alterations elicited by retinyl palmitate, and the association between topical application of retinoids and enhancement of photocarcinogenesis. A diverse array of cosmetic products currently contain retinyl palmitate. These cosmetic products include moisturizing preparations, night skin care products, cleansing products, suntan preparations, bath soaps and detergents and skin fresheners. Data available through the FDA's Voluntary Cosmetics Registration Program indicate that the number of formulations containing retinyl palmitate has increased from 355 in April 1992 to 667 in May 2000. Currently, cosmetic products containing retinyl palmitate are being marketed aggressively for rejuvenation of the skin. The continuing demand for these cosmetic products by a population interested in maintaining a youthful appearance will predictably result in a continuing increase in products containing retinyl palmitate.

Topical application of retinyl palmitate is a pragmatic strategy for loading the skin with retinol (vitamin A). Cosmetic formulations containing retinyl palmitate are substantially more stable than those containing retinol. Furthermore, retinyl palmitate readily penetrates into the epidermis and dermis. *In vitro* measurements of retinyl palmitate's percutaneous absorption indicate that 18% of retinyl palmitate, topically applied in acetone, penetrates human skin within 30 hrs. Percutaneous absorption of retinyl palmitate in currently marketed cosmetic products may be still greater due to the considerable efforts of cosmetics formulators to maximize the effectiveness of products containing retinyl palmitate and retinol. Studies indicate that absorbed retinyl palmitate is readily hydrolyzed to retinol by cutaneous esterases. In addition, skin contains the enzymes required for further metabolism of retinol to retinaldehyde and retinoic acid, and some studies have shown that levels of retinoic acid in the skin can increase following topical application of retinyl palmitate or retinol.

Many of the biochemical and histological alterations in skin elicited by topical application of the pharmacologic agent, retinoic acid, are also observed after treatment of skin with retinyl palmitate and retinol. Biochemical changes include substantial elevation of cellular retinoic acid binding protein, retinoic acid 4-hydroxylase, and collagen synthesis. In addition, levels of matrix metalloproteinases decrease following topical application retinol. Histological changes include epidermal hyperplasia, epidermal thickening, and increased fibroblast growth. These cutaneous changes elicited by retinyl palmitate and retinol are similar to biochemical and histological alterations following topical exposure to retinoic acid.

The biochemical and histological changes in skin triggered by topical application of retinyl palmitate and retinol may be essential for many of the benefits claimed for these cosmetic products. Attendant risks include those associated with retinoids, such as retinoic acid, added to drug products. One risk is developmental toxicity. There is a report that topically applied retinol fails to increase the serum levels of retinol in humans, and therefore presents little risk for developmental toxicity. However, the details of this study are not available. Currently, a study, funded by FDA's Office of Women's Health, is being conducted in the Center for Food Safety

and Applied Nutrition to determine the effect of topically applied retinol on systemic levels of retinol. If substantial elevation of systemic retinol is observed, the second phase of this study will examine directly associated developmental toxicity. A second risk associated with topical exposure to retinoids is enhancement of photocarcinogenesis. Experimental studies have indicated that topically applied retinoic acid can, under some conditions of testing, enhance photocarcinogenesis. The effects of topically applied retinol or retinyl palmitate on photocarcinogenesis have not yet been evaluated.

TABLE OF CONTENTS

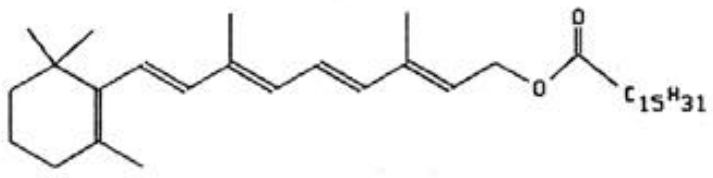
SUMMARY			**i**
1.0	**BASIS FOR NOMINATION**		**1**
2.0	**INTRODUCTION**		**1**
	2.1	**Chemical Identification**	**1**
	2.2	**Physical-Chemical Properties**	**1**
	2.3	**Stability**	**2**
3.0	**PRODUCTION PROCESSES**		**3**
4.0	**REGULATORY STATUS**		**3**
5.0	**OCCURRENCE IN COSMETICS**		**4**
6.0	**BIOCHEMICAL AND HISTOLOGICAL EFFECTS ON SKIN**		**4**
	6.1	**Percutaneous Absorption and Metabolism**	**4**
	6.2	**Biochemical and Histological Cutaneous Changes**	**5**
7.0	**TOXICOLOGICAL ISSUES**		**5**
	7.1	**Developmental Toxicity**	**5**
	7.2	**Photocarcinogenesis**	**6**
	7.3	**Human Studies**	**11**
8.0	**REQUESTED STUDIES**		**12**
9.0	**REFERENCES**		**14**

1.0 BASIS FOR NOMINATION

Retinyl palmitate was selected by the Center for Food Safety and Applied Nutrition for phototoxicity and photocarcinogenicity testing based on the increasingly widespread use of this compound in cosmetic retail products for use on sun-exposed skin, the biochemical and histological cutaneous alterations elicited by retinyl palmitate, and the association between topical application of retinoids and enhancement of photocarcinogenesis.

