Statement from Dr Des Fernandes about Retinyl Palmitate

This is my statement concerning the reports that retinyl palmitate could be implicated in the earlier onset of skin cancers.

First of all we have to recognise that this is not a problem created by the NIH. A consumer-oriented body (EWG?) in the USA has instigated a terror campaign because they feel the FDA is not responding to information given them by the NIH.

I have not come across any public statement from the research workers demanding that retinyl palmitate be banned. This document is to help you get perspective of this polemic.

Briefly: I think the proper response to any questions about the safety of Retinyl palmitate should be answered by saying that Dr Des Fernandes views the research with great suspicion because it is done on animals and not humans.

In summary my opinion:

These studies have been done in mice, not in humans whose skin behaves differently. Retinyl Palmitate protects us from sunlight at the same time and clinically the protection heavily outweighs the negative potential.

High stores of Retinyl Palmitate seem to protect us from getting skin cancer. Vitamin A is well known as an anticancer drug for many cancers and if we use any form of oral vitamin A we automatically raise the Retinyl Palmitate levels in the skin.

High oral doses of vitamin A specifically reduce the chances of squamous cell carcinoma in humans.

Every cosmetic vitamin A ingredient (except ret propionate) is automatically converted mainly into Retinyl Palmitate.

All the known clinical research on cosmetic forms of vitamin A reduces the signs of photoaging and photoaging is the first step in the generation of skin cancer.

The FDA and COLIPA have not banned Retinyl Palmitate

Retinyl Palmitate and light exposure - Research

Retinyl palmitate is being researched for what happens to its photo-degraded by products. Here are some references:[1]

Qingsu Xia >, Jun Jie Yin >, Shu-Hui Cherng >, 1 >, Wayne G. Wamer >, Mary Boudreau >, Paul C. Howard > and Peter P. Fua. UVA photoirradiation of retinyl
Abstract

We have previously reported that photoirradiation of retinyl palmitate (RETINYL PALMITATE) in ethanol with UVA light results in the formation of photodecomposition products, including 5,6-epoxy-RETINYL PALMITATE and anhydroretinol (AR). Photoirradiation in the presence of a lipid, methyl linoleate, induced lipid peroxidation, suggesting that reactive oxygen species (ROS) are formed. In the present study, we employ an electron spin resonance (ESR) spin trap technique to provide direct evidence as to whether or not photo irradiation of RETINYL PALMITATE by UVA light produces ROS. Photo irradiation of RETINYL PALMITATE by UVA in the presence of 2,2,6,6- tetramethylpiperidine (TEMP), a specific probe for singlet oxygen, resulted in the formation of TEMPO, indicating that singlet oxygen was generated. Both 5,5-dimethyl N- oxide pyrroline (DMPO) and 5-tert- butoxycarbonyl 5-methyl-1-pyrroline N-oxide (BMPO) are specific probes for superoxide. When photoirradiation of RETINYL PALMITATE was conducted in the presence of the DMPO or BMPO, ESR signals for DMPO- were obtained. These results unambiguously confirmed the formation of superoxide radical anion.

Consistent with a free radical mechanism, there was a near complete and time-dependent photodecomposition of RETINYL PALMITATE and its photodecomposition products. ESR studies on the photoirradiation of 5,6-epoxy-RETINYL PALMITATE and AR indicate that these compounds exhibit similar photosensitizing activities as RETINYL PALMITATE under UVA light.

They have done further research that confirms that retinyl palmitate is broken down and may cause DNA damage in laboratory tests. They used continuous UVA light at 50J/cm² comparable with normal sun-exposure on an average day. (2)
Retinyl palmitate (RP) is an ester of retinol (vitamin A) and the predominant form of retinol found endogenously in the skin. We have previously reported that photoirradiation of RP with UVA light resulted in the formation of anhydroretinol (AR), 5,6-epoxyretinyl palmitate (5,6-epoxy-RP) and other photodecomposition products. While AR was formed through an ionic photodissociation mechanism, 5,6-epoxy-RP was formed through a light-mediated, free radical-initiated chain reaction. In the current study, the phototoxicity of RP, AR and 5,6-epoxy-RP in human skin Jurkat T-cells with and without light irradiation was determined using a fluorescein diacetate assay. Under similar conditions, the Comet assay was used to assess damage to cellular DNA. Nuclear DNA was not significantly damaged when the cells were irradiated by UVA plus visible light in the absence of a retinoid; however, when the cells were illuminated with UVA plus visible light in the presence of either RP, 5,6-epoxy-RP or AR (50, 100, 150 and 200 mM), DNA fragmentation was observed. Cell death was observed for retinoid concentrations of 100
mM or higher. When treated with 150 mM of RP, 5,6-epoxy-RP or AR, cell death was 52, 33 and 52%, respectively. These results suggest that RP and its two photodecomposition products, AR and 5,6-epoxy-RP, induce DNA damage and cytotoxicity when irradiated with UVA plus visible light. We also determined that photoirradiation of RP, AR and 5,6-epoxy-RP causes single strand breaks in supercoiled phi chi 174 plasmid DNA. Using a constant dose of UVA light (50 J/cm²), the level of DNA cleavage was highest in the presence of AR, followed by 5,6-epoxy-RP, then RP. The induced DNA strand cleavage was inhibited by NaN₃. These results suggest that photoirradiation of RP, 5,6-epoxy-RP and AR with UVA light generates free radicals that initiate DNA strand cleavage.

To confuse one, Fu, working at the same toxicology institute has also written papers about the importance of vitamin A in skin. [3]

The skin is similar to other organs in how it absorbs, stores, and metabolizes vitamin A. However, because of the anatomical location of skin and the specialized physiological roles it plays, there are ways in which the skin is rather unique. The stratified structure of the epidermis results from the orchestration of retinoid-influenced cellular division and differentiation. Similarly, many of the physiological responses of the skin, such as dermal aging, immune defense, and wound healing, are significantly affected by retinoids. While much is known about the molecular events through which retinoids affect the skin’s responses, more remains to be learned. Interest in the effects of retinol, retinyl palmitate, and other retinoids on the skin, fueled in part by the promise of improved dermatologic and cosmetic products, will undoubtedly make the effects of retinoids on skin a subject for continued intense investigation.

Sorg has written another article on the safety of vitamin A that also uses mice and gets different impression. These mice did not get skin cancer. Vitamin A exerts a photoprotective action in skin by absorbing ultraviolet B radiation: Antille Tran Sorg et al. J Invest Dermatol 2003 pages 1163-7

Retinyl esters, a storage form of vitamin A, concentrate in the epidermis, and absorb ultraviolet radiation with a maximum at 325 nm. We wondered whether these absorbing properties of retinyl esters might have a biologically relevant filter activity. We first used an in vitro model to assess the photoprotective properties of retinyl palmitate. We then applied topical retinyl palmitate on the back of hairless mice before exposing them to 1 J per cm² ultraviolet B, and assayed the levels of thymine dimers produced in epidermal DNA 2 h following ultraviolet B exposure. Finally, we applied topical retinyl palmitate or a sunscreen on the buttocks of human volunteers before exposing them to four minimal erythema
doses of ultraviolet B; we assayed the levels of thymine dimers produced 2 h following ultraviolet B exposure, and determined the intensity of erythema 24 h after ultraviolet B. In vitro, retinyl palmitate was shown to be as efficient as the commercial filter octylmethoxycinnamate in preventing ultraviolet-induced fluorescence or photobleaching of fluorescent markers. The formation of thymine dimers in mouse epidermis was significantly inhibited by topical retinyl palmitate. In human subjects, topical retinyl palmitate was as efficient as a sun protection factor 20 sunscreen in preventing sunburn erythema as well as the formation of thymine dimers. These results demonstrate that epidermal retinyl esters have a biologically relevant filter activity and suggest, besides their pleomorphic biologic actions, a new role for vitamin A that concentrates in the epidermis.

Saurat and his team (who promote retinaldehyde) have written a lot about how all the versions of vitamin A become retinyl palmitate and yet they still
specifically protect the skin from aging, photodamage. See the article I have previously sent out by Saurat on Skin, sun and vitamin A from ageing to cancer.[4]

I believe that there are 7 major reasons why we should not lose faith in retinyl palmitate as an important molecule for cosmetic products. Here is the detailed report. I will highlight the references in Blue and smaller font.

These studies have been done in mice, not in humans whose skin behaves differently. The research is on murine cells and one cannot extrapolate it to humans. There are differences in fundamental cell processes in stem cells between humans and mice.

One should notice that this research is from the NIH.

Differences between human and mouse embryonic stem cells.
Stem Cell Section, Laboratory of Neurosciences, National Institute on Aging, NIH, Baltimore, MD 21224, USA.

