

May 28, 2010

The Honorable Margaret Hamburg, M.D.  
Commissioner  
Food and Drug Administration  
10903 New Hampshire Ave.  
Building 1 Room 2217  
Silver Spring, MD 20993-0002

Dr. Linda S. Birnbaum  
Director  
National Institute of Environmental Health Sciences /  
National Institutes of Health, and National Toxicology Program  
P.O. Box 12233  
Research Triangle Park, NC 27709

**Re: Pressing Need to Expedite Photocarcinogenicity Assessment for Sunscreen Ingredient Retinyl Palmitate**

Dear Commissioner Hamburg and Dr. Birnbaum:

We are writing to commend the scientists at the Center for Phototoxicology of the National Toxicology Panel (NTP) and Food and Drug Administration (FDA) National Center for Toxicological Research (NCTR) for outstanding research to help illuminate factors driving the rising skin cancer rates in the United States. We urge you to assess rapidly the data generated by the center's investigation into whether retinyl palmitate, a vitamin A derivative and common ingredient in sunscreen products, is toxic and carcinogenic in the presence of sunlight.

Ten years ago FDA nominated retinyl palmitate for testing to determine whether the compound has photocarcinogenic effects. That possibility was suggested by a series of studies conducted by FDA and academic scientists since 1985. In a document supporting the nomination, the National Toxicology Panel cited FDA's concerns about the use of RP in skin care products (NTP 2000):

**“Retinyl palmitate was selected by the [FDA's] 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.”**

Since that nomination, FDA researchers have published 17 studies and science reviews on the toxicity and chemistry of retinyl palmitate on the skin. According to FDA scientists, the study

findings suggest that retinyl palmitate breaks down in sunlight to photomutagenic compounds, forms free radicals in the presence of UVA and UVB radiation and “[causes] events that affect a large segment of the chromosome” (e.g., Mei et al. 2005, 2006; Xei et al. 2006, see Addendum).

This research has culminated in the center’s completion of a one-year photocarcinogenicity study of retinyl palmitate. The study method, which is based on rodent testing, is currently the state-of-the-art technique for establishing whether a compound is carcinogenic in the presence of sunlight.

Key study data have been published on NTP’s website (NTP 2009), but your agencies’ final assessment of the work has not yet been made public.

Our review of the publicly available data suggests that your completion of this assessment could not be more urgent. The data show that tumors and lesions developed as much as 21 percent more rapidly in lab animals coated in a retinyl palmitate (RP)-laced cream (at concentrations of 0.1 percent to 0.5 percent), compared to control animals treated with an RP-free cream. Both groups were exposed to the equivalent of nine minutes of bright sunlight each day for up to a year. The differences are statistically significant and dose-dependent (EWG 2010).

The dramatically accelerated development of tumors and lesions in retinyl palmitate-treated animals, compared to untreated animals, has potentially significant implications for public health, which is why EWG raised concerns about the chemical in our 2010 review of sunscreen products (EWG 2010). Sunscreen makers have added retinyl palmitate and related forms of vitamin A to 41 percent of sunscreens on the market this year, according to EWG analysis of ingredient labels for nearly 500 products.

We are concerned that sunscreen industry consultants are attempting to downplay the relevance of the federal study. First, according to recent media reports, they disregard FDA’s body of research on retinyl palmitate. As well, they misstate the basic purpose of laboratory toxicity studies that rely on non-human animals. For instance, a dermatologist who consults for a wide range of prominent sunscreen companies was quoted as saying that it was “very premature to even cast doubt about the safety of this chemical,” on grounds that rodent studies are not applicable to humans.

As the FDA points out, “testing for photocarcinogenicity in humans is unethical; animal testing has been used as a surrogate.” As you well know, FDA, NTP and other scientific institutions are working to develop sorely needed non-animal methods for toxicity testing. Until reliable non-animal models are available, animal tests are established, state-of-the-art methods for evaluating toxicity. FDA acknowledges uncertainties in applying the test results to humans (FDA 2003). But given currently available methods, NTP cancer studies like the RP study conducted by the center are considered the “gold standard” for assessing human carcinogenicity risks (Ball 2009; Bucher 2002). FDA’s Guidance for Photosafety recommends the methods and species (hairless mouse) used by the center (FDA 2003). Scientists from the renowned MD Anderson Cancer Center have noted that “SKH1 [hairless] mice are the most widely used in dermatologic

research... tumors induced in these mice resemble, both at the morphologic and molecular levels, UVR-induced skin malignancies in man” (Benavides 2009).