2.0 INTRODUCTION

All-*Trans*-Retinyl Palmitate
CASRN 79-81-2
RTECS NO. VH6860000



2.1 Chemical Identification

The following synonyms are used for retinyl palmitate:

Aquasol A
Arovit
Axerophtol palmitate
Optovit-A
Retinol hexadecanoate
Retinol palmitate
Retinyl palmitate
Vitamin A palmitate

(The Cosmetic, Toiletry and Fragrance Association, 1999)

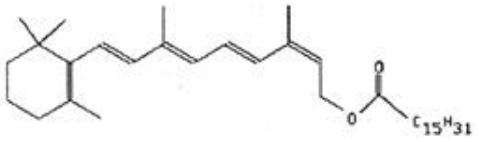
2.2 Physical-Chemical Properties of All-*Trans*-Retinol and Retinyl Palmitate

Property	Retinol	Retinyl palmitate
Formula[1]	$C_{20}H_{30}O$	$C_{36}H_{60}O_2$
Formula weight[1]	286.46	524.88
Melting point (^oC)[1]	63-64	28-29
UV Absorption[1,2]		
λ_{max}	325	326
$E^{1\%}_{1cm}$	1820	960
ε	52,140	50,390
Fluorescence[1]		
Excitation λ_{max}	325	325
Emission λ_{max}	470	470
Solubility[3]		
Water	insol.	insol.
Glycerol	insol.	insol.
Ethanol	sol.	sol.
Chloroform	sol.	sol.
Ether	sol.	sol.

[1]Olsen, J. A. (1991).
[2]In isopropanol. Values are similar in ethanol but differ in chloroform and other solvents.
[3]Cosmetic Ingredient Review (1987).

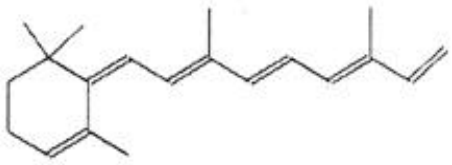
2.3 Stability

Retinoids have limited chemical and photochemical stability. The chemical stability of all-*trans*-retinyl palmitate and all-*trans*-retinol is strongly dependent on environmental factors such as solvent, temperature and availability of oxygen (Ji and Seo, 1999). There are multiple pathways for decomposition of all-*trans*-retinol and all-*trans*-retinyl palmitate. One significant pathway involves thermal isomerization of the all-*trans* isomer of retinol or retinyl palmitate to the appropriate 13-*cis* isomer:



13-*cis*-retinyl palmitate

Thermal isomerization is favored in lipophilic solvents and emulsions containing high compositions of oils (Ji and Seo, 1999). A second significant pathway for decomposition of retinol is dehydration leading to anhydro-retinol:



anhydro-retinol

Dehydration is favored in solvents and emulsions containing high compositions of water, and is further accelerated by oxygen and surfactants (Ji and Seo, 1999). Anhydro-retinol has also been observed as a decomposition product of retinyl palmitate (McBee *et al.*, 2000). A third general pathway for chemical decomposition of all-*trans*-retinol and all-*trans*-retinyl palmitate is oxidative degradation, leading to a complex mixture of degradation products (Samokyszyn and Marnett, 1990). Studies have shown that retinyl palmitate is significantly more chemically stable than retinol (Semenzato *et al.*, 1997; Ihara *et al.*, 1999).

Additionally, retinoids have limited photochemical stability. Pathways for photochemical decomposition include photoisomerization, photodimerization and photooxidation (Mousseron-Canet, 1971; Mousseron-Canet *et al.*, 1966; Dillon *et al.*, 1996). *In vitro* and *in vivo* studies have demonstrated that retinol is significantly more photochemically stable than retinyl palmitate (Ihara, *et al.*, 1999; Tang *et al.*, 1994; Sorg *et al.*, 1999).

3.0 PRODUCTION PROCESSES

Several processes have been developed for the synthesis of retinol and its esters. Two major synthetic procedures are those of Hoffmann-La Roche and of the Badische Anilin-und Soda-Fabrik (BASF). The Roche procedure involves as a key intermediate a C14 aldehyde and further requires the efficient reduction of acetylenic to olefinic bonds near the end of the synthesis. The BASF procedure depends heavily on the Wittig reaction, by which a phosphonium ylid reacts with an aldehyde or ketone to give an olefin and phosphine oxide (Olsen, 1991). Retinol is esterified by several procedures. An imidazolide method is used widely because of its nonacidic reaction conditions which stabilize retinol (Frickel, 1984).

4.0 REGULATORY STATUS

Retinyl palmitate has been found GRAS (generally recognized as safe) as a food additive when used in accordance with good manufacturing practices (Code of Federal Regulations, 2000a). Retinyl palmitate has also been approved for use in over the counter and prescription drugs (Food and Drug Administration, 1994; Food and Drug Administration, 1999)

5.0 OCCURRENCE IN COSMETIC FORMULATIONS

The number of cosmetic retail products containing retinyl palmitate has increased rapidly in the last two decades. Data available from FDA's Voluntary Cosmetics Registration Program, compiled in accordance with Title 21 part 270.4 (d)(1) of the Code of Federal Regulations (Code of Federal Regulations, 2000b), indicate that 102 cosmetic formulations in 1981, 355 cosmetic formulations in 1992 and 667 formulations in 2000 contained retinyl palmitate. Retail product categories containing retinyl palmitate include moisturizing preparations, skin care preparations, night skin care preparations, lipsticks, suntan gels and preparations, makeup preparations, and bath soaps and detergents (Cosmetic, Toiletry and Fragrance Association, 1999). The concentration of retinyl palmitate varies substantially amongst formulations in different product categories. The Cosmetic Ingredient Review (CIR) Expert Panel, the cosmetic industry's self-governing body for evaluating the safety of cosmetic ingredients, concluded a review of safety data for retinol and retinyl palmitate in 1987 (Cosmetic Ingredient Review, 1987). The CIR Expert Panel found that retinol and retinyl palmitate were safe as cosmetic ingredients in the practices of use and concentration ranges used (up to 13%) at that time.