Abstract
We compared gene expression profiles of mouse and human ES cells by immunocytochemistry, RT-PCR, and membrane-based focused cDNA array analysis. Several markers that in concert could distinguish undifferentiated ES cells from their differentiated progeny were identified. These included known markers such as SSEA antigens, OCT3/4, SOX-2, REX-1 and TERT, as well as additional markers such as UTF-1, TRF1, TRF2, connexin43, and connexin45, FGFR-4, ABCG-2, and Glut-1. A set of negative markers that confirm the absence of differentiation was also developed. These include genes characteristic of trophoectoderm, markers of germ layers, and of more specialized progenitor cells. While the expression of many of the markers was similar in mouse and human cells, significant differences were found in the expression of vimentin, beta-III tubulin, alpha-fetoprotein, eomesodermin, HEB, ARNT, and FoxD3 as well as in the expression of the LIF receptor complex LIFR/IL6ST (gp130). Profound differences in cell cycle regulation, control of apoptosis, and cytokine expression were uncovered using focused microarrays. The profile of gene expression observed in H1 cells was similar to that of two other human ES cell lines tested (line I-6 and clonal line-H9.2) and to feeder-free subclones of H1, H7, and H9, indicating that the observed differences between human and mouse ES cells were species-specific.
rather than arising from differences in culture conditions.

To my mind this article clearly illustrates why it is very suspect to extrapolate the results of the Vitamin A - skin cancer on mice to humans in a direct fashion.
Opinion: Comparative biology of mouse versus human cells: modelling human cancer in mice
Annapoorni Rangarajan & Robert A. Weinberg

Abstract
Laboratory mice have represented a powerful experimental system for understanding the intricacy of human cancer pathogenesis. Indeed, much of our current conceptualization of how tumorigenesis occurs in humans is strongly influenced by mouse models of cancer development. However, an emerging body of evidence indicates that there are fundamental differences in how the process of tumorigenesis occurs in mice and humans. What are these species-specific differences and how do they affect the use of mice as models of human tumour pathogenesis?

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The whole genetic development of cancer in each species shows important and distinct differences. Such differences raise very serious questions. Confusion about the validity of these tests in mice is increased when one looks at even older studies done on Swiss Mice where they found that both retinoic acid and retinyl palmitate inhibited the development of skin cancers.[5]

Abdel-Galil 1984: Two retinoids (13-cis-retinoic acid and retinyl palmitate) have been shown to exert a good preventive effect in chemically induced papillomas and carcinomas of the skin in female Swiss mice; this effect was investigated over a period of 23 weeks. The tumors were induced by repeated topical application of 3-methylcholanthrene (0.3% MCA, dissolved in acetone; 14 applications). Retinyl palmitate (RP; 6 mg in 0.1 ml acetone/mouse; 10 applications) and 13-cis-retinoic acid (RA; 3 mg in 0.1 ml acetone/mouse; 10 applications) were also administered topically for the 3rd to 9th week from the start of the experiment. This investigation gave evidence for the fact that both the retinoids did not only inhibit the development of skin papillomas but had also a marked effect on skin carcinomas.

There is more conflicting evidence when scientists tested retinyl palmitate for genotoxicity in hamsters and found no evidence of genetic damage from retinyl
palmitate applied to the skin and then exposed to UV. [6] Genetic damage is fundamental to the development of cancer. If there is no demonstrable genotoxicity then the chances for cancer are almost nil.

Dufour 2009: Retinyl palmitate (RP), an ingredient of cosmetic and medical skin-care preparations, has been reported to be photo-genotoxic/photo-clastogenic in mouse lymphoma cells (Tk locus) as well as in human Jurkat T-cells, as measured by use of the
comet assay. Given that these results were obtained under exploratory conditions, we re-investigated the photo-genotoxicity of RP following a protocol consistent with current recommendations for photo-genotoxicity testing of drugs and chemicals. We tested RP in Chinese hamster ovary (CHO) cells in the dark (standard chromosome aberration test) under pre-irradiation (UVA irradiation of cells and subsequent treatment with RP) or simultaneous irradiation (irradiation of cells and RP together, standard photo-genotoxicity protocol) conditions. UVA irradiation was applied at 350 and 700mJ/cm² with the high UV dose targeted to produce a small increase in the incidence of structural chromosome aberrations (CA) in cells not treated with RP. RP was tested up to and above its limit of solubility in the culture medium (20-40μg/mL). There was no overt cytotoxicity under dark or different irradiation conditions. Treatment of cells with RP in the dark, as well as treatment under pre- or simultaneous irradiation conditions failed to produce biologically significant increases in the incidence of CA, whereas the positive control substances 4-nitroquinolone and 8-methoxypsoralene produced significantly positive effects in the dark or under simultaneous irradiation, respectively. Overall, our results failed to confirm the reported positive photo-genotoxic effects, and suggest that they may have been due to the test conditions, i.e. high irradiation doses, high cytotoxicity or re-irradiation of photo-products. In conclusion, our data suggest that, under standard conditions for testing photo-genotoxicity, RP had no in vitro genotoxic or photo-genotoxic potential and is therefore unlikely to pose a local or systemic genotoxic or photo-genotoxic risk.

Some more research shows that retinyl palmitate specifically, and not cis-retinoic acid, reduces the incidence of papillomas in a different strain of mice[7]. This is exactly opposite of what the FDA research says. Who is right? I believe that these studies give clues to the development of tumors in mice but each strain of mouse has a different response.

Helen L. Gensler2, Ronald R. Watson, Satoru Moriguchi3 and G. Tim Bowden
Effects of Dietary Retinyl Palmitate or 13-cis-Retinoic Acid on the Promotion of Tumors in Mouse Skin1 Cancer Research 47, 967-970, February 15, 1987
Department of Radiation Oncology and Cancer Center [H. L. G., G. T. B.] and Department of Family and Community Medicine [R. R. W., S. M.], University of Arizona Health Sciences Center, Tucson, Arizona 85724

The present study was designed to determine the effects of dietary 13-cis-retinoic acid and retinyl palmitate on mouse skin tumor promotion by 12-O-tetradecanoylphorbol-13-acetate (TPA). Female CD-1 mice were initiated with 150
nmol of 7,12-dimethylbenz(a)anthracene and promoted twice weekly with 8 nmol of TPA. Diets supplemented with retinyl palmitate to yield 60,000 or 200,000 IU or 700,000 for 5 wk followed by 350,000 IU per kg of diet (700,000/350,000) fed to mice during tumor promotion resulted in 9%, 37%, and 65% inhibition of the papilloma yield, respectively, at 21 wk of promotion. Although topical applications of 13-cis-retinoic acid have been almost as effective as retinoic acid in preventing the appearance of mouse skin tumors, dietary 13-cis-retinoic acid at 200,000 or 700,000 IU per kg of diet resulted in no reduction in papilloma yield but did result in a dose-dependent decrease in the tumor burden (weight of tumors per mouse). Therefore, dietary retinyl palmitate yielded a dose-dependent inhibition of the number and weight of tumors promoted by TPA, whereas dietary 13-cis-retinoic acid resulted in a decrease in weight but not in number of tumors promoted by TPA.

Sorg also found conflicting evidence in hairless mouse. [8]
We showed in a recent study that topical retinyl palmitate prevented UV-B-induced DNA damage and erythema in humans. Given that retinyl palmitate is a precursor of retinoic acid, the biological form of vitamin A that acts through nuclear receptors, we wondered whether these protective effects toward UV-B exposure were either receptor dependent or linked to other properties of the retinoid molecule such as its spectral properties. We determined the epidermal retinoid profile induced by topical retinoic acid in hairless mice and analyzed its effect on markers of DNA photodamage (thymine dimers) and apoptosis following acute UV-B exposure; we compared these effects to those induced by other natural topical retinoids (retinaldehyde, retinol and retinyl palmitate) which do not directly activate the retinoid receptors. We then analyzed the direct action of these retinoids on UV-B-induced DNA damage and apoptosis in cultured A431 keratinocytes. Topical retinoic acid significantly decreased (approximately 50%) the number of apoptotic cells, as well as the formation of thymine dimers in the epidermis of mice exposed to acute UV-B. Interestingly, the other topical retinoids decreased apoptosis and DNA damage in a similar way. On the other hand, neither retinoic acid nor the other retinoids interfered with the apoptotic process in A431 keratinocytes exposed to UV-B, whereas DNA photodamage was slightly decreased. We conclude that the decrease of apoptotic cells in hairless mouse epidermis following topical retinoids and UV-B irradiation reflects a protection of the primary targets of UV-B (DNA) by a mechanism independent of the activation of retinoid nuclear receptors, rather than a direct inhibition of apoptosis.

In humans we know that contrary findings have been shown to be true: low levels of retinyl esters (90% of which is retinyl palmitate) are predictive for getting skin cancer. (see below reference 12) The NIH study deals with retinyl palmitate - It would be extreme to believe that ANYTHING containing retinyl palmitate is detrimental when this is the natural storage form of ~80% of the vitamin A in our skin.