The literature shows that since 2002, FDA scientists have also studied retinyl palmitate with non-animal laboratory assays, including cellular and mechanistic studies, and with short-term animal studies. Your current study of the possible photocarcinogenicity of retinyl palmitate, using rodents, is based on a significant body of science that has deployed a variety of testing methods.

Some industry consultants may not be aware that the center’s testing is done in lieu of unethical human testing or that animals susceptible to cancer are selected to reduce the number of animals needed for testing. We are concerned that the broader dermatology community may not be fully aware of the relevance of the center’s important work.

As demonstrated by the media response to our report, the public and medical community are expressing immense interest in the safety of retinyl palmitate, especially in suncare products. With this letter we urge you to expedite the final review of the retinyl palmitate study data and provide guidance to consumers, physicians, and the industry about Vitamin A-based products.

Fully 10 years have passed since FDA scientists determined they had sufficient data to initiate research on the possible health hazards of retinyl palmitate, an effort that has culminated in the key photocarcinogenicity study now before us. EWG, like many scientists and health professionals around the country, is eagerly awaiting the final publication of your conclusions. We urge you to place high priority on its timely release. In the meantime, given the public health implications of the data you have published, and the industry’s use of RP in hundreds of suncare products *before* the government has completed its safety review, EWG is recommending that consumers avoid sunscreen containing retinyl palmitate.

Sincerely yours,

Kenneth A. Cook  
President

Copy: Dr. Paul Howard, Director, NTP/NCTR Center for Phototoxicology

## References

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NTP (National Toxicology Program). 2009. Pathology Tables, Survival and Growth Curves from NTP Long-Term Studies. TR-568 All-trans-retinyl palmitate. National Toxicology Program. Available: <http://ntp.niehs.nih.gov/index.cfm?objectid=555571BB-F1F6-975E-76F2BC5E369EB6F7> webpage last updated on July 15, 2009

## ADDENDUM

### 17 FDA/NTP STUDIES AND SCIENCE REVIEWS OF VITAMIN A PHOTOTOXICITY, PHOTOMUTAGENICITY, AND RELATED ISSUES OF ITS CHEMISTRY ON THE SKIN, PUBLISHED SINCE 2002

**2009** – Mei N, Chen T, Godar DE, Moore MM. **UVA-induced photomutagenicity of retinyl palmitate.** Comment on: *Mutat Res.* 2009 Jan 10;672(1):21-6. *Mutat Res.* 2009 Jun-Jul;677(1-2):105-6; author reply 107-8. Epub 2009 May 27.

Division of Genetic and Reproductive Toxicology, National Center for Toxicological Research, Jefferson, AR 72079, USA. [nan.mei@fda.hhs.gov](mailto:nan.mei@fda.hhs.gov)

**2009** – Mei N, Hu J, Xia Q, Fu PP, Moore MM, Chen T. Cytotoxicity and mutagenicity of retinol with ultraviolet A irradiation in mouse lymphoma cells. *Toxicol In Vitro*. 2010 Mar;24(2):439-44. Epub 2009 Oct 14.

Division of Genetic and Reproductive Toxicology, National Center for Toxicological Research, Jefferson, AR 72079, USA. nan.mei@fda.hhs.gov.

“Vitamin A (all-trans-retinol; retinol) is an essential human nutrient and plays an important role in several biological functions. However, under certain circumstances, retinol treatment can cause free radical generation and induce oxidative stress. In this study, we investigated photocytotoxicity and photomutagenicity of retinol using L5178Y/Tk(+/-) mouse lymphoma cells concomitantly exposed to retinol and ultraviolet A (UVA) light... **[The] results suggest that retinol is mutagenic when exposed to UVA in mouse lymphoma cells through a clastogenic mode-of-action.**”

**2007** – Yin JJ, Xia Q, Fu PP. UVA photoirradiation of anhydroretinol--formation of singlet oxygen and superoxide. *Toxicol Ind Health*. 2007 Nov;23(10):625-31.