6.0 BIOCHEMICAL AND HISTOLOGICAL EFFECTS ON SKIN

6.1 Percutaneous Absorption and Metabolism

The percutaneous absorption and metabolism of retinyl palmitate have been examined both *in vitro* and *in vivo*. Boehnlein *et al.*, 1994, have shown that about 18% of retinyl palmitate, applied topically in acetone to excised human skin, penetrates in 30 hrs after application. In addition, approximately 44% of the absorbed retinyl palmitate was hydrolyzed to retinol. In a clinical study, Duell *et al.*, 1997 found that topical application of a cream containing 0.6% retinyl palmitate resulted in elevated levels of retinol in skin measured 48 hrs and 72 hrs after application. No increases in levels of retinyl palmitate were noted, indicating efficient hydrolysis of retinyl palmitate to retinol by cutaneous esterases.

There is evidence that the enzymes required for sequential metabolism of retinol to retinaldehyde and retinoic acid are present in skin. Oxidation of retinol to retinaldehyde, catalyzed by cytosolic retinol dehydrogenase, is the rate-limiting step in the production of retinoic acid (Kim *et al.*, 1992). Alcohol dehydrogenases (class I and class IV) which actively catalyze oxidation of retinol have been found in mouse skin (Haselbeck *et al.*, 1997) and human keratinocytes (Siegenthaler *et al.*, 1990). Retinaldehyde dehydrogenase activity, needed for conversion of retinaldehyde to retinoic acid, has also been found in skin (Siegenthaler *et al.*, 1990b; Randolph and Simon, 1993). In spite of the presence of cutaneous enzymes required for conversion of retinol to retinoic acid, few studies have directly demonstrated biotransformation of topically applied retinol to retinoic acid in skin (Connor and Smit, 1987; Bailly *et al.*, 1998).

6.2 Biochemical and Histological Cutaneous Changes

Topical application of retinyl palmitate or retinol results in biochemical changes characteristically produced by retinoic acid. Topical application of retinoic acid induces increases in retinoic acid 4-hydroxylase and cellular retinoic acid binding proteins (Roos *et al.*,

1998). Topically applied retinyl palmitate and retinol have been shown to induce similar effects in human skin. Duell *et al.*, 1997 reported that topically applied 0.6% retinyl palmitate produces an increase in retinoic acid 4-hydroxylase, an enzyme required for limiting levels of retinoic acid through catabolic metabolism. The elevation seen after treatment with 0.6% retinyl palmitate was similar to the increase elicited by 0.025% retinoic acid. Cellular retinoic acid binding protein-II is essential for transport of retinoic acid from cytoplasm to the nucleus. Topically applied retinol (0.4%) was found to increase levels of cellular retinoic acid binding protein-II similar to the increase induced by 0.025% retinoic acid (Kang *et al.*, 1995). The effect of retinol on retinoid responsive genes in human skin has been examined by Varani *et al.*, 2000. These investigators examined expression of collagen-degrading matrix metalloproteinase genes whose expression is known to be inhibited by retinoic acid (Fisher *et al.*, 1996). Topical application of 1% retinol for 7 days resulted in a substantial reduction in metalloproteinase gene expression with concomitant increases in collagen accumulation.

Significant histological changes in skin are induced by topical application of retinyl palmitate or retinol. Studies in animal models indicate that topically applied retinyl palmitate or retinol induces epidermal hyperplasia and thickening (Counts *et al.*, 1988; González *et al.*, 1997). Similarly, topical application of retinyl palmitate or retinol to human skin results in epidermal hyperplasia and thickening (Duell *et al.*, 1997; Kang *et al.*, 1995).

7.0 TOXICOLOGICAL ISSUES

7.1 Developmental Toxicity

The increasing use of retinyl palmitate and retinol in cosmetic products has raised concerns about potential developmental toxicity. Recently, it has been reported that topical application of retinol does not result in changes in constitutive plasma levels of retinol and, therefore, does not increase the risk for developmental toxicity (Ries and Hess, 1999). The effect of topically applied retinol on systemic levels of retinol is now being investigated in a project sponsored by FDA's Office of Women's Health. In the first phase of this project, conducted in the Center for Food Safety and Applied Nutrition, percutaneous absorption of retinol is being examined in an animal model (rat). If systemic levels of retinol are found to increase, attendant increases in developmental toxicity will be examined in the study's second segment.

7.2 Photocarcinogenesis

The effect of topically applied retinyl palmitate and retinol on photocarcinogenesis has not been determined. However, several studies have appeared in which the effect of topically applied retinoic acid on photocarcinogenesis is investigated (Table 2). The similarities between the biochemical and histological effects of topically applied retinyl palmitate and retinoic acid on skin suggests that these studies are relevant for assessing the need for testing the effects of retinyl palmitate on photocarcinogenesis.