The clinical benefits in humans far outweigh these preliminary findings in mice. We have to think rationally about this data, and the overwhelming preponderance of peer-reviewed clinical evidence supporting the use of topical Retinyl Palmitate. [9]

Retinyl palmitate protects us from sunlight at the same time and clinically the protection heavily outweighs the negative potential. [10] Retinyl esters, a storage form of vitamin A, concentrate in the epidermis, and
absorb ultraviolet radiation with a maximum at 325 nm. We wondered whether these absorbing properties of retinyl esters might have a biologically relevant filter activity. We first used an in vitro model to assess the photoprotective properties of retinyl palmitate. We then applied topical retinyl palmitate on the back of hairless mice before exposing them to 1 J per cm² ultraviolet B, and assayed the levels of thymine dimers produced in epidermal DNA 2 h following ultraviolet B exposure. Finally, we applied topical retinyl palmitate or a sunscreen on the buttocks of human volunteers before exposing them to four minimal erythema doses of ultraviolet B; we assayed the levels of thymine dimers produced 2 h following ultraviolet B exposure, and determined the intensity of erythema 24 h after ultraviolet B. In vitro, retinyl palmitate was shown to be as efficient as the commercial filter octylmethoxycinnamate in preventing ultraviolet-induced fluorescence or photobleaching of fluorescent markers. The formation of thymine dimers in mouse epidermis was significantly inhibited by topical retinyl palmitate. In human subjects, topical retinyl palmitate was as efficient as a sun protection factor 20 sunscreen in preventing sunburn erythema as well as the formation of thymine dimers. These results demonstrate that
epidermal retinyl esters have a biologically relevant filter activity and suggest, besides their pleomorphic biologic actions, a new role for vitamin A that concentrates in the epidermis.

High stores of retinyl palmitate seem to protect us from getting skin cancer. Vitamin A is well known as an anticancer drug for many cancers and if we use any form of oral vitamin A we automatically raise the RP levels in the skin. Retinyl palmitate is the safety mechanism to ensure that we have sufficient supplies when supply from liver stores runs low.[11]


Vitamin A and its active metabolites are important for growth and differentiation of a variety of cells, mainly in mucosa-associated epithelia, where they exhibit a wide spectrum of activities. Vitamin A, stored as retinyl esters (REs), is delivered from liver stores into the bloodstream as retinol bound to retinol binding protein. This process is regulated homeostatically, ending up in a more or less constant plasma retinol level. In situations of a high vitamin A demand (e.g., inflammation, diseases, prenatal period), this supply can be insufficient because of delayed production of retinol binding protein, leading to local deficiencies and impairment of structure and function in the respective tissues.

This delay may be overcome by cellular RE stores. Several cell types, including buccal mucosa cells, can take up RE. Retinyl palmitate is taken up when it is applied topically to either metaplastically mutated rat vaginal epithelium (as a gel) or to human meta- and dysplastic bronchial epithelia (via inhalation) that have a vitamin A deficiency. In rats and humans, the modified epithelia can be normalized, at least in part. In conclusion, topically applied retinyl esters may be a promising therapy for local retinol deficiencies and may reverse the morphological alterations of the epithelium in tissues that are vitamin A deficient.

And [12]

Decreased retinyl ester concentrations in UV-induced murine squamous cell carcinomas Berne, B. Torma, H. Staberg, B. Mikkelsen, S Vahlquist, A. Vahlquist is a great vitmain A researcher)
Acta Derm Venereol 1989 Vol 69 page 503-8
Squamous cell carcinomas were induced in hairless mice by repeated irradiations with UVB (280-320 nm, total dose 30 J/cm2) plus UVA (320-400 nm, total dose
168 J/cm²). The irradiated animals and non-irradiated controls were fed on diets with or without vitamin A supplementation (20,000 IU/kg). At the appearance of tumours, 30 to 43 weeks after the last irradiation, the vitamin A (retinol plus retinyl ester) concentrations in the serum, liver, epidermis and tumours and the retinol esterifying activities in microsomes from epidermis and tumours were measured. The liver and epidermal vitamin A concentrations were 2-3 times higher in vitamin A supplemented than in unsupplemented animals, but did not differ between tumour-bearing animals and non-irradiated controls receiving identical diets. The vitamin A concentration in the tumours was significantly lower than in perilesional epidermis. The largest difference (p less than 0.001) between the tumour and epidermal values was observed in the vitamin A supplemented group. The low vitamin A content of the tumours was entirely due to a marked (2 to 6-fold) reduction in the retinyl ester fraction. In contrast, the retinol content of the tumours was increased to twice that of normal epidermis. The activity of the esterifying enzyme, acyl-CoA:retinol acyltransferase (EC 2.3.1.76), was unchanged. The reason for the reduced retinyl ester concentration thus remains unclear. Still, it is possible that a disturbed interconversion of retinol to retinyl esters plays a role in murine photo-carcinogenesis.
And from the doyen of vitamin A research, Vahlquist, [13] to show the importance of maintaining high retinyl esters in the skin to minimise skin cancer


Keratinocytes from three different layers of epidermis (stratum basale, stratum spinosum, and stratum granulosum/corneum) were shown by high-performance liquid chromatography to contain retinol, 3,4-didehydroretinol and several fatty acyl esters thereof. The concentration of unesterified congeners increased 1.8-2.8 times from the inner to the outer layers of epidermis, while the corresponding increase in fatty acyl esters was 4.0-6.5 times. Together the esters represented 71% of the total vitamin A content in stratum granulosum/corneum as compared to 54% in stratum basale. The in situ synthesis of fatty acyl esters of retinol and 3,4-didehydroretinol (vitamin A2) was studied by addition of [3H]retinol to organ-cultured human breast skin. The radioactive compounds appearing in the epidermis after 48 h were, in order of abundance, retinyl esters, retinol, 3,4-didehydroretinyl esters, and 3,4-didehydroretinol. Studies at the subcellular level demonstrated the highest esterifying activity in the microsomal fraction. The enzyme catalyzing the reaction, acyl CoA:retinol acyltransferase (ARAT; EC 2.3.1.76), had a pH optimum of 5.5-6.0, which differs from that of ARAT in other tissues. ARAT activities in microsomes from different layers of epidermis were similar, but, owing to a presumed pH gradient in upper epidermis, the in vivo esterification of vitamin A may be enhanced in terminally differentiating keratinocytes. The mean ARAT activities in basal cell carcinomas and squamous cell carcinomas were less than 50% of the control values, and the relative amounts of retinyl esters were significantly lower than normal. We suggest that the esterification of vitamin A may also be of importance in relation to pathologic keratinocyte differentiation.

Here’s an explanation of the role played by retinyl esters.[14]

Guo and team in 2001 in Cancer Research, also feels that something interferes in the formation of retinyl esters in cancer cells:

Reduced level of retinyl esters and vitamin A in human renal cancers.

Clinical and preclinical studies suggest that retinoids can inhibit the growth of a small percentage of human renal cancers (RCs), although the majority of RCs both in vitro and in vivo are retinoid resistant. Our recent studies indicate that the metabolism of retinol to retinyl esters is greatly reduced in human carcinoma cell lines of the oral cavity, skin, and breast as compared with their normal epithelial counterparts, suggesting that human carcinoma cells are retinoid deficient relative to normal epithelial cells. We considered whether retinoid resistance in RCs was
related to an abnormality in retinoid metabolism. The metabolism of [3H]retinol and of [3H]retinoic acid (RA) was examined in RC cell lines and normal human kidney (NK) epithelial cells cultured in media, in RA, or in RA plus IFN-alpha. The expression of LRAT (lecithin:retinol acyltransferase) was assessed by Northern and Western analysis. Retinol and retinyl ester levels were determined in tissue samples of normal human kidney and renal cell carcinoma. NK cells esterified all of the 50 nM [3H]retinol in which they were cultured. In contrast, six of the seven RC cell lines metabolized only trace amounts of [3H]retinol to [3H]retinyl esters. Consistent with this relative lack of [3H]retinol esterification by the tumor cells, the tumor cells exhibited LRAT transcripts of aberrantly low sizes relative to those in normal epithelial cells. Moreover, the NK cells expressed abundant levels of LRAT protein by Western analysis, whereas the RC cells did not express LRAT protein. When samples of human kidney tumor tissue were compared with samples of normal kidney tissue from patients who had undergone surgery for primary RC, the normal kidney tissues contained much higher levels of retinol and retinyl esters (approximately 0.5-2 microg/gram wet weight) than the tumor tissues in all seven patients examined. Culture of the RC lines in IFN-alpha plus all-trans-RA, a combination therapy used clinically, resulted in higher intracellular levels of [3H]retinol and [3H]retinyl esters. The metabolism of [3H]RA was also examined in these RC lines versus
NK cells. Although the NK epithelial cells metabolized [3H]RA, the majority of the RC lines metabolized [3H]RA at a much slower rate. Most of the RC lines metabolized only 10-30% of the 50 nM [3H]RA over 6 h of culture. These data indicate that RCs both in vitro and in vivo are retinol and retinyl ester deficient relative to the normal human kidney, and they suggest that the aberrant differentiation of the neoplastic renal cells results in part from a defect in retinoid metabolism.

