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National Toxicology Program (NTP)
Technical Reports Peer Review Panel

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Dear members of the Technical Reports Peer Review Panel:

These comments are provided on behalf of the Environmental Working Group (EWG), a non-profit public health research and advocacy organization based in Washington, DC. Our work focuses on the human and environmental health impact of potentially toxic chemicals in consumer products, so we reviewed with great interest the National Toxicology Program (NTP) Draft Technical Report on the Photococarcinogenesis Study of Retinoic Acid and Retinyl Palmitate in SKH-1 Mice (simulated solar light and topical application study), released for public comments in December 2010 (NTP 2010).

As you know, this compound is a common ingredient in skin creams and cosmetics, sunscreens and other personal care products. Your assessment of its potential to increase skin tumor risk in the presence of sunlight will be of great importance to public health.

This meticulous study is a culmination of a long-term research program on retinyl palmitate photogenotoxicity and photocarcinogenecity at the NTP/National Center for Toxicological Research (NCTR) Center for Phototoxicology. NTP initiated the program in 2000 in response to the decision of the Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition to nominate retinyl palmitate for phototoxicity and photocarcinogenicity testing. The FDA selected retinyl palmitate for further study "*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*" (NTP 2000).

EWG strongly agrees with the conclusion of the draft NTP report that retinyl palmitate "*enhanced the photocarcinogenicity activity of SSL [solar-simulating light] and UVB in SKH-1 mice based upon earlier onsets and increased multiplicities of in-life skin lesions and increased incidences and multiplicities of squamous cell neoplasms*" (NTP 2010). Our comments focus on three major points:

- The experimental protocol in the NTP/NCTR study was appropriately chosen to answer the question of topical retinyl palmitate toxicity posed by the FDA.
- The overall weight of evidence collected by the study supports the finding of retinyl palmitate photococarcinogenicity.

- The findings of the draft report are in agreement with evidence in the research database on the photogenotoxicity and photocarcinogenicity of retinoid compounds.

Details and rationale for our comments are provided below.

1. The experimental protocol in the NTP/NCTR study was appropriate.

The FDA's decision to nominate retinyl palmitate (RP) for NTP testing specifically sought to address, through the use of a widely accepted animal model, potential human health risks that might be associated with the growing use of this compound in cosmetic and personal care products. The one-year study protocol developed by NTP/NCTR scientists involved SKH-1 hairless mice, the laboratory animal model most commonly used in photocarcinogenesis research because "tumors induced in these mice resemble, both at the morphologic and molecular levels, UVR-induced skin malignancies in man" (Benavides 2009). The NTP approach was based on the study designs reported by P.D. Forbes and colleagues using simulated solar light (Forbes 2003). While a variety of experimental approaches has been developed for studying photocarcinogenicity in the SKH-1 model, the NTP protocol adequately addresses the question of retinyl palmitate photocarcinogenecity. This protocol was proven effective in a 1-year study on glycolic acid and salicylic acid described below (NTP 2007) as well as in a pilot 13-week retinyl palmitate study (Yan 2007).

In the NTP study, groups of 36 male and 36 female SKH-1 mice were irradiated five days per week (Monday through Friday, in the morning) for 40 weeks with solar-simulating light (SSL) with a UVA/UVB ratio of 20.5:1, similar to natural sunlight. Two levels of light intensity were tested, 6.75 and 13.7 mJ CIE/cm2, equivalent to 0.3 and 0.6 of a minimal erythemal (sunburn) dose (MED). According to an FDA publication, 0.6 MED is equivalent to nine minutes of unprotected skin UV exposure on a sunny day with a UV index of 10 on the World Health Organization UV scale (WHO 2002; Yan 2007). The mice received topical applications of control cream or creams containing 0.1%, 0.5%, 1.0%, or 2.0% (w/w) retinyl palmitate on the dorsal skin region in the afternoon on the days of irradiance exposures. These doses of retinyl palmitate correspond to real-life concentrations found in personal care products on the market (Cosmetics Ingredient Review 2009; NTP 2000).