Studies of the effects of topically applied retinoic acid on photocarcinogenesis differ greatly in significant aspects of study design including: vehicle chosen; amount of retinoic acid applied and schedule for application; animal model used; and spectral distribution and dose of incident UV

radiation. The potential effect of the chosen vehicle on a study's outcome has been noted (Kligmann, 1987). This investigator observed that organic solvents, such as methanol, may cause sub-clinical irritation which could alter the effects of retinoids on skin and result in enhancement of photocarcinogenesis. This issue can not be definitively addressed since none of the studies currently available include a UV-irradiated control group receiving no topical treatment. However, irritation induced by topical treatment with organic solvents may not alone explain the differences observed, since studies in which methanol is used as a vehicle (e.g., Davies and Forbes, 1988) and a study in which a lotion serves as a vehicle (Hartmann and Teelman, 1981) demonstrate enhancement of photocarcinogenesis by topically applied retinoic acid. In addition, it has been observed that vehicles formulated without antioxidants differ substantially from marketed products and allow decomposition of retinoids (Kligmann, 1987). However, the studies outlined in Table 2 suggest no clear association between inclusion of antioxidants in topical formulations and a study's outcome. Additionally, the importance of pigmentation in the response of the animal model to UV radiation and retinoic acid has been suggested (Kligmann, 1987). A recent study, however, indicates that topically applied retinoic acid enhances photocarcinogenesis in both lightly pigmented and albino mice, although the enhancement of photocarcinogenesis is smaller in pigmented mice (Halliday *et al*., 2000). Finally, the spectral distribution and dose of incident UV radiation may play an important role in the outcome of photocarcinogenesis studies. A pattern is evident for studies in which solar simulating UV radiation is used at dose levels less than the human minimal erythema dose (Forbes *et al.*, 1979; Forbes *et al.*, 1981; Davies and Forbes, 1988; Hartmann and Teelman, 1981; Halliday *et al.*, 2000). Topically applied retinoic acid was found to enhance photocarcinogenesis in these studies. In contrast, topically applied retinoic acid is found to inhibit, or not affect, photocarcinogenesis in studies using UV radiation from unfiltered sources (Epstein and Grekin, 1981; Kligmann and Kligmann, 1981; Kligmann and Kligmann, 1981b). These radiation sources emit UVC radiation not present in terrestrial sunlight (Brown *et al.*, 2000). In addition, the doses of UV radiation in studies employing unfiltered sources exceed the human minimal erythema dose. While our current knowledge of the effects of retinoic acid on photocarcinogenesis may not allow a mechanistic explanation for the differences in outcome for studies employing lower UV doses from solar simulators and higher doses from unfiltered sources, it may be observed that the use of solar simulating UV radiation at low doses more closely resembles conditions encountered by individuals using retinoid-containing products.

Retinoic acid is currently used in a number of dermal drug products such as Retin-A® cream (containing 0.1%, 0.05% or 0.025% tretinoin, i.e. all-*trans*-retinoic acid) for acne treatment and Renova® (containing 0.05% all-*trans*-retinoic acid) as an adjunctive agent for mitigation of fine wrinkles, mottled hyperpigmentation and tactile roughness of facial skin (Physicians' Desk Reference, 2000). The results of experimental photocarcinogenesis studies are mentioned in the following precautions for use of these products:

"Studies in hairless albino mice suggest that tretinoin may accelerate the tumorigenic potential of weakly carcinogenic light from a solar simulator. In other studies, when lightly pigmented hairless mice treated with tretinoin were exposed to carcinogenic doses of UVB light, the incidence and rate of development of skin tumors were reduced. Due to significantly different experimental conditions, no strict comparison of these disparate data is possible. Although the

Table 2. Effects of Topically Applied Retinoids on Photocarcinogenesis

Topical Treatment	Treatment Regimen	UV Radiation	Animal	Effect on Photocarcinogenesis	Reference
0.3% retinoic acid in cream	10 months UV, 3 times/wk. Each irradiation followed by topical application of retinoic acid for first 4.4 months.	1.38 J/cm^2 UVC + UVB from hot quartz lamp	albino hairless mouse	Enhancement. Toxicity high (33% lethality).	Epstein, 1977.
0.05%, 0.025%, 0.005% retinoic acid in 59.5% ethanol/ 39.5 % PEG + 1% BHT	45 wks UV, 3 times/wk. Each irradiation followed by topical application (166, 83 or 16.6 nmole/mouse) after each irradiation.	1.25 J/cm^2 UVC + UVB from hot quartz lamp	albino hairless mouse	Inhibition (0.05%). No effect (0.025%, 0.005%)	Epstein and Grekin, 1981.
0.01%, 0.001% retinoic acid in methanol	2 wks daily topical treatment pre-irradiation (33 or 3.3 nmole/mouse, then 28 wks UV, 7 times per week with retinoic acid applied (33 or 3.3 nmole/mouse) after each irradiation.	~1/4 MED (human) from a solar simulator	Skh-1 mouse (albino, hairless)	Enhancement	Forbes *et al.*, 1979.
0.01% retinoic acid in methanol	6 wks UV pre-treatment 5 times/wk, then retinoic acid (33 nmole/mouse) alone 3 times/wk for 20 wks.	~1/2 MED (human) from FS20 fluorescence bulbs. UVC present also.	Skh-1 mouse (albino, hairless)	Enhancement	Forbes *et al.*, 1981.
0.01% retinoic acid in methanol	40 wks UV, 3 times/wk. Retinoic acid applied (33 nmole/mouse) after each irradiation.	~1/2 MED (human) from a solar simulator	cryptothrix mouse (some albino and some lightly pigmented)	Enhancement	Davies and Forbes, 1988.