Center for Food Safety and Applied Nutrition, US Food and Drug Administration, College Park, Maryland 20740, USA. junjie.yin@fda.hhs.gov

“Anhydroretinol is a metabolite of vitamin A (retinol) and a major photodecomposition product of retinyl palmitate and retinyl acetate. Anhydroretinol is biologically active, inducing cell death in lymphoblastoid cells, prevention of N-methyl-N-nitrosourea-induced mammary cancer, and inhibition of cell growth in lymphocytes. In the present study, electron spin resonance (ESR) spin-trap techniques were employed to explore the mechanism of lipid peroxidation initiation... **Our overall results provide evidence that photoirradiation of anhydroretinol with UVA light generates reactive oxygen species**, e.g. singlet oxygen and superoxide, which mediate the induction of lipid peroxidation.”

**2007** – Yan J, Xia Q, Wamer WG, Boudreau MD, Warbritton A, Howard PC, Fu PP. Levels of retinyl palmitate and retinol in the skin of SKH-1 mice topically treated with retinyl palmitate and concomitant exposure to simulated solar light for thirteen weeks. *Toxicol Ind Health*. 2007 Nov;23(10):581-9.

National Center for Toxicological Research, US Food and Drug Administration, Jefferson, Arkansas, USA.

“Retinyl esters account for more than 70% of the endogenous vitamin A found in human skin, and retinyl palmitate is one of the retinyl esters in this pool. Human skin is also exposed to retinyl palmitate exogenously through the topical application of cosmetic and skin care products that contain retinyl palmitate. In this study, the accumulation of retinyl palmitate and generation of retinol in the skin of male and female SKH-1 mice that received repeated topical applications of creams containing 0.0%, 0.1%, 0.5%, 1.0%, 5.0%, 10%, or 13% of retinyl palmitate 5 days a week for a period of 13 weeks were

studied. Because products containing retinyl palmitate are frequently applied to sun-exposed skin, and because it is well established that exposure to sunlight and UV light can alter cutaneous levels of retinoids, mice in this study were additionally exposed 5 days a week to simulated solar light... **Our results indicate that topically applied retinyl palmitate may alter the normal physiological levels of retinyl palmitate and retinol in the skin of SKH-1 mice and may have a significant impact on vitamin A homeostasis in the skin.**”

2007 – Fu PP, Xia Q, Yin JJ, Cherng SH, Yan J, Mei N, Chen T, Boudreau MD, Howard PC, Wamer WG. Photodecomposition of vitamin A and photobiological implications for the skin. *Photochem Photobiol.* 2007 Mar-Apr;83(2):409-24.

National Center for Toxicological Research, US Food and Drug Administration, Jefferson, AR, USA. peter.fu@fda.hhs.gov  
“Vitamin A (retinol), an essential human nutrient, plays an important role in cellular differentiation, regulation of epidermal cell growth and normal cell maintenance. In addition to these physiological roles, vitamin A has a rich photochemistry. Photoisomerization of vitamin A, involved in signal transduction for vision, has been extensively investigated. The biological effects of light-induced degradation of vitamin A and formation of reactive species are less understood and may be important for light-exposed tissues, such as the skin. Photochemical studies have demonstrated that excitation of retinol or its esters with UV light generates a number of reactive species including singlet oxygen and superoxide radical anion. **These reactive oxygen species have been shown to damage a number of cellular targets, including lipids and DNA. Consistent with the potential for damaging DNA, retinyl palmitate has been shown to be photomutagenic in an in vitro test system. The results of mechanistic studies were consistent with mutagenesis through oxidative damage.** Vitamin A in the skin resides in a complex environment that in many ways is very different from the chemical environment in solution and in in vitro test systems. **Relevant clinical studies or studies in animal models are therefore needed to establish whether the pro-oxidant activity of photoexcited vitamin A is observed in vivo, and to assess the related risks.**”

2007 – Fu PP, Xia Q, Boudreau MD, Howard PC, Tolleson WH, Wamer WG. Physiological role of retinyl palmitate in the skin. *Vitam Horm.* 2007;75:223-56.