The effectiveness of this study protocol and its ability to distinguish between positive and negative results has been clearly demonstrated in another study carried out by NTP/NCTR scientists, *Photocarcinogenesis Study of Glycolic Acid and Salicylic Acid* (NTP 2007). Taking into consideration the survival data, time-to-tumor data and the pathology results, the NTP concluded that glycolic acid did not alter the photocarcinogenesis of simulated solar light and salicylic acid was photoprotective (NTP 2007).

Furthermore, there is a recognized increase in tumor incidence in SSL-exposed animals treated with control cream compared to SSL-exposed animals that did not receive any cream treatment, a phenomenon observed in the retinyl palmitate study (NTP 2010); the glycolic and salicylic acid study (NTP 2007); as well as other studies in this animal model (Jacobs 2004; Lu 2009). Despite

the elevated background associated with the application of the control cream, NTP/NCTR researchers found enhanced photocarcinogenicity associated with retinyl palmitate or retinoic acid application, but no such effects associated with the application of glycolic acid or salicylic acid. This result clearly demonstrates that the SKH-1 study protocol used by the NTP can reveal compound-specific effects which makes it valid for assessing retinyl palmitate photococarcinogenicity.

2. The weight of evidence from the study supports the finding of retinyl palmitate photococarcinogenicity.

The NTP report analyzed the study data in multiple ways, examining mean survival time among exposed animals; in-life median skin lesion onset; in-life skin lesion incidence; multiplicity of in-life skin lesions and different tumor types; as well as incidence rates of skin lesions and tumors. It reached the following conclusions:

- In both sexes, all dose groups and at all levels of UV exposure, daily application of creams containing RP significantly 1) decreased survival time; 2) sped up the onset of skin lesion development; and 3) increased the number of squamous neoplasms per animal.

- Retinyl palmitate exposure in combination with simulated sunlight increased the number of in-life skin lesions and the number of focal atypical hyperplasias per animal in six of eight dose groups.

- In both male and female animals exposed to retinyl palmitate in combination with the lower dose of simulated sunlight, there were significant dose-related increases in the incidence of squamous cell carcinoma. At the higher dose of UV light exposure, however, NTP determined that the high rate of lesions and tumor incidence in the control cream group appeared to overwhelm any increase in RP-exposed animals and precluded detection of a statistically significant increase, an effect consistent with the high sensitivity of the SKH-1 mouse model to UV photocarcinogenicity (Benavides 2009).

EWG agrees with the NTP interpretation of the study data and finds that the overall weight of evidence strongly supports the NTP's conclusion of retinyl palmitate photococarcinogenicity.

3. The findings of the draft report are in agreement with the research database on the photogenotoxicity and photocarcinogenicity of retinoid compounds.

In earlier research, FDA scientists have shown that UV-exposed retinyl palmitate and other retinoids form free radicals and cause DNA mutations (Cherng 2005; Mei 2006; Mei 2010; Yan 2005). The photomutagenic effects of retinyl palmitate and other retinoids suggest a plausible mechanism by which a combination of RP and sunlight exposure could increase the onset, incidence and multiplicity of skin tumors. Other mechanisms could be also involved, such as increased cell division and hyperplasia which have been detected in the NTP research (NTP

2010) as well as other studies on retinoid compounds (Sorg 2006). Notably, in studies with human volunteers, retinol and retinyl palmitate induced skin hyperplasia (Duell 1997).

The NTP research also agrees with the preponderance of studies conducted by university and industry researchers pointing to the likelihood of photocarcinogenicity risks associated with topically applied retinoids. While there is diversity among the findings in the literature (So 2004), seven studies conducted between 1977 and 2008 found evidence of retinoic acid photocarcinogenicity (Epstein 1977; Forbes 1979; Forbes 1981; Davies 1988; Hartmann 1981; Halliday 2000; Lerche 2008). Overall, as noted by NTP (2000), in studies in which solar-simulating UV radiation was used at dose levels less than the human minimal erythema dose, topically applied retinoic acid generally enhanced photocarcinogenesis. In contrast, studies using UV radiation from unfiltered sources that emit UVC radiation not present in terrestrial sunlight (Brown 2000) at levels exceeding the human minimal erythema dose found that topically applied retinoic acid did not affect or inhibit photocarcinogenesis (Epstein and Grekin, 1981; Kligmann and Kligmann, 1981a; Kligmann and Kligmann, 1981b).