Table 2. (continued) Effects of Topically Applied Retinoids on Photocarcinogenesis

Topical Treatment	Treatment Regimen	UV Radiation	Animal	Effect on Photocarcinogenesis	Reference
0.01% retinoic acid in methanol	40 wks UV, 3 times/wk. Retinoic acid applied (33 nmole/mouse) after each irradiation.	~1/2 MED (human) from a solar simulator	fuzzy rat (albino)	Enhancement	Davies and Forbes, 1988.
0.001% retinoic acid in 70% ethanol/30% propylene glycol	30 wks UV, 3 times/ wk application of retinoic acid (3.3 nmole/ mouse) followed each irradiation. Topical application continued 15 wks after irradiation ceased.	0.18 J/cm^2 UVB (~6 human MED) from FS20 sunlamp. UVC also present.	Skh-2 mouse (pigmented, hairless).	No effect on tumor latency, tumor yield or tumor progression.	Kligmann and Kligmann, 1981.
0.001% retinoic acid in 70% ethanol/30% propylene glycol	30 wks UV only, 3 times/wk; application of retinoic acid (3.3 nmole/mouse) only wks 31-45 (after UV treatment ended).	0.18 J/cm^2 UVB (~6 human MED) from FS20 sunlamp. UVC also present.	Skh-2 mouse (pigmented, hairless).	No effect on tumor latency, tumor yield or tumor progression.	Kligmann and Kligmann, 1981.

Table 2. (continued) Effects of Topically Applied Retinoids on Photocarcinogenesis

Topical Treatment	Treatment Regimen	UV Radiation	Animal	Effect on Photocarcinogenesis	Reference
0.05% retinoic acid in ethanol/propylene glycol + BHT.	18 wks application of retinoic acid, then 20 wks UV with application of retinoic acid after each irradiation, then 15 wks application of retinoic acid (after UV treatment ended).	0.012 J/cm^2 UVB (~4 human MED). UVC also present.	Skh-2 mouse (pigmented, hairless).	Inhibition	Kligmann and Kligmann, 1981b.
0.01%, 0.001% retinoic acid in lotion.	28 wks UV, 3 times/wk. Each irradiation followed by application of retinoic acid.	~ _ MED from solar simulator.	Fü-alb Hr/Hr mouse (albino, hairless).	Enhancement	Hartmann and Teelman, 1981.
0.001% retinoic acid in acetone	4 wks. UV, 5 times/wk. One group received topical retinoic acid (3.4 nmole/mouse) immediately after irradiation, one group received 5 applications (3.4 nmole/mouse) 0,1,2,3 and 4 hr after irradiation. Mice observed 52 wks.	3 J/cm^2 UVB (day 1), 6 J/cm^2 UVB (day 2), 9 J/cm^2 UVB (thereafter) from filtered FS40 fluorescent bulbs.	Skh-1 mouse (albino, hairless).	Inhibition. The inhibition greater for group receiving 5 applications.	Connor *et al.*, 1983.

Table 2. (continued) Effects of Topically Applied Retinoids on Photocarcinogenesis

Topical Treatment	Treatment Regimen	UV Radiation	Animal	Effect on Photocarcinogenesis	Reference
0.05% retinoic acid in ethanol/DMSO/acetone (1/1/6)	25 wks UV, 5 times/wk for first 4 wks and 3 times/wk thereafter. Topical application of retinoic acid (34 nmole/mouse) immediately after irradiation.	First 4 wks. 0.10 J/cm^2 UVB (~1/3 MED) 5 times/wk. from solar simulator, daily dose increased 20%/wk next 4 wks., daily dose remained same last 17 wks.	Skh-1 albino and Skh-2 lightly pigmented hairless mice.	Enhancement	Halliday *et al.*, 2000.

significance of these studies to man is not clear, patients should avoid or minimize exposure to sun (Physicians' Desk Reference, 2000)."

Two studies examining the effects of dietary supplementation with retinol and retinyl palmitate on photocarcinogenesis are summarized in Table 3. Both studies involved pre-feeding mice the appropriate retinoid prior to UV irradiation. Gensler *et al.*, 1990 supplemented the diets with retinyl palmitate 30 times greater than the amount in the basal diet. The group receiving this supplemented diet had a 41% reduction in tumor burden compared to the group receiving basal diet. Mikkelsen *et al.*, 1998 examined the effects of dietary supplementation with retinol on photocarcinogenesis. The group receiving a low level of dietary supplementation (0.5 mg retinol/kg diet) had a 42% reduction in incidence of tumors elicited by combined irradiation with UVA and UVB compared to the group receiving a high level of dietary supplementation (5 mg retinol/kg diet). When tumors were induced by UVB radiation alone, the group receiving the low level of dietary supplementation had a 38% reduction in incidence of tumors compared to the group given the higher level of dietary supplementation with retinol.