In another article Guo points out this problem again in Carcinogegensis page 1925-33 2000 [15]

Esterification of all-trans-retinol in normal human epithelial cell strains and carcinoma lines from oral cavity, skin and breast: reduced expression of lecithin:retinol acyltransferase in carcinoma lines
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When exogenous [3H]retinol (vitamin A) was added to culture medium, normal human epithelial cells from the oral cavity, skin, lung and breast took up and esterified essentially all of the [3H]retinol within a few hours. As shown by [3H]retinol pulse–chase experiments, normal epithelial cells then slowly hydrolyzed the [3H]retinyl esters to [3H]retinol, some of which was then oxidized to [3H]retinoic acid (RA) over a period of several days. In contrast, cultured normal human fibroblasts and human umbilical vein endothelial cells (HUVEC) did not esterify significant amounts of [3H]retinol; this lack of [3H]retinol esterification was correlated with a lack of expression of lecithin:retinol acyltransferase (LRAT) transcripts in normal fibroblast and HUVEC strains. These results indicate that normal, differentiated cell types differ in their ability to esterify retinol. Human carcinoma cells (neoplastically transformed epithelial cells) of the oral cavity, skin and breast did not esterify much [3H]retinol and showed greatly reduced LRAT expression. Transcripts of the neutral, bile salt-independent retinyl ester hydrolase and the bile salt-dependent retinyl ester hydrolase were undetectable in all of the normal cell types, including the epithelial cells. These experiments suggest that
Retinoid-deficiency in the tumour cells could develop because of the lack of retinyl esters, a storage form of retinol.

High oral doses of vitamin A specifically reduce the chances of squamous cell carcinoma and basal cell carcinoma as well as a number of other cancers.\[16\]

As indicated above, in some cases the effects of retinoids appear to be species-specific. Although retinyl acetate and 4-HPR are ineffective in preventing mammary cancer induced by DMBA or occurring spontaneously in mice, these retinoids prevent carcinogen-induced mammary cancer in rats. In contrast, retinoids have modest chemopreventive activity for bladder cancer in various strains of both mice and rats and may have some therapeutic and preventive effects in human bladder. Retinyl palmitate is reported to reduce the incidence of esophageal lesions in hamsters; however, retinyl acetate may increase the incidence of esophageal tumors in rats. Although 13-cis-RA reduces the incidence of spontaneous thymic lymphomas in AKR mice and C57BI/10W mice exposed to X rays and has some therapeutic effect on myelodysplastic syndromes in
humans, 4-HPR may enhance leukemic progression in patients with this syndrome. For treatment of this syndrome, selection of the proper retinoid appears to be important.

Topically applied retinyl palmitate reduces the incidence of cervical cancer in hamsters, and topically applied RA has a therapeutic effect on cervical dysplasia in humans.

Retinamides have a modest chemopreventive effect against pancreatic cancer in rats dosed with azaserine; these compounds are reported both to increase and to decrease the incidence of pancreatic cancer in hamsters. Retinoids may, or may not, be carcinogen-specific in different species. Some are effective in preventing mammary cancer in rats, regardless of which carcinogen is used. Applied to mouse skin, retinoids are active with either DMBA or BP as the carcinogen and 12-tetradecanoyl phorbol-13-acetate (TPA) as the promoter. Nevertheless, retinoids are not effective in preventing skin papillomas and carcinomas caused by UV light. There is no comparable system for humans, although retinoids demonstrate activity against basal cell carcinomas, squamous cell carcinomas, and actinic keratoses on the skin of humans. Fewer bladder tumors develop in rats dosed with HO-BBN when they are put on diets containing certain retinoids, but those dosed with FANFT are not affected. Similarly, retinyl acetate is reported to be active against liver tumors induced by 3′-MeDAB but not against those induced by aflatoxin B1. In contrast, forestomach carcinomas induced in hamsters by either DMBA or BP are prevented by retinyl palmitate. The route of administration of retinoids may also be important. (ABSTRACT TRUNCATED AT 400 WORDS)

Sorg also gives a probable answer why retinyl esters need to be associated with antioxidants. [17]

Vitamins A and E are present in mammalian skin. Although the main circulating form of vitamin A in the blood is retinol, the epidermis stores it as retinyl esters. The epidermis can be easily loaded with high amounts of vitamin A by topical application of either retinol or retinaldehyde, two well-tolerated precursors of the biologically active retinoic acid, while topical alpha-tocopherol loads the epidermis with vitamin E. The probable physiological function of epidermal vitamin E is to contribute to the antioxidant defense of the skin, whereas that of epidermal vitamin A (retinol and retinyl esters) is not yet well understood. Besides being a precursor for retinoic acid, vitamin A also has a free radical scavenging potential. Due to their physical properties, vitamins A and E absorb ultraviolet (UV) light in
the region of solar spectrum that is responsible for most of the deleterious biological effects of the sun. In the mouse, topical vitamin A has been shown to prevent the UV-induced epidermal hypovitaminosis A, while topical vitamin E prevents oxidative stress and cutaneous and systemic immunosuppression elicited by UV. Thus constitutive epidermal vitamins A and E appear complementary in preventing UV-induced deleterious cutaneous and systemic effects, and these properties can be reinforced by topical application of retinol or retinaldehyde and topical alpha-tocopherol.

Every cosmetic vitamin A ingredient (except retinyl propionate and retinyl acetate) is automatically converted mainly into retinyl palmitate and then metabolised to retinol, retinaldehyde and eventually into retinoic acid. Topically applied retinyl palmitate is converted into retinol. In human skin, 44% of the absorbed retinyl palmitate was hydrolyzed to retinol. This shows that use of retinyl palmitate in cosmetic formulations may result in significant delivery of retinol into the skin.[18]

Retinyl palmitate, a widely used ingredient in cosmetic products, is promoted for its beneficial effects on the appearance of skin. Previous studies suggest that enzymes are available in skin to metabolize this ingredient during skin absorption. Esterase activity hydrolyzes retinyl palmitate to retinol (vitamin A), which is oxidized in many tissues to retinoic acid primarily by alcohol dehydrogenase. The activities of esterase and alcohol
dehydrogenase were characterized in hairless guinea pig skin by using flow-through diffusion cells and radiolabeled model compounds (methyl salicylate and benzyl alcohol) previously shown to be metabolized by these enzymes. Methyl salicylate was hydrolyzed by esterase to a greater extent in viable skin than in nonviable skin. Glycine conjugation of salicylic acid and benzoic acid occurred only in viable skin. The metabolism of methyl salicylate and benzyl alcohol occurred to a greater extent in male guinea pig skin than in female guinea pig skin. The percutaneous absorption of both radiolabeled compounds was similar in viable and nonviable skin. About 30 and 18% of topically applied retinyl palmitate were absorbed from an acetone vehicle by hairless guinea pig skin and human skin, respectively. Less than 1% of the applied dose of this lipophilic compound diffused from skin into the receptor fluid. Retinol was the only detectable metabolite of retinyl palmitate in both hairless guinea pig and human skin. In human skin, 44% of the absorbed retinyl palmitate was hydrolyzed to retinol. The use of retinyl palmitate in cosmetic formulations may result in significant delivery of retinol into the skin.

All the known clinical research on cosmetic forms of vitamin A reduce the signs of photoaging. Photoaging is the first step in the generation of skin cancer. The FDA and COLIPA have not banned retinyl palmitate. See document below from Personal Care Products Council.

Company Logo

January 20, 2011

Lori D. White, Ph.D., PMP
NTP Designated Federal Officer NIEHS/NIH
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whiteld@niehs.nih.gov

Dear Dr. White:
The following comments are submitted on behalf of the Personal Care Products Council in response to the National Toxicology Program (NTP) Board of Scientific Counselors Technical Report Subcommittee’s review of “NTP Technical Report on the Photocarcinogenesis Study of Retinoic Acid and Retinyl Palmitate in SKH-1 Mice”,

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Based in Washington, D.C., the Council is the leading national trade association representing the $250 billion global cosmetic and personal care products industry. Founded in 1894, the Council’s more than 600 member companies manufacture, distribute, and supply the vast majority of finished personal care products marketed in
the United States. As the makers of a diverse range of products that millions of consumers rely on everyday, from sunscreens, toothpaste, and shampoo to moisturizer, lipstick, and fragrance, member companies are global leaders committed to product safety, quality, and innovation. The Council was previously known as the Cosmetic, Toiletry, and Fragrance Association (CTFA).