The NTP study employed moderate levels of simulated sunlight that correspond to a mild sunlight exposure scenario for both higher and lower SSL doses. NTP also used realistic retinyl palmitate concentrations similar to those found in personal care products. Finally, NTP utilized a research protocol and statistical methods whose effective performance has been demonstrated by earlier research (Howard 2002; NTP 2007; NTP Statistical Methods Working Group 2004). These three key aspects of the NTP protocol allowed the study to detect clear and unambiguous photococarcinogenic activity of retinyl palmitate in this mouse model.

In conclusion, EWG expresses strong support for the high-quality NTP/NCTR study, which provides the best, most detailed source of data currently available on the phototoxicity of retinyl palmitate. Together with other retinyl palmitate toxicity studies carried out by FDA scientists over the last decade, this research will help fill the long-standing data gap on the safety of retinyl palmitate in personal care products used on sun-exposed skin.

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References:

Benavides F, Oberyszyn TM, VanBuskirk AM, Reeve VE, Kusewitt DF. 2009. The hairless mouse in skin research. J Dermatol Sci 53(1): 10-8.

Brown, DB, Peritz AE, Mitchell DL, Chiarello S, Uitto J, Gasparro FP. 2000. Common Fluorescent Sunlamps Are An Inappropriate Substitute for Sunlight. Photochem. Photobiol. 72: 340-344.

Cherng SH, Xia Q, Blankenship LR, Freeman JP, Wamer WG, Howard PC, et al. 2005. Photodecomposition of retinyl palmitate in ethanol by UVA light-formation of

photodecomposition products, reactive oxygen species, and lipid peroxides. Chem Res Toxicol 18(2): 129-38.
Cosmetics Ingredient Review. 2009. CIR Compendium Containing Abstracts, Discussions, and Conclusions of CIR Cosmetic Ingredient Safety Assessments. Available: http://www.cir-safety.org/
Davies RE and Forbes PD. 1988. Retinoids and Photocarcinogenesis. J. Toxicol.-Cut.& Ocular Toxicol. 7(4): 241-253.
Duell EA, Kang S, Voorhees JJ. 1997. Unoccluded retinol penetrates human skin in vivo more effectively than unoccluded retinyl palmitate or retinoic acid. J Invest Dermatol 109(3): 301-5.
Epstein J H. 1977. Chemicals and Photocarcinogenesis. Aust. J. Dermatol. 18: 57-61.
Epstein JH, Grekin DA. 1981. Inhibition of Ultraviolet-Induced Carcinogenesis by All-Trans-Retinoic Acid. J. Invest. Dermatol. 76: 178-180.
Forbes PD, Urbach F, Davies RE. 1979. Enhancement of Experimental Photocarcinogenesis by Topical Retinoic Acid. Cancer Lett. 7: 85-90.
Forbes PD. 1981. Photocarcinogenesis: An Overview. J. Invest. Dermatol. 77: 139-143.
Hartmann HR, Teelmann K. 1981. The Influence of Topical and Oral Retinoid Treatment on Photocarcinogenicity in Hairless Albino Mice. In: Orfanos, C. E. et al. eds. Retinoids: Advances in Basic Research and Therapy. Berlin: Springer-Verlag, pp. 447-451.
Forbes PD, Beer JZ, Black HS, Cesarini JP, Cole CA, Davies RE, et al. 2003. Standardized protocols for photocarcinogenesis safety testing. Front Biosci 8: d848-54.
Halliday GM, Robertson BO, Barnetson RSC. 2000. Topical Retinoic Acid Enhances, and a Dark Tan Protects, from Subedemal Solar-Simulated Photocarcinogenesis. J. Invest. Dermatol. 114: 923-927.
Howard P, Sams RL, II, Bucher JR, Allaben WT. 2002. Phototoxicology and Photocarcinogenesis at the U.S. Food and Drug Administration's National Center for Toxicological Research. J Food Drug Anal 10(4): 252-57.
Jacobs, A.C., Brown, P.C., Chen, C., Ellis, A., Farrelly, J., and Osterberg, R. (2004). CDER photosafety guidance for industry. Toxicol. Pathol. **32** (Suppl. 2), 17-18.
Kligmann LH, Kligman, AM. 1981a. Lack of Enhancement of Experimental Photocarcinogenesis by Topical Retinoic Acid. Arch. Dermatol. Res. *270:* 453-462.
Kligmann LH, Kligman, AM. 1981b. Lack of Enhancement of Experimental Photocarcinogenesis by Retinoic Acid In: Retinoids: Advances in Basic Research and Therapy, Eds: Orfanos, C.E. *et al.*, Springer-Verlag, Berlin. pp. 447-451.
Lu YP, Lou YR, Xie JG, Peng Q, Shih WJ, Lin Y, et al. 2009. Tumorigenic effect of some commonly used moisturizing creams when applied topically to UVB-pretreated high-risk mice. J Invest Dermatol 129(2): 468-75.
Mei N, Xia Q, Chen L, Moore MM, Fu PP, Chen T. 2005. Photomutagenicity of retinyl palmitate by ultraviolet a irradiation in mouse lymphoma cells. Toxicol Sci 88(1): 142-9.
Mei N, Hu J, Xia Q, Fu PP, Moore MM, Chen T. 2010. Cytotoxicity and mutagenicity of retinol with ultraviolet A irradiation in mouse lymphoma cells. Toxicol In Vitro 24(2): 439-44.
NTP. 2000. All-Trans-Retinyl Palmitate [CASRN 79-81-2]. Nomination for National Toxicology Program testing. Available: http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/RetinylPalmitate.pdf
NTP. 2007. NTP Draft Technical Report on the Photococarcinogenesis Study of Glycolic Acid And Salicylic Acid (CAS Nos. 79-14-1 and 69-72-7) in SKH-1 Mice (Simulated Solar Light And

