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October 27, 2000

Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane, rm. 1061
Rockville, MD 20857

RE: Docket 99D-5435 (Guidance for Industry on Photosafety Testing)

Dear Sirs:

These additional comments are submitted regarding Draft Guidance for Industry on Photosafety Testing. The PhRMA (letter dated April 10, 2000) assessed the Skh mouse as a photobiological model for humans. Attached is a more complete analysis of photobiologically important differences between rodent and humans, entitled "The Skh mouse model in photobiology."

The Kaidbey-Kligman photomaximization test is inappropriate given our current knowledge of photobiology (Caswell and Stephens, Photoderm Photoimmunol Photomed 1999; 15: 146). The test proposed by Bioskin (letter dated April 3, 2000) is an improvement over the Kaidbey-Kligman photomaximization test. However, the Bioskin test is inappropriate because it fails to account for test materials that absorb in the wavelengths being irradiated. For example, the test proposed by Bioskin is inappropriate for testing a sunscreen active. The sunscreen active would grossly attenuate the quantity of ultraviolet radiation. Any revised phototest should accommodate the test material's potential to absorb the ultraviolet radiation.

Sincerely,



Michael Caswell, Ph.D.

99D-5435

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Abstracts

A Reassessment of the Photomaximization Procedure. Caswell M and Stephens T, Stephens & Associates, Inc., 3310 Keller Springs Road, Suite 130, Carrollton, TX 75006

Photoallergic Contact Dermatitis (PACD) is an enhanced acquired immunologically mediated reactivity dependent on exposure to ultraviolet or visible radiation. Radiation induces the formation of photoproducts which are processed to an antigen by skin antigen presenting cells (APCs). UVA radiation causes most cases of PACD, although UVB and light may cause some cases.

Photomaximization procedures have been developed to identify topical photocontact sensitizers. Many of these procedures involve the use of UVB radiation to generate photoproducts and to enhance the delivery of the topical agents to viable skin tissue. One procedure suggests irradiating the skin with 3 MEDs twice weekly for three weeks.

Over-exposure to UVB induces a loss of APCs from the skin which contributes to immunosuppression. One study reported that $144\text{mJ}/\text{cm}^2$ for four consecutive days results in complete depletion of HLA-DR positive cells in the skin, while $72\text{mJ}/\text{cm}^2$ for four consecutive days results in 35% depletion of HLA-DR positive cells in the skin.

These results suggest the need for a new photomaximization procedure. This new procedure should develop photoproducts using UV and/or visible radiation, while avoiding the induction of immunosuppression. This poster will discuss strategies for improving the detection of photocontact allergens.

The Skh Mouse Model in Photobiology

The mouse is a valuable research tool for photobiologists. The Skh-1 (Skh:hairless-1) and Skh-2 (Skh:hairless-2) strains have been used extensively in determining the effects of UV radiation on skin. The Skh-1 mouse is albino and hairless; the Skh-2 is pigmented and hairless. Either mouse is easy to house and to handle. Because both the Skh-1 and Skh-2 animals are hairless, no shaving or clipping is needed to expose the animal to UV radiation. Mouse skin is easily handled for histology and for *in vitro* experimentation. Because the mice reproduce rapidly, large numbers are available for research. The mouse model predictably generates squamous cell carcinoma following UV exposure, so it can be used to generate large amounts of data for statistical analysis.

Despite all of these benefits of the Skh mice,¹ research has gradually been published suggesting that the response to UV radiation by Skh-1 and by Skh-2 mice is unlike the response to UV radiation by humans. The skin on the mouse is much thinner than that of humans.² The Skh mouse skin epidermis is only about 3 microns thick with a stratum corneum composing about 20% of the epidermis.³ Human skin epidermis is about 100 microns thick with a stratum corneum about 15 microns thick. This difference between mouse and human skin is important because the type and amount of UV radiation reaching the viable tissue will differ. Of the UV radiation reaching viable tissue, UVB will be more attenuated in human skin than in mouse skin. Like humans, Skh-1 mouse skin thickens in response to UV radiation.⁴

The mouse is a haired nocturnal animal. Generally, it hides during daylight hours and comes out of hiding during the dark. Even if it were to venture out during daylight hours, hair would protect its skin from UV radiation. One would not expect the mouse to have developed biochemical adaptations to UV radiation.

Unlike Skh-1 mice, humans are known to respond to UV exposure by developing pigmentation in their integument. This allows the sun tanner to expose himself to increasing amounts of UV.⁵ The Skh-1 mouse is an albino animal, so it does not produce melanin as a protection against further UV exposure. In this regard, it may be an appropriate model for albino humans.⁶ The Skh-2 mouse does produce melanin, but the melanin fails to be protective.⁷

The Skh-1 mouse generates almost exclusively squamous cell carcinoma in response to overexposure of UV radiation.⁸ Humans, on the other hand, generate predominately (ca. 60%) basal cell carcinoma.^{9 10 11 12} This difference is important because squamous cell carcinoma and basal cell carcinoma likely have a different UV-induction process.^{13 14} Using the Skh-1 mouse model to make public health recommendations regarding UV exposure for humans fails to acknowledge the risk of basal cell carcinoma, the largest contributor to human skin cancer.

The Skh-1 mouse has little or no photoreactivation capacity to repair cyclobutane pyrimidine dimers,¹⁵ the main photoproducts induced by sunlight.¹⁶ Photoreactivation is a

well-studied common method for humans to repair UV-induced damage to pyrimidines