National Center for Toxicological Research, Food and Drug Administration, Jefferson, Arkansas 72079, USA.  
“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.”

**2006** – Mei N, Xia Q, Chen L, Moore MM, Chen T, Fu PP. Photomutagenicity of anhydroretinol and 5,6-epoxyretinyl palmitate in mouse lymphoma cells. *Chem Res Toxicol.* 2006 Nov;19(11):1435-40.

Division of Genetic, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, Arkansas 72079, USA. nan.mei@fda.hhs.gov. Retinyl palmitate (RP) is frequently used as an ingredient in cosmetics and other retail products. “We previously reported that, under UVA light irradiation, RP is readily decomposed into multiple products, including anhydroretinol (AR) and 5,6-epoxyretinyl palmitate (5,6-epoxy-RP). We also determined that combined treatment of mouse lymphoma cells with RP and UVA irradiation produced a photomutagenic effect. In this study, we evaluated the photomutagenicity of AR and 5,6-epoxy-RP, in L5178Y/Tk+/- mouse lymphoma cells. Treatment of cells with AR or 5,6-epoxy-RP alone at 10 and 25 microg/mL for 4 h did not show a positive mutagenic response. However, because these doses did not induce the required amount of cytotoxicity for mouse lymphoma assay, we are unable to determine whether or not these two compounds are mutagenic. Treatment of cells with 1-25 microg/mL AR or 5,6-epoxy-RP under UVA light (315-400 nm) for 30 min (1.38 mW/cm<sup>2</sup>) produced a synergistic photomutagenic effect. At 10 microg/mL (37.3 microM) AR with UVA exposure, the mutant frequency (MF) was about 3-fold higher than that for UVA exposure alone, whereas the MF for 25microg/mL (46.3microM) of 5,6-epoxy-RP + UVA was approximately 2-fold higher than that for UVA exposure alone. Compared with previous results for RP + UVA treatment, the potency of the induced phototoxicity and photomutagenicity was AR > RP > 5,6-epoxy-RP. To elucidate the underlying photomutagenic mechanism, we examined the loss of heterozygosity (LOH) at four microsatellite loci spanning the entire chromosome 11 for mutants induced by AR or 5,6-epoxy-RP. Most mutants lost the Tk+ allele, and more than 70% of the chromosome damage extended to 38 cM in chromosome length. AR + UVA induced about twice as many mutants that lost all four microsatellite markers from the chromosome 11 carrying the Tk+ allele as RP + UVA or 5,6-epoxy-RP + UVA. **These results suggest that two of RP's photodecomposition products are photomutagenic in mouse lymphoma cells, causing events that affect a large segment of the chromosome.”**

**2006** – Yan J, Xia Q, Webb P, Warbritton AR, Wamer WG, Howard PC, Boudreau M, Fu PP. Levels of retinyl palmitate and retinol in stratum corneum, epidermis and dermis of SKH-1 mice. *Toxicol Ind Health.* 2006 Apr;22(3):103-12.

National Center for Toxicological Research, U.S. Food and Drug Administration,  
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“Vitamin A (retinol) regulates many biological functions, including epidermal cell growth. Retinyl palmitate (RP) is the major esterified form of retinol and the predominant component of retinoids in the skin; however, how endogenous levels of RP and retinol in the skin are affected by the age of the animal remains unknown. Furthermore, the levels of retinol and RP in the various skin layers – the stratum corneum, epidermis and dermis of skin - have not been reported. In this paper, we report the development of a convenient method for separation of the skin from SKH-1 female mice into the stratum corneum, epidermis, and dermis and the determination of the levels of RP and retinol in the three fractions by HPLC analysis. The total quantities of RP and retinol from the stratum corneum, epidermis, and dermis are comparable to those extracted from the same amount of intact skin from the same mouse. There was an age-related effect on the levels of RP and retinol in the skin and liver of female mice. An age-related effect was also observed in the stratum corneum, epidermis, and dermis. The levels of RP and retinol were highest in the epidermis of 20-week-old mice, and decreased when the age increased to 60- and 68-weeks. The total amount of RP at 20 weeks of age was found to be 1.52 ng/mg skin, and decreased about 4-fold at 60- and 68-weeks of age. A similar trend was found for the effects of age on the levels of retinol.”