7.3 Human studies

A diverse group of studies have been conducted to determine the effects of retinyl palmitate on a variety of health conditions, involving numerous routes of administration. Some recent representative examples include the following. In an inhalation study, retinyl palmitate was used as a means of Vitamin A supplementation in preschool children, increasing levels of serum retinol and retinol-binding protein (Biesalski *et al.*, 1999). Supplementation with retinyl palmitate was also assessed in mother-to-child transmission of HIV-1, but was found to have no effect (Coutsoudis *et al.*, 1999). Mixed results have been reported for the use of retinyl palmitate as a chemotherapeutic agent. In a study by Redlich *et al.* (1999) in high risk smokers, a combination of beta-carotene and retinyl palmitate resulted in a 28% increase in the incidence of lung cancer. In intervention studies in patients with head and neck cancer, van Zandwijk *et al.* (2000) showed no benefits from treatment with retinyl palmitate, while immune functions were improved in patients using a combination of retinyl palmitate, cisplatin, 5-fluorouracil and Thymopentin (Recchia *et al.*, 1999).

8.0 REQUESTED STUDIES

A study of the photocarcinogenesis of retinyl palmitate, under conditions relevant to the use of retinyl palmitate in cosmetics, is requested. Additional mechanistic studies are needed to establish the relevance of the results obtained in the selected animal model. These additional studies could include *in vivo* assessment of the photogenotoxicity of topically applied retinyl palmitate and biochemical studies of the effects of topically applied retinyl palmitate on the skin of the experimental model.

Table 3. Effects of Dietary Retinoids on Photocarcinogenesis

Dietary Supplementation	Treatment Regimen	UV Radiation	Animal	Effect on Photocarcinogenesis	Reference
66 mg retinyl palmitate/kg diet (120 IU/g).	UV during wks 18 to 42 of dietary regime, 5 times/wk.	0.83 J/cm^2 UVB from FS40 lamps (UVC also present).	Lightly pigmented C3H/HeN mice.	Inhibition.	Gensler *et al.*, 1990.
0.5 and 5 mg retinol/kg diet (0.5-10.9 IU/g).	Supplemented diets fed 1 month prior to irradiation. Mice were then irradiated daily for 18 wks while continuing supplemented diet. Mice observed up to 60 wks.	Some groups received UVB only (26 J/cm^2 over 18 wks.) from FS40 fluorescent bulbs (UVC also present). Some groups received UVA + UVB (30 J/cm^2) from FS40 and TL40 bulbs.	Oslo/Bom lightly pigmented hairless mice.	Enhancement (5 mg/kg) Inhibition (0.5 mg/kg)	Mikkelsen *et al.*, 1998.

9.0 REFERENCES

Bailly, J., M. Crettaz, M. H. Schifflers and J. P Marty (1998) *In Vitro* Metabolism by Human Skin and Fibroblasts of Retinol, Retinal and Retinoic Acid. **Exp. Dermatol.** *7:* 27-34.

Biesalski H., R. Reifen, P. Furst and M. Edris (1999) Retinyl Palmitate Supplementation by Inhalation of an Aerosol Improves Vitamin A Status of Preschool Children in Gondar. **British J. Nutrition** *82*: 179-182.

Boehnlein, J., A. Sakr, J. L. Lichtin and R. L. Bronaugh (1994) Characterization of Esterase and Alcohol Dehydrogenase Activity in Skin. Metabolism of Retinyl Palmitate to Retinol (Vitamin A) During Percutaneous Absorption. **Pharm. Res.** *11(8):* 1155-1159.

Brown, D. B., A. E. Peritz, D. L. Mitchell, S. Chiarello, J. Uitto and F. P. Gasparro (2000) Common Fluorescent Sunlamps Are An Inappropriate Substitute for Sunlight. **Photochem. Photobiol.** *72:* 340-344.

Code of Federal Regulations (2000a) Title 21, Part 182.5930. Washington, D.C.

Code of Federal Regulations (2000b) Title 21, Part 720.4 (d)(1). Washington, D.C.

Connor, M. J., N. J. Lowe, J. H. Breeding and M. Chalet (1983) Inhibition of Ultraviolet-B Skin Carcinogenesis by All-*trans*-Retinoic Acid Regimens That Inhibit Ornithine Decarboxylase Induction. **Cancer Res.** *43:* 171-174.

Connor, M. J. and M. H. Smit (1987) The Formation of All-*trans*-Retinoic Acid from All-*trans*-Retinol in Hairless Mouse Skin. **Biochem. Pharmacol.** *36:* 919-924.

Cosmetic Ingredient Review (1987) Final Report on the Safety Assessment of Retinyl Palmitate and Retinol. **J. Am. Coll. Toxicol.** *6(3):* 279-320.

The Cosmetic, Toiletry and Fragrance Association (1999) **International Cosmetic Ingredient Dictionary and Handbook, Eighth Edition 2000**, Eds: Wenninger, J., Canterbery, R.C. and McEwen, G.N. Jr., CTFA, Washington, D.C., p. 1279.

Counts, D. F., F. Skreko, J. McBee and A. G. Wich (1988) The Effect of Retinyl Palmitate on Skin Compositiion and Morphometry. **J. Soc. Cosmet. Chem.** *39:* 235-240.

Coutsoudis, A., K. Pillay, E. Spooner, L. Kuhn and H. Coovadia (1999) Randomized Trial Testing the Effect of Vitamin A Supplementation on Pregnancy Outcomes and Early Mother-to-Child HIV-1 Transmission in Durban, South Africa. **AIDS** *13*: 1517-1524.