Topical Application Study). NTP TR 524. NIH Publication No. 07-4472. Available: http://ntp.niehs.nih.gov/?objectid=883A96DC-F1F6-975E-72D9D9CF9FB7337C
NTP. 2010. NTP Draft Technical Report on the Photococarcinogenesis Study of Retinoic Acid and Retinyl Palmitate [CAS Nos. 302-79-4 (All-Trans-Retinoic Acid) and 79-81-2 (All-Trans-Retinyl Palmitate)] in SKH-1 Mice (Simulated Solar Light And Topical Application Study). Scheduled Peer Review Date: January 26, 2011. NTP TR 568. NIH Publication No. 11-5910. Available: http://ntp.niehs.nih.gov/index.cfm?objectid=A73F2BD6-F1F6-975E-789930D86CD3C2E1
NTP Statistical Methods Working Group. 2004. Report on Analysis of Tumor Data in Photocarcinogenesis Studies. Available: http://ntp.niehs.nih.gov/ntp/Meetings/2004/Phototox_Report1.pdf
So PL, Lee K, Hebert J, Walker P, Lu Y, Hwang J, et al. 2004. Topical tazarotene chemoprevention reduces Basal cell carcinoma number and size in Ptch1+/- mice exposed to ultraviolet or ionizing radiation. Cancer Res 64(13): 4385-9.
Sorg O, Antille C, Kaya G, Saurat JH. 2006. Retinoids in cosmeceuticals. Dermatol Ther 19(5): 289-96.
World Health Organization (2002) Global Solar UV Index: A Practical Guide. Geneva: WHO. Available: http://www.unep.org/pdf/Solar_Index_Guide.pdf
Yan J, Xia Q, Cherng SH, Wamer WG, Howard PC, Yu H, et al. 2005. Photo-induced DNA damage and photocytotoxicity of retinyl palmitate and its photodecomposition products. Toxicol Ind Health 21(7-8): 167-75.
Yan J, Xia Q, Wamer WG, Boudreau MD, Warbritton A, Howard PC, et al. 2007. 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 23(10): 581-9.