**2006** – Yan J, Wamer WG, Howard PC, Boudreau MD, Fu PP. Levels of retinyl palmitate and retinol in the stratum corneum, epidermis, and dermis of female SKH-1 mice topically treated with retinyl palmitate. *Toxicol Ind Health*. 2006 May;22(4):181-91.

National Center for Toxicological Research, US Food and Drug Administration, Jefferson, AR 72079, USA.

“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.**”



**2006** – Xia Q, Yin JJ, Wamer WG, Cherng SH, Boudreau MD, Howard PC, Yu H, Fu PP. Photoirradiation of retinyl palmitate in ethanol with ultraviolet light--formation of photodecomposition products, reactive oxygen species, and lipid peroxides. *Int J Environ Res Public Health*. 2006 Jun;3(2):185-90.

National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, AR 72079, USA.

We have previously reported that photoirradiation of retinyl palmitate (RP), a storage and ester form of vitamin A (retinol), with UVA light resulted in the formation of photodecomposition products, generation of reactive oxygen species, and induction of lipid peroxidation. In this paper, we report our results following the photoirradiation of RP in ethanol by an UV lamp with approximately equal UVA and UVB light. The photodecomposition products were separated by reversed-phase HPLC and characterized spectroscopically by comparison with authentic standards. The identified products include: 4-keto-RP, 11-ethoxy-12-hydroxy-RP, 13-ethoxy-14-hydroxy-RP, anhydroretinol (AR), and trans- and cis-15-ethoxy-AR. Photoirradiation of RP in the presence of a lipid, methyl linoleate, resulted in induction of lipid peroxidation. Lipid peroxidation was inhibited when sodium azide was present during photoirradiation which suggests free radicals were formed. **Our results demonstrate that, similar to irradiation with UVA light, RP can act as a photosensitizer leading to free radical formation and induction of lipid peroxidation following irradiation with UVB light.**

**2005** – Xia Q, Yin JJ, Cherng SH, Wamer WG, Boudreau M, Howard PC, Fu PP. UVA photoirradiation of retinyl palmitate--formation of singlet oxygen and superoxide, and their role in induction of lipid peroxidation. *Toxicol Lett*. 2006 May 5;163(1):30-43. Epub 2005 Dec 27.

National Center for Toxicological Research, U.S. Food and Drug Administration, Department of Biochemical Toxicology, HFT-110, 3900 NCTR Road, Jefferson, AR 72079, USA.

“We have previously reported that photoirradiation of retinyl palmitate (RP) in ethanol with UVA light results in the formation of photodecomposition products, including 5,6-epoxy-RP 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 photoirradiation of RP by UVA light produces ROS. Photoirradiation of RP 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 RP was conducted in the presence of the DMPO or BMPO, ESR signals for DMPO- $\cdot$ OOH or BMPO- $\cdot$ OOH 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 RP and its photodecomposition products. ESR

studies on the photoirradiation of 5,6-epoxy-RP and AR indicate that these compounds exhibit similar photosensitizing activities as RP under UVA light.”

**2005** – Mei N, Xia Q, Chen L, Moore MM, Fu PP, Chen T. Photomutagenicity of retinyl palmitate by ultraviolet a irradiation in mouse lymphoma cells. *Toxicol Sci.* 2005 Nov;88(1):142-9. Epub 2005 Aug 17.

Division of Genetic and Reproductive Toxicology, National Center for Toxicological Research, FDA, Jefferson, Arkansas 72079, USA.