Davies, R. E. and P. D. Forbes (1988) Retinoids and Photocarcinogenesis. **J. Toxicol.-Cut. & Ocular Toxicol.** *7(4): 241-253.*

Dillon, J., E. R. Gaillard, P. Bilski, C. F. Chignell and K. J. Reszka (1996) The Photochemistry of the Retinoids As Studied by Steady-State and Pulsed Methods. **Photochem. Photobiol.** *63:* 680- 685.

Duell, E. A., S. Kang and J. J. Voorhees (1997) Unoccluded Retinol Penetrates Human Skin *In Vivo* More Effectively Than Unoccluded Retinyl Palmitate or Retinoic Acid. **J. Invest. Dermatol.** *109:* 301-305.

Epstein, J. H. (1977) Chemicals and Photocarcinogenesis. **Aust. J. Dermatol.** *18:* 57-61.

Epstein, J. H. and D. A. Grekin (1981) Inhibition of Ultraviolet-Induced Carcinogenesis by All-Trans-Retinoic Acid. **J. Invest. Dermatol.** *76:* 178-180.

Fisher, G. J., S. C. Datta, H. T. Talwar, Z. Q. Wang, J. Varani, S. Kang and J. J. Voorhees (1996) Molecular Basis of Sun-Induced Premature Skin Ageing and Retinoid Antagonism. **Nature (London)** *379:* 335-339.

Food and Drug Administration (1994) OTC Drug Review Ingredient Status Report (http://www.fda.gov/cder/otc/ingred.pdf).

Food and Drug Administration (1999) Prescription and Over-the-Counter Drug Product List-19th Edition (http://www.fda.gov/cder/rxotcdpl/pdpl_0699.htm).

Forbes, P. D., F. Urbach and R. E. Davies (1979) Enhancement of Experimental Photocarcinogenesis by Topical Retinoic Acid. **Cancer Lett.** *7:* 85-90.

Forbes, P. D. (1981) Photocarcinogenesis: An Overview. **J. Invest. Dermatol.** *77:* 139-143.

Frickel, F. (1984) Chemistry and Physical Properties of Retinoids. In: **The Retinoids, Volume 1**, Eds: M. Sporn, A. Roberts, and D. Goodman. Academic Press, FL., pp. 7-145.

Gensler, H. L., M. Aickin and Y. M. Peng (1990) Cumulative Reduction of Primary Tumor Growth in UV-Irradiated Mice by the Combination of Retinyl Palmitate and Canthaxanthin. **Cancer Lett.** *53:* 27-31.

González, S., M. V. Alcaraz, F. Diaz, T. J. Flotte, I. P. de Vargas, R. R. Anderson and N. Kollias (1997) DNA Ploidy Changes in Rhino Mouse Skin Induced by All-Trans Retinoic Acid and Retinol. **Skin Pharmacol.** *10:* 135-143.

Halliday, G. M., B. O. Robertson and R. St. C. Barnetson (2000) Topical Retinoic Acid Enhances, and a Dark Tan Protects, from Subedemal Solar-Simulated Photocarcinogenesis. **J. Invest. Dermatol.** *114:* 923-927.

Hartmann, H. R. and K. Teelmann (1981) The Influence of Topical and Oral Retinoid Treatment on Photocarcinogenicity in Hairless Albino Mice. In: Orfanos, C. E. et al. eds. **Retinoids: Advances in Basic Research and Therapy.** Berlin: Springer-Verlag, pp. 447-451.

Haselbeck, R. J., H. L. Ang and G. Duester (1997) Class IV Alcohol/Retinol Dehydrogenase Localization in Epidermal Basal Layer: Potential Site of Retinoic Acid Synthesis During Skin Development. **Devel. Dynamics** *208:* 447-453.

Ihara, H., N. Hashizume, N. Hirase, R. Suzue (1999) Esterification Makes Retinol More Labile to Photolysis. **J. Nutr. Sci. Vitaminol.** *45:* 353-358.

Ji, H.-G. and B. Seo (1999) Retinyl Palmitate at 5% in a Cream: Its Stability, Efficacy and Effect. **Cosmetics and Toiletries** *114:* 61-68.

Kang, S., E. A. Duell, G. J. Fisher, S. C. Datta, Z.-Q. Wang, A. P. Reddy, A. Tavakkol, J. Y. Yi, C. E. M. Griffiths, J. T. Elder and J. J. Voorhees (1995) Application of Retinol to Human Skin *In Vivo* Induces Epidermal Hyperplasia and Cellular Retinoid Binding Proteins Characteristic of Retinoic Acid But Without Measurable Retinoic Acid Levels or Irritation. **J. Invest. Dermatol.** *105:* 549-556.

Kim, , C.-I., M. A. Leo and C. S. Lieber (1992) Retinol Forms Retinoic Acid Via Retinal. **Arch. Biochem. Biophys.** *294:* 388-393.

Kligmann, L. H. and A. M. Kligman (1981) Lack of Enhancement of Experimental Photocarcinogenesis by Topical Retinoic Acid. **Arch. Dermatol. Res.** *270:* 453-462.