NTP BSC Technical Report Review Panel Charge
Retinyl palmitate is approved by U.S. Food and Drug Administration (FDA) as a food GRAS nutrient and as an over-the-counter (OTC) and prescription drug. To achieve premarket approval, FDA, which is the U.S. regulatory authority for retinyl palmitate when used as a drug, in foods and in cosmetics, required extensive and rigorous premarket testing. It is important that NTP Technical Report (TR) panels recognize that NTP is not a regulatory authority. We were therefore encouraged to note that NTP’s charge to the panel is focused and crisp: (1) peer review the scientific and technical elements of the study and its presentation; (2) determine whether the study’s experimental design and conduct support the NTP’s conclusions regarding the carcinogenic activity of the substance tested. Retinyl Palmitate Nomination

In November, 2000, the FDA’s Center for Food Safety and Applied Nutrition (CFSAN) nominated Retinyl Palmitate (RP) to the National Toxicology Program requesting “
photocarcinogenesis study of retinyl palmitate, under conditions relevant to the use of retinyl palmitate in cosmetics” – and—“mechanistic studies to establish the relevance of the results obtained in the selected animal model”. In the draft TR 568 report, made available in December, 2010, the nomination rational and testing request states “--- 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 RP, and the association between topical application of retinoids and enhancement of photocarcinogenesis”.

While the term “sunscreen” is not mentioned, we believe it may be implied.

The NTP RP protocol was not properly constructed to test sunscreens or sun blockers containing RP.

Time Line
We understand the FDA nominated RP to the NTP in 2000, that the one (1) year photocarcinogenesis study was begun in 2003 and that the on-site pathology was not completed until mid-2006. While it is recognized that delays can occur in any study, we question the two-year delay in pathology completion, since the 1% and 2% RP animals did not have pathology performed, and especially the four-year delay from pathology completion to the availability of the draft RP TR in December 2010. Because of the reported study flaws in the TR 568 report, we wonder if NTP had concerns about the adequacy of the study or ever considered not bringing the study forward.

Protocol Design
In the standard UVR SKH-1 protocol designed by Forbes, animals receive test agent followed by UVR exposures on Monday, Wednesday and Friday and receive UVR exposures followed by test agent on Tuesday and Thursday, a routine followed for 40 weeks with an additional 15 week no dose/no UVR monitoring; endpoints are typically time to lesion formation (specified size) and/or lesion multiplicity. The exposure protocol for this study was different in that treatment with UVR was in the morning 5 days a week and treatment with test agents was in the afternoon 5 days a week then, after 40 weeks
of treatment, the mice were held without treatment for additional 12 weeks prior to sacrifice. The exposure protocol for the TR 568 report was selected to “mimic human use where people are exposed to sunlight during the afternoon then use the retinoid-containing creams at night”. We wonder how the change in the exposure protocol from the widely accepted Forbes standard protocol could have influenced the outcome of these studies.

Reasons for Removal
Tables 4 and 5 in TR 568 show that in groups with control cream and RP, the main reason for animals exiting the study was Skin Lesion ≥ 10 mm. However, in the Preliminary Pathology Tables presented on the NTP web site the cause for removal was listed as “harvest.” We believe that removal criteria other than tumors ≥10 mm may have been used when determining whether or not to remove animals under the “harvest” terminology (e.g. due to severe toxicity). It would benefit the reader if the Standard Operating Procedure (SOP) for animal removal were included in the appendices of TR568 since the removals were considered to be non-censored animals (known lifetime) and the vast majority of animals exposed to sunlight/cream or sunlight/cream + RP were in the “removed” category. Therefore the criteria for selecting
animals for removal from this experiment were considered non-censored in spite of the fact that many were removed due to toxicity (and thus should have been classified as censored animals).

Note that the issue of censoring is of more than academic interest. For example, one might have comparable numbers of tumors in each of two dose groups but if animals were removed sooner in the first group, it would have a higher tumor rate than the second because the first group would have fewer animal-years. At a more general level, the report notes that the survival analyses presented are in fact survival-removal analyses. So what exactly does removal mean? A full understanding of the data in this study must be accompanied by a detailed discussion of the removal criteria, and the reader would also be assisted by a discussion of the relative numbers of animals removed by removal criterion.

Statistical Analysis and Confounders
Our overall impression is that the statistical analyses applied in TR 568 are appropriate, and that the signals that the test system is generating may appear reasonable to the reader not familiar with the nuances of photo-cocarcinogenesis bioassays. However, we note that the difference between the response of control cream plus UVR compared to UVR only is unacceptably dramatic (see Example 1 this document). We also note that 1% and 2% RP formulations appear toxic even in the absence of UVR (Figures 8 and 9) and assume that this is the reason that neither 1% nor 2% RP animals appear in the pathology evaluations. To us, this should have resulted in study termination.

Males all low sun with no cream

Example 1 Time on Test (TOT) Data Male survival with Low-Sun treatment (6.75 mJ.CIE/cm2) for No Cream (NC - Green); Control Cream (PH7 - Turquoise); Low RP
(LRP - Red); and Mid RP (MRP - Blue). Note the magnitude of the shift to the left for
the Control Cream (Turquoise line) compared to the No Cream group (Green Line).

Since the shift to the left is dramatic and unacceptable – can this really be an adequate study?
A first concern is that there is no way to estimate the effects of RP independent from the effects of the control cream which indeed is a major problem. What would be the effects, if any, of RP administered in a control cream that did not by itself act as a promoter? One can only speculate.

A second issue is the test system itself. TR568 says that 1% and 2% RP levels caused severe skin irritation requiring animal removal, even in the absence of exposure to UVR. However, those levels have reportedly been used without such irritation in other published peer-reviewed studies. So are the 1% and 2% RP levels toxic because too high of a dose was selected, because of a property of the SKH-1 mice used in this study, or because of an effect of the interaction of RP with a component of the control cream, such as diisopropyl adipate? Again, one can only speculate.

Third, we believe it is inappropriate to use time to tumor formation and/or tumor multiplicity data from animals that exhibit toxicity and 1) were removed from the experiment early, and 2) were excluded from pathology examination (1% and 2% RP animals).

Finally, no amount of statistical sophistication or manipulation can legitimately estimate main effects in the presence of large interactions. For example, the Cox Hazard Ratios between cream and various levels of RP do not represent independent RP effects. Rather they represent the effect of the cream, the effect of RP, and the effect of the unknown but also possibly they represent large interaction between the cream and RP. We believe it is simply irresponsible to attempt to present such analyses without caveats concerning the fact that the degree to which such differences exist is unknown and in fact cannot be estimated with the available data.
A control vehicle must be known not to enhance or prevent a particular biological event; it is only a carrier of the test agent or used to simulate a particular manipulation of the test animal. If it is noted that the control vehicle elicits the same biological response that is to be measured in a study, then reasonable scientists would consider the experiment flawed and the study would be repeated using a non-reactive control vehicle or abandoned.

For this particular study, it is difficult to imagine how, 1) once it was noted that control cream animals were developing comparable numbers of tumors to the test agent animals at the same UVR dosage and/or 2) noted that animals were experiencing severe toxicity reactions requiring removal as “Harvest” (preliminary NTP Pathology Tables), that this study was allowed to proceed for the entire one (1) year duration of the experiment. Indeed, the fact that the 1% and 2% RP dosed animals were in such poor condition as to preclude pathological examination is a strong statement that this experiment was flawed and should have been terminated.

Topically applied vehicle control formulations may include water, emollients, moisturizers, ointments, creams, salves and balms. It is known that, depending on the formulation mixture, all may increase or decrease test agent absorption, change the
optical properties such that UVR penetration is enhanced or reduced or support chemical reactions between the test agent and a control formulation component. This is why it is important to test the vehicle control formulation independently to assure it does not enhance the biological event that the test is measuring. This study suffers from that oversight.

NTP has conducted many properly designed, well managed and accurately reported hazard identification studies over the years that have contributed to public health and found utility by the regulatory community. Unfortunately, for reasons discussed above, the TR 568 study does not measure up to NTP standards. Therefore, we believe that the only reasonable call that NTP can support for TR 568 is: Inadequate Study of Carcinogenic Activity.

UVA / UVB Studies
In a separate experiment the NTP tested RP (1.0%) in female SKH-1 mice in the presence and absence of UVA or UVB irradiation. This study utilized the same control cream and thus suffers from the same experimental flaws noted with the UVR study. We believe the results from the UVA / UVB study can only be viewed as “observational” and certainly cannot be utilized in any capacity to support the NTP call for the one (1) year photo-cocarcinogenesis study.

Initiation/Promotion/Progression UVR/SKH-1 Animal Model
The SKH-1 / UVR protocol design is a (x) staged initiation-promotion- progression design model. A slope shift to the left could be due to (1) photo-activated production to a bioactive chemical, (2) modulation of UVR-induced genotoxicity, (3) simple enhanced promotion of UVR-initiated cells, (4) test agent acting additively/synergistically with UVR, (5) simple phototoxicity, (6) immune suppression, (7) interaction between control cream and test agent,(8) altered apoptosis, (9) a combination of the above, or (10) other unknown mechanisms. The variability in outcome when testing various substances,
including the retinoids, using this animal model is quite large, as even noted in this TR. Study results are more often a consequence of protocol design, test agent purity, exposure times, test agent and UVR application sequence and the type of control vehicle utilized.

Moreover, UVR is, by itself, the initiator and the promoter (it is a “complete” carcinogen) as nicely demonstrated in the UVR dose curves published in this TR; this by itself is a confounder when attempting to interpret study outcomes. Clearly, extrapolation concerns must also exist when considering animal vs. human differences in test agent response, UVR response and/or response to the combination of test agent/UVR. So the question for the FDA becomes: how does one even begin to understand what a slope shift to the left means in the presence of a test agent and UVR, in a regulatory framework? That is, how does FDA measure the “risk to human health” from such animal studies? Furthermore, can the FDA really regulate an animal photo-cocarcinogen “promoter”?

Given those questions, we note with interest that the FDA Center for Drug Evaluation and Research (CDER) has recently published, “Guidance for Industry M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing
Authorization for Pharmaceuticals” which in Note 6, states: “Testing for photocarcinogenicity in rodents using currently available models (e.g., hairless rodent) is not considered useful in support of pharmaceutical development and generally is not recommended. We wonder if this same policy is also embraced by CFSAN and other FDA product centers. Summary

There was what we believe an unusual 11-year delay from FDA nomination to NTP reporting the results from this one (1) year photo-cocarcinogenesis study (2000 – 2011) and speculate that the delay may have been driven by NTP questioning the adequacy of the study and debating the merits of bringing this study forward for a public peer review.

The NTP used a protocol design different from the accepted Forbes design which was, we believe, not adequately justified in TR 568 and furthermore was an untested design at the beginning of the RP photo-cocarcinogenesis study. The impact on the outcome of the TR 568 is uncertain.

The UVA and UVB studies suffer from the same confounder’s that the UVR study does (active control cream) and can only be viewed as observational in nature and should not be used in any manner in supporting the call for TR 568.

No reasonable scientist would have continued a study so obviously flawed by the presence of a reactive control cream that alone dramatically changed the slope of the response. Moreover, the obvious toxic response to RP dosing in the presence and absence of UVR was another reason to terminate the study.

It is impossible to determine the independent action of RP on the development of skin tumors or tumor multiplicity. Additionally, it is difficult to imagine how any U.S or international regulatory body could use such data in a risk assessment or for formulating any reasonable risk management decision.

Finally, the only reasonable call that the NTP can support for this study is: Inadequate Study of Carcinogenic Activity. Sincerely,

John E. Bailey, Ph.D. Executive Vice President Science
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This next section is not an official Company document and will be featured in a book that Dr Ernst Eiselen and I will write about skin care.
Topical products formulated by Dr Des Fernandes have only been tested on humans right from the beginning when Dr Fernandes started his research with retinyl palmitate creams in 1987. We only know what it does to humans and cannot guess what it would do to animals. As a result Dr Fernandes has accumulated evidence over many hundreds or personal patients in South Africa with more than ten years of daily use of retinyl palmitate based creams (in all his formulated skin care products). If we
look at patients that have used retinyl palmitate daily for more than 20 years in South Africa, then Dr Fernandes has seen many hundreds of patients. If we look only at the patients who have been using retinyl palmitate based topical skin care creams then possibly the number of people whom Dr Fernandes personally looked after from 1987 onwards would be in the region of 300 people. Dr Fernandes noticed was that people with established skin cancer who regularly came in every few months or maybe every year for excision of basal cell carcinomas or squamous cell carcinoma, started to make appointments only for checking of their skin. I only realised this after treating the patients for about three years (by 1991) but have subsequently noticed that as people use higher and higher doses of retinyl palmitate creams, their keratoses became less evident. Many Doctors and Skin Care Therapists around the world have also experienced this result. If retinyl palmitate caused skin cancer then surely Dr Fernandes would have seen some cases. We have histology that shows skin changes after using retinyl palmitate based creams for at least six months. Some specimens clearly show signs typically seen in people who have or who will soon get a skin cancer and yet the same skin taken just 1 to 2 mm away after at least six months of retinyl palmitate creams, shows a total reversal that astounded the pathologists. Why would living human skin cells have such a different outcome from what the particular NIH studies suggest? From Dr Fernandes point of view, the reason is that retinyl palmitate in humans at the concentrations used, do not pose a risk for skin cancer but actually have the opposite effect despite being used with a sunscreen and being exposed to the South African sun.

A paper from 2008 basically repeats my experience” by Watson et al [19]

http://www3.interscience.wiley.com/journal/119388347/abstract

Repair of photoaged dermal matrix by topical application of a cosmetic ‘antiageing’ product

BACKGROUND: Photoaged skin is characterized by coarse and fine wrinkles. The mechanism of wrinkle formation appears to involve changes to components of the dermal extracellular matrix. Topical treatment with all-trans retinoic acid (RA) can repair photoaged dermal matrix; this is regarded as the ‘gold standard’ against which repair agents are judged. To date, little is known regarding the ability of over-the-counter ‘antiageing’ products to repair photoaged skin. OBJECTIVES: We used a modified occluded patch test to ascertain whether topical applications of cosmetic ‘antiageing’ products are able to repair photoaged human skin.

METHODS: Commercially available test products [basic moisturizer, ‘antiageing’ cream containing different active complex levels (6% active: lipopentapeptide, white lupin peptides, antioxidants, retinyl palmitate; 2% active: lipopentapeptide, white lupin peptides, antioxidants)] were applied under occlusion for 12 days prior to biopsy and histological assessment in photoaged volunteers (n=9). RA was used
as a positive control. RESULTS: In agreement with previous studies, the patch-test study revealed that RA produced significant fibrillin-1 deposition in the papillary dermis (P<0.01) but had little effect on procollagen I or matrix metalloproteinase-1 expression. The 6% total active complex formulation, however, increased the deposition of fibrillin-1 and procollagen I (P<0.01, P<0.05, respectively).

CONCLUSIONS: This study indicates that in an in vivo 12-day patch test an over-the-counter cosmetic product can induce changes in photoaged dermal extracellular matrix, which are indicative of repair.

The same research workers who have reported on the photo-degradation products have confirmed the physiological changes. [20]
Levels of retinyl palmitate and retinol in the stratum corneum, epidermis, and dermis of female SKH-1 mice topically treated with retinyl palmitate by Yan et al 2006 - just for interest from Toxicol Ind Health pages 181-91

Retinyl esters are the storage form of vitamin A in skin, and retinyl palmitate (RP) accounts for the majority of the retinyl esters endogenously formed in skin. RP is also obtained exogenously through the topical application of cosmetic and skin care products that contain RP. There is limited information on the penetration and distribution of RP and vitamin A within the stratified layers of the skin. The purpose of these studies was to determine the time course for accumulation and disappearance of RP and retinol in the stratified layers of skin from female SKH-1 mice that received single or repeated topical applications of creams containing 0.5 or 2% of RP. We developed an HPLC method with detection limits of 5.94 and 1.62 ng, to simultaneously quantify the amount of RP and retinol, respectively, in skin samples. Our results showed that RP rapidly diffuses into the stratum corneum and epidermal skin layers within 24 h following the application of RP-containing creams. Of the three skin layers, the highest level of RP and retinol per weight unit (ng/mg) at all time points was found in the epidermis. Levels of RP and retinol were lowest in the dermal layer and intermediate in the stratum corneum. The levels of RP and retinol in the separated skin layers and in the intact skin decreased with time, but levels of RP remained higher than control values for a period of up to 18 days. Our results indicate that the application of RP to mouse skin alters the normal physiological levels of RP and retinol in the skin.

Cancer Biology

I believe that we have to look at studies in humans and the study in Arizona to discover whether vitamin A can protect one from skin cancers first of all showed that supplementation of vitamin A by mouth raised the skin retinyl palmitate significantly.[21]

The retinoid skin cancer prevention (SKICAP) trials are a set of double-blind, randomized, placebo-controlled clinical trials. The SKICAP-actinic keratoses (AK) trial tests the hypothesis that daily supplementation of retinol (25,000 IU) for 5 years reduces the incidence of skin cancers in high-risk individuals, those with a history of greater than ten clinically or pathologically diagnosed AK and, at most, one prior pathologically confirmed cutaneous squamous cell carcinoma (SCC) or basal cell carcinoma (BCC). The SKICAP-SCC/BCC (S/B)
trial tests the hypothesis that daily supplementation of retinol (25,000 IU) or 13-cis-retinoic acid (5 or 10 mg) for 3 years reduces skin cancer incidence in very high-risk individuals, those with a history of at least four pathologically confirmed SCCs or BCCs. Between 1984 and 1988, 2800 participants were enrolled at two clinics on the SKICAP-AK trial; and between 1985 and 1990, a total of 719 participants were enrolled at four clinics on the SKICAP-S/B trial. The initial recruitment strategy was referral by dermatologists, but low accrual necessitated the use of other strategies to achieve enrollment goals, which included involving additional clinics and using paid trial-specific advertisements in print and electronic media. Thirteen % of the SKICAP-AK participants and 36% of the SKICAP-S/B participants were enrolled through dermatologist referral, whereas paid advertisements resulted in enrollment of 87% of SKICAP-AK and 43% of SKICAP-S/B participants. A population-based skin cancer registry was used to identify and enroll the remaining 21% of the SKICAP-S/B participants.

These people —trialists— have been walking around under Arizona’s sun for many years (the research started 1988) and their skin levels of vitamin A are much higher than people who would use a vitamin A based
sunscreen. I believe this is very clear evidence that retinyl palmitate does not cause acceleration of tumours.

In fact later publications have shown that by supplementing with 75,000 i.u. vitamin A every day they were able to reduce the incidence of squamous cell carcinoma. [22]

PURPOSE: Previously, we reported the results of a Phase III, placebo-controlled trial in 2297 randomized participants with moderately severe actinic keratoses wherein 25000 IU/day vitamin A caused a 32% risk reduction in squamous cell skin cancers. We hypothesized that dose escalation of vitamin A to 50000 or 75000 IU/day would be both safe and more efficacious in skin cancer chemoprevention. EXPERIMENTAL DESIGN: One hundred and twenty-nine participants with severely sun-damaged skin on their lateral forearms were randomized to receive placebo or 25000, 50000, or 75000 IU/day vitamin A for 12 months. The primary study end points were the clinical and laboratory safety of vitamin A, and the secondary end points included quantitative, karyometric image analysis and assessment of retinoid and rexinoid receptors in sun-damaged skin. RESULTS: There were no significant differences in expected clinical and laboratory toxicities between the groups of participants randomized to placebo, 25000 IU/day, 50000 IU/day, and 75000 IU/day. Karyometric features were computed from the basal cell layer of skin biopsies, and a total of 22600 nuclei from 113 participants were examined, showing statistically significant, dose-response effects for vitamin A at the 25000 and 50000 IU/day doses. These karyometric changes correlated with increases in retinoic acid receptor alpha, retinoic acid receptor beta, and retinoid X receptor alpha at the 50000 IU/day vitamin A dose. CONCLUSIONS: The vitamin A doses of 50000 and 75000 IU/day for 1 year proved safe and equally more efficacious than the 25000 IU/day dose and can be recommended for future skin cancer chemoprevention studies.

One has to bear in mind that whatever vitamin A we consume or apply to the surface of the skin becomes retinyl palmitate in the skin.

We know that carotenoids make the skin sun resistant. [23]

Excessive exposure to solar radiation, especially ultraviolet A (UVA: 320-400 nm) and ultraviolet B (UVB: 290-320 nm) radiation, may induce UV-carcinogenesis and erythema in the skin. Although the protective effects of carotenoids against skin lesions are still unclear, beta-carotene has been proposed as an oral sun protectant. The purpose of this study was to determine the magnitude of the protective effects of oral alpha- and beta-carotene supplementation for 24 weeks on UVA- and UVB-induced erythema in humans. While being exposed to UVA and UVB radiation, 22 subjects (11 men and 11 women) were supplemented with natural carotenoids for 24 weeks. Each day for the first 8 weeks, subjects were given 30 mg of natural carotenoids containing 29.4 mg of beta-carotene, 0.36 mg of alpha-carotene, and traces of other carotenoids in vegetable oil. The natural carotenoid dose was progressively raised by 30-mg increments, at every 8 weeks, from 30 mg
to 90 mg. Small areas (1 cm²) of the skin were exposed to increasing doses of UV light (16-42 mJ/cm²) to determine the minimal erythema dose (MED). MED was defined as a uniform pink color with well-defined borders. MED readings were obtained by visual inspection 24 hr postirradiation. Blood samples taken during supplementation were used to determine alpha- and beta-carotene serum levels and for a lipid peroxidation analysis. During natural carotenoid supplementation, the MED of solar simulator radiation increased significantly (P<0.05). After 24 weeks of supplementation, serum beta-carotene levels were increased from 0.22 μg/ml (95% CI; 0.16-0.27) to 1.72 μg/ml (95% CI; 1.61-1.83). Similarly, alpha-carotene serum levels increased from 0.07 μg/ml (95% CI; 0.048-0.092) to 0.36 μg/ml (95% CI; 0.32-0.40). Serum lipid peroxidation was significantly (P<0.05) inhibited in a dose-dependent manner during natural carotenoid supplementation. The present data suggest that supplementation with natural carotenoids may partially protect human skin from UVA- and UVB-induced erythema, although the magnitude of the protective effect is modest.
We use beta-carotene and lutein as well as other antioxidants in our creams and that definitely makes the skin more sun-resistant. Ultraviolet radiation (UVR) promotes skin cancer development by mutagenic, immunosuppressive, and oxidative-stress-inducing mechanisms; however, certain antioxidants may counteract and prevent UVR-induced photodamage. Lutein is a xanthophyll carotenoid with potent antioxidant activity. Because reactive oxygen species (ROS) are believed to have a role in UVR-induced skin damage, we investigated whether lutein can modify UVR effects including the tissue swelling response to midrange UVR (280-320 nm, ultraviolet B (UVB) radiation) and UVR suppression of contact hypersensitivity (CHS) in both the local and the systemic models of UV-induced immunosuppression. We found that compared to mice fed the standard laboratory diet, mice fed dietary lutein demonstrated significant inhibition of ear swelling owing to UVB radiation. Mice exposed to 1700 J per m² UVB radiation four times at daily intervals and then sensitized to dinitrofluorobenzene at the site of irradiation showed a decreased CHS response upon challenge. This suppression by UVB radiation was significantly inhibited by lutein feeding. When UVB radiation was given at a single dose of 10,000 J per m² to inhibit the induction of CHS at a distant, nonirradiated site, no effect of lutein was seen. Finally, lutein accumulated in the skin of mice following diet supplementation and was shown to decrease ROS generation following UVR exposure. Thus, lutein modulates the skin’s response to UVR and may contribute to the defense against some of the deleterious effects of solar radiation.

It's a complex story and that's why you should also understand conflicting evidence from Offord [25]. This is one of the reasons why I have always insisted that we have to have the widest antioxidant protection. One will particularly notice the carnosic acid (rosemary extract) that is in the some of the topical formulations by Dr Fernandes.

The photoprotective potential of the dietary antioxidants vitamin C, vitamin E, lycopene, beta-carotene, and the rosemary polyphenol, carnosic acid, was tested in human dermal fibroblasts exposed to ultraviolet-A (UVA) light. The carotenoids were prepared in special nanoparticle formulations together with vitamin C and/or vitamin E. Nanoparticle formulations, in contrast to dimethylsulphoxide, stabilized lycopene in the cell culture medium and allowed efficient cellular uptake. The presence of vitamin E in the formulation further increased the stability and cellular uptake of lycopene. UVA irradiation of the human skin fibroblasts led to a 10-15-fold rise in metalloproteinase 1 (MMP-1) mRNA. This rise was suppressed in the presence of low microM concentrations of vitamin E, vitamin C, or carnosic acid but not with beta-carotene or lycopene. Indeed, in the presence of 0.5-1.0 microM beta-carotene or lycopene, the UVA-induced MMP-1 mRNA was further increased by 1.5-2-fold. This increase was totally suppressed when vitamin E was included in the nanoparticle formulation. Heme-oxygenase 1 (HO-1) mRNA expression was strongly induced by UVA irradiation but none of the antioxidants inhibited this effect at the
concentrations used in this study. Indeed, beta-carotene or lycopene (0.5-1.0 microM) led to a further 1.5-fold rise in the UVA-induced HO-1 mRNA levels. In conclusion, vitamin C, vitamin E, and carnosic acid showed photoprotective potential. Lycopene and beta-carotene did not protect on their own but in the presence of vitamin E, their stability in culture was improved and the rise in MMP-1 mRNA expression was suppressed, suggesting a requirement for antioxidant protection of the carotenoids against formation of oxidative derivatives that can influence the cellular and molecular responses.

On the other hand, a study using standard low doses of vitamin A and general antioxidants showed no protective effect from oral supplementation. [26]

Our objective was to examine prospectively the intake of vitamins A (including retinol and total vitamin A), C and E; folate; total carotene; and several individual carotenoids (alpha-carotene, beta-carotene, beta-cryptoxanthin and lutein/zeaxanthin) in relation to incidence
of SCC of the skin in 2 large cohorts of men and women. We used a prospective cohort study design with up to 14 years of follow-up in women and 10 years in men. Diet was measured with FFQs every 2-4 years; cases of SCC of the skin were ascertained on biennial questionnaires and confirmed by medical records. Participants were female nurses and male health professionals, from the Nurses’ Healthy Study and the Health Professionals Follow-up Study in the United States, without a history of any cancer in 1982 (n = 85,944 women) and 1986 (n = 43,867 men). Follow-up response was achieved for over 90% of potential person-years. Relative risks and 95% confidence intervals for development of SCC of the skin are reported. We recorded 369 cases of SCC in women and 305 cases in men. After multivariate adjustment for various known behavioral, sun-exposure and sun-sensitivity risk factors for SCC, there were no significant inverse associations between these dietary factors and SCC incidence. No evidence was found that vitamins A, C and E; folate; or carotenoids play an important protective role against incident SCC.

I believe that if retinyl palmitate creates any free radicals in cells they will be minimised by the antioxidant cohort in the topical formulations and will not become genotoxic. We also have evidence that vitamin A may suppress malignant cells. [27]

[Modulation of Involucrin and Envelope Competence in Human Keratinocytes by Hydrocortisone, Retinyl Acetate, and Growth Arrest1] Polly R. Cline and Robert H. Rice2 Charles A. Dana Laboratory of Toxicology, Harvard School of Public Health, Boston, Massachusetts 02115

Involucrin accumulation and ionophore-assisted envelope formation, markers of keratinocyte differentiation, were found to be highly dependent on culture conditions in the malignant epidermal keratinocyte line, SCC-13, derived from a human squamous cell carcinoma. In confluent cultures, approximately one-half of the cells were competent to form envelopes when grown in medium without hydrocortisone or retinyl acetate supplementation. Addition of hydrocortisone to the medium during growth resulted in up to 90% competence, while addition of retinyl acetate instead resulted in as low as 10% competence. Hydrocortisone partially antagonized the effect of retinyl acetate when both agents were added together. Involucrin levels, measured by radioimmunoassay, were modulated essentially in parallel with envelope competence under the various conditions tested. When the cells were grown in medium supplemented with hydrocortisone, the levels shortly after confluence were over 50-fold higher than in sparse cultures. Regardless of hydrocortisone or retinyl acetate addition, less than 1% of the cells

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were competent in sparse cultures of growing cells, but up to 90% exhibited this property after growth arrest in serum-free medium containing hydrocortisone. High levels of competence were correlated with cessation of cell division but not with loss of colony-forming efficiency; under optimal conditions, two-thirds of the cells were capable of both envelope formation and colony initiation. Normal human epidermal cells showed a 4- to 5-fold increase in envelope competence from sparse to confluent culture but were insensitive to the suppressive effect of retinyl acetate. The results suggest that some potential differentiated character of malignant keratinocytes may be suppressed in vivo by physiological agents such as vitamin A.

I have long pointed out that retinyl palmitate has the unique advantage of being a sunscreen at the same time as being essential nutrition for cells. [28] Retinol and Retinyl Ester Epidermal Pools Are Not Identically Sensitive to UVB Irradiation and Anti-Oxidant Protective Effect.
Background: UV irradiation can deplete epidermal vitamin A, thus the hypothesis that UV-induced depletion of vitamin A in sun-exposed skin is involved in the pathogenesis of skin cancers and skin ageing. Objectives: In this study we addressed two questions: (1) Are retinol (ROL) and retinyl esters (RE) - the two predominant forms of vitamin A - equally sensitive to the action of UVB, and (2) could the depletion be prevented by anti-oxidants? Methods: Hairless mice were irradiated with a single UVB dose, corresponding to the maximum of ROL and RE absorption. Retinoid content, enzyme activities catalysing the esterification of ROL (ARAT and LRAT) and the hydrolysis of RE (REH), as well as retinol-binding protein (CRBP-1) expression were determined in the epidermis.

Results: A single UVB dose induced a rapid, dose-dependent decrease in both ROL and RE in the epidermis of hairless mice, with partial replenishment after 24 h. The dose-response curve for ROL showed a high sensitivity to UV at doses not exceeding 200 mJ/cm², followed by a plateau, whereas RE underwent a continuous dose-dependent decrease at UVB doses up to 1 J/cm². A topical anti-oxidant mixture containing 0.5% ascorbate, 0.25% tocopherol and 0.25% melatonin failed to protect epidermal RE from UVB-induced depletion, whereas it did prevent ROL depletion. ARAT and REH, as well as CRBP-1, were not affected by UVB in these conditions. Conclusion: Vitamin A storage in the epidermis comprises two forms, ROL and RE, that do not show similar sensitivity to acute UVB exposure. ROL stores comprise a UVB-resistant (possibly by CRBP) portion and a UVB-sensitive portion that can be protected by anti-oxidants. RE stores do not show such a pattern.

The very fact that retinyl palmitate is a sunscreen is what has started this controversy of interpreting results from research. We know that sunscreens generate other radicals or photodecomposition products except for the sunscreens that are not yet permitted in USA. In Europe and other countries one can use sunscreens that are more effective and do not create free radicals. Incidentally while we are on this point, why has this self-righteous body never asked for the banning of Oxybenzone (benzophenone -3) that you will see on virtually all sunscreens in the USA. It is the one molecule amongst the sunscreens that definitely generates free radicals.
I found an older paper supporting this idea that cancers appear sooner. These are respected researchers but they used a special variety of mice which do not grow any hair and are not similar to human skin. The article is 12 years old.


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Vitamin A and its derivatives (retinoids) exert modulatory effects on epithelial differentiation and are used therapeutically against skin cancers, but the role of dietary vitamin A in ultraviolet (UV)-induced carcinogenesis is far from clear. To study this process, 220 hairless mice were given diets containing low (0.3-0.6 mg/kg; A-) or high (4-6 mg/kg; A+) amounts of retinol, which resulted after 2 months in an approximately 4-fold
difference in liver and skin vitamin A levels as determined by HPLC. Commencing after 1 month of diet, daily irradiations with UVB (280-320 nm) or UVAB (280-380 nm) were given to 176 of the animals for 18 weeks (cumulative doses of UVB and UVA: 26 J/cm2 and 168 J/cm2, respectively). The first skin tumours, known to be squamous cell carcinomas, appeared after 35 weeks in the UVAB-irradiated A+ animals and 5-6 weeks later in the other groups. After one year the frequency of tumour-bearing animals was 49-63% in the A+ groups and 28-39% in the A- groups (P = 0.003). Two months later the corresponding figures were 66-72% and 50-53%, respectively (P = 0.014). Disregarding the effect of dietary vitamin A, there was no difference in the final tumour incidence between UVB- and UVAB-irradiated animals. The epidermal vitamin A content at 72 h post-irradiation was approximately 60% lower in A+ animals and approximately 10% lower in A- animals compared with the non-irradiated controls. Rather than protecting against skin cancer, a diet rich in vitamin A seems to facilitate UV carcinogenesis in hairless mice. A possible explanation is that photodecomposition of excessive vitamin A generates short-lived intermediates that may act as photosensitizers during cutaneous carcinogenesis.

In summary, what I have tried to explain is the relevance of the information that is being bandied about. Cellular metabolism is a jigsaw puzzle and if we look at one piece of the puzzle in isolation we cannot get the full picture. When that piece is fitted into place with its proper neighbours we see that it contributes but does not dominate.

NIH studies are important studies but as has been pointed out, everything is biased. Even this document, which is biased towards pointing out the protective effects of retinyl palmitate! They are searching for the effects of a single molecule and not what happens in a normal well-nourished cell.

The restriction of scientific method, as well as the practical impossibility, prevents them from looking at whole, living human cells responding to natural light.

I believe that this long statement of explanation gives you clear evidence that retinyl palmitate is a safe ingredient. It is naturally in the skin at slightly lower levels than they tested, and in someone like me who takes high retinyl acetate supplements, the levels are certainly higher than they tested. No one would suggest that I am going to develop a skin cancer and I have been applying retinyl palmitate to my skin for 24 years and supplementing orally for 16 years at high dose. My skin is also rich in many antioxidants that I believe help the vitamin A to be safe and achieve healthy skin. The extensive research, the clinical results and the histology point to retinyl palmitate being an effective and safe normaliser of skin.

Des Fernandes
References