Retinyl palmitate (RP), a storage form of vitamin A, is frequently used as a cosmetic ingredient, with more than 700 RP-containing cosmetic products on the U.S. market in 2004. There are concerns for the possible genotoxicity and carcinogenicity of RP when it is exposed to sunlight. To evaluate the photomutagenicity of RP in cells when exposed to ultraviolet A (UVA) light, L5178Y/Tk+/- mouse lymphoma cells were treated with different doses of RP alone/or in the presence of UVA light. Treatment of the cells with RP alone at the dose range of 25-100 microg/ml did not increase mutant frequencies (MFs) over the negative control, whereas treatment of cells with 1-25 microg/ml RP under UVA light (82.8 mJ/cm<sup>2</sup>/min for 30 min) produced a dose-dependent mutation induction. The mean induced MF (392 x 10<sup>(-6)</sup>) for treatment with 25 microg/ml RP under UVA exposure was about threefold higher than that for UVA alone (122 x 10<sup>(-6)</sup>), a synergistic effect. To elucidate the underlying mechanism of action, we examined the mutants for loss of heterozygosity (LOH) at four microsatellite loci spanning the entire chromosome 11, on which the Tk gene is located. The mutational spectrum for the RP + UVA treatment was significantly different from the negative control, but not significantly different from UVA exposure alone. Ninety four percent of the mutants from RP + UVA treatment lost the Tk+ allele, and 91% of the deleted sequences extended more than 6 cM in chromosome length, indicating clastogenic events affecting a large segment of the chromosome. **These results suggest that RP is photomutagenic in combination with UVA exposure in mouse lymphoma cells, with a clastogenic mode-of-action.**

**2005** – Yan J, Xia Q, Cherng SH, Wamer WG, Howard PC, Yu H, Fu PP. Photo-induced DNA damage and photocytotoxicity of retinyl palmitate and its photodecomposition products. *Toxicol Ind Health.* 2005 Sep;21(7-8):167-75.

National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, AR, USA. [yu@ccaix.jsums.edu](mailto:yu@ccaix.jsums.edu)

“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 microM), DNA fragmentation was observed.** Cell death was observed for retinoid concentrations of 100 microM or higher. When treated with 150 microM 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/cm<sup>2</sup>), 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 NaN<sub>3</sub>. **These results suggest that photoirradiation of RP, [and compounds RP breaks down into, in the presence of UV radiation] 5,6-epoxy-RP and AR with UVA light generates free radicals that initiate DNA strand cleavage.”**

**2005** – Tolleson WH, Cherng SH, Xia Q, Boudreau M, Yin JJ, Wamer WG, Howard PC, Yu H, Fu PP. Photodecomposition and phototoxicity of natural retinoids. *Int J Environ Res Public Health*. 2005 Apr;2(1):147-55.

National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, AR 72079, USA.

“Sunlight is a known human carcinogen. Many cosmetics contain retinoid-based compounds, such as retinyl palmitate (RP), either to protect the skin or to stimulate skin responses that will correct skin damaged by sunlight. However, **little is known about the photodecomposition of some retinoids and the toxicity of these retinoids and their sunlight-induced photodecomposition products on skin. Thus, studies are required to test whether topical application of retinoids enhances the phototoxicity and photocarcinogenicity of sunlight and UV light.** Mechanistic studies are needed to provide insight into the disposition of retinoids in vitro and on the skin, and to test thoroughly whether genotoxic damage by UV-induced radicals may participate in any toxicity of topically applied retinoids in the presence of UV light. This paper reports the update information and our experimental results on photostability, photoreactions, and phototoxicity of the natural retinoids including retinol (ROH), retinal, retinoid acid (RA), retinyl acetate, and RP (Figure 1).”

**2005** – Cherng SH, Xia Q, Blankenship LR, Freeman JP, Wamer WG, Howard PC, Fu PP. Photodecomposition of retinyl palmitate in ethanol by UVA light-formation of photodecomposition products, reactive oxygen species, and lipid peroxides. *Chem Res Toxicol*. 2005 Feb;18(2):129-38.