Kligmann, L. H. and A. M. Kligman (1981b) Lack of Enhancement of Experimental Photocarcinogenesis by Retinoic Acid.In: **Retinoids: Advances in Basic Research and Therapy,** Eds: Orfanos, C.E. *et al.*, Springer-Verlag, Berlin. pp. 447-451.

Kligmann, L. H. (1987) Retinoic Acid and Photocarcinogenesis-A Controversy. **Photodermatology** *4:* 88-101.

McBee, J. K., V. Kuksa, R. Alvarez, A. R. de Lera, O. Prezhdo, F. Haeseleer, I. Sokal and K. Palczewski (2000) Isomerization of *all-trans*-Retinol to *cis*-Retinol in Bovine Retinal Pigment Epithelial Cells: Dependence on the Specificity of Retinoid-Binding Proteins. **Biochemistry** *39:* 11370-11380.

Mikkelsen, S., B. Berne, B. Staberg and A. Vahlquist (1998) Potentiating Effect of Dietary Vitamin A on Photocarcinogenesis in Hairless Mice. **Carcinogenesis** *19(4)*: 663-666.

Mousseron-Canet, M., J.-C. Mani, C. Favie and D. Lerner (1966) On the Photochemical Isomerization of Vitamin A. **Compt. Rend.** *262:* 153-155.

Mousseron-Canet, M. (1971) Photochemical Transformation of Vitamin A. In: **Vitamins and Coenzymes,** Methods in Enzymology, Vol. 18 (Part C), Eds.: D. B. McCormick and L. D. Wright , Academic Press, NY, pp. 591-615.

Olsen, J. A. (1991) Vitamin A. In: **Handbook of Vitamins**, Ed: L. J. Machlin, Marcel Dekker, Inc., NY, p. 8.

Physicians' Desk Reference (2000) Medical Economics Company, Inc., Montvale, NJ.

Randolph, R. K. and M. Simon (1993) Characterization of Retinol Metabolism in Cultured Human Epidermal Keratinocytes. **J. Biol. Chem.** *268:* 9198-9205.

Recchia F., G. Sica, S. De Filippis, M. Rosselli, P. Pompili, G. Saggio and S. Rea (1999) Chemo-immunotherapy in Advanced Head and Neck Cancer. **Anticancer Res.** *19(1B)*: 773-777.

Redlich, C., J. Chung, M. Cullen, W. Blaner, A. van Bennekum and L. Berglund (1999) Effect of Long-term Beta-carotene and Vitamin A on Serum Cholesterol and Triglyceride Levels among Participants in the Carotene and Retinol Efficacy Trial (CARET). **Atherosclerosis** *145*: 425-432.

Ries, G. and R. Hess (1999) Retinol: Safety Considerations for Its Use in Cosmetic Products. **J. Toxicol.-Cut. & Ocular Toxicol.** *18(3):* 169-185.

Roos, T. C., F. K. Jugert, H. F. Merk and D. B. Bickers (1998) Retinoid Metabolism in the Skin. **Pharm. Rev.** *50:* 315-333.

Samokyszyn, V. M. and L. J. Marnett (1990) Inhibition of Microsomal Lipid Peroxidation by 13-*cis*-Retinoic Acid. In: **Retinoids, Part B, Cell Differentiation and Clinical Applications,** Methods in Enzymology, Vol. 190, Ed.: L. Packer, Academic Press, NY, pp. 281-288.

Semenzato, A., L. Bovenga, L. Faiferri, R. Austria and A. Bettero (1997) Stability of Vitamin A Propionate in Cosmetic Formulations. **Seifen, Oele, Fette, Wächse** *123:* 151-154.

Siegenthaler, G., J.-H. Saurat and M. Ponec (1990) Reinol and Retinal Metabolism. Relationship to the State of Differentiation of Cultured Human Keratinocytes. **Biochem. J.** *268:* 371-378.

Siegenthaler, G., D. Gumowski-Sunek, J. H.. Saurat (1990b) Metabolism of Natural Retinoids in Psoriatic Epidermis. **J. Invest. Dermatol.** *98:* 47s-48s.

Sorg, O., C. Tran, P. Carrauz, L. Didierjean, and J.-H. Saurat (1999) Retinol and Retinyl Ester Epidermal Pools Are Not Identically Sensitive to UVB Irradiation and Anti-Oxidant Protective Effect. **Clin. Lab. Invest.** *199:* 302-307.

Tang, C., A. R. Webb, R. M. Russell and M. F. Holick (1994) Epidermis and Serum Protect Retinol But Not Retinyl Esters from Sunlight-Induced Photodegradation. **Photodermatol. Photoimmunol. Photomed.** *10:* 1-7.

van Zandwijk, N., O. Dalesio, U. Pastorina, N. de Vries, H. van Tinteren (2000) EUROSCAN, a Randomized Trial of Vitamin A and *N*-acetylcysteine in Patients with Head and Neck Cancer or Lung Cancer. **J. National Cancer Institute** *92*: 977-986.

Varani, J., R. L. Warner, M. Gharaee-Kermani, S. H. Phan, S. Kang, J. Chung, A. Wang, S. C. Datta, G. J. Fisher and J. J. Voorhees (2000) Vitamin A Antagonizes Decreased Cell Growth and

Elevated Collagen-Degrading Matrix Metalloproteinases and Stimulates Collagen Accumulation in Naturally Aged Human Skin. **J. Invest. Dermatol.** *114:* 480-486.