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“Photodecomposition of retinyl palmitate (RP), an ester and the storage form of vitamin A (retinol), in ethanol under UVA light irradiation was studied. The resulting photodecomposition products were separated by reversed-phase HPLC and identified by spectral analysis and comparison with the chromatographic and spectral properties of synthetically prepared standards. The identified products include 5,6-epoxy-RP, 4-keto-RP, 11-ethoxy-12-hydroxy-RP, 13-ethoxy-14-hydroxy-RP, anhydroretinol (AR), palmitic acid, ethyl palmitate, and four tentatively assigned cis and trans isomeric 15-ethoxy-ARs. AR was formed as a mixture of all-trans-AR, 6Z-cis-AR, 8Z-cis-AR, and 12Z-cis-AR with all-trans-AR predominating. 5,6-Epoxy-RP, 4-keto-RP, 11-ethoxy-12-hydroxy-RP, and 13-ethoxy-14-hydroxy-RP were also formed from reaction of RP with alkylperoxy radicals generated by thermal decomposition of 2,2'-azobis(2,4-dimethylvaleronitrile). Formation of these photodecomposition products was inhibited in the presence of sodium azide (NaN<sub>3</sub>), a free radical inhibitor. These results suggest that formation of 5,6-epoxy-RP, 4-keto-RP, 11-ethoxy-12-hydroxy-RP, and 13-ethoxy-14-hydroxy-RP from photoirradiation of RP is mediated by a light-initiated free radical chain reaction. AR and the isomeric 11-ethoxy-ARs were not formed from reaction of RP with alkylperoxy radicals generated from 2,2'-azobis(2,4-dimethylvaleronitrile), and their formation was not inhibited when NaN<sub>3</sub> was present during the photoirradiation of RP. We propose that these products were formed through an ionic photodissociation mechanism, which is similar to the reported formation of AR through ionic photodissociation of retinyl acetate. RP and all its identified photodecomposition products described above (i) were not mutagenic in *Salmonella typhimurium* tester strains TA98, TA100, TA102, and TA104 in the presence and absence of S9 activation enzymes, (ii) were not photomutagenic in *Salmonella typhimurium* TA102 upon UVA irradiation, and (iii) did not bind with calf thymus DNA in the presence of microsomal metabolizing enzymes. These results suggest that RP and its decomposition products are not genotoxic; however, photoirradiation of RP, 5,6-epoxy-RP, and AR with UVA light in the presence of methyl linoleate resulted in lipid peroxide (methyl linoleate hydroperoxides) formation. The lipid peroxide formation was inhibited by dithiothreitol (DTT) (free radical scavenger), NaN<sub>3</sub> (singlet oxygen and free radical scavenger), and superoxide dismutase (SOD) (superoxide scavenger) but was enhanced by the presence of deuterium oxide (D<sub>2</sub>O) (enhancement of singlet oxygen lifetime). **These results suggest that photoirradiation of RP, 5,6-epoxy-RP, and AR by UVA light generated reactive oxygen species** resulting in lipid (methyl linoleate) peroxidation.

**2003** – Fu PP, Cheng SH, Coop L, Xia Q, Culp SJ, Tolleson WH, Wamer WG, Howard PC. Photoreaction, phototoxicity, and photocarcinogenicity of retinoids. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev.* 2003 Nov;21(2):165-97.

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“Sunlight is a human carcinogen. Many retinoid-containing cosmetics are used to protect damages caused by sunlight irradiation. Since retinol is thermally unstable and retinyl

palmitate (RP) is relatively more stable, RP is also widely used as an ingredient in cosmetic formulations. **In general, little is known about the photodecomposition of retinoids and the toxicity of retinoids and their photodecomposition products on the skin's responses to sunlight.** This review focuses on the update information on photoreactions, phototoxicity, and photocarcinogenicity of the natural retinoids including retinol, retinal, retinoid acid (RA), retinyl acetate, and RP.

**2002** – Fu PP, Howard PC, Culp SG, Xia Q, Webb PJ, Blankenship LR, et al. 2002. Do topically applied skin creams containing retinyl palmitate affect the photocarcinogenicity of simulated solar light? *J Food Drug Anal* 10: 262-68.

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**“Retinyl palmitate (all-trans-retinyl palmitate; RP) was nominated in 2001 by the U.S. Food and Drug Administration’s Center for Food Safety and Applied Nutrition (CFSAN) to the National Toxicology Program (NTP) as a high priority compound for phototoxicity and photocarcinogenicity studies at the National Center for Toxicological Research (NCTR). Studies with SKH-1 hairless mice are required to test whether topical application of RP enhances the phototoxicity and photocarcinogenicity of simulated solar light and UV light.** Mechanistic studies are needed to provide insight into the disposition of RP *in vitro* and on the skin of mice, and to test thoroughly whether genotoxic damage by UV-induced radicals may participate in any toxicity of topically applied RP in the presence of UV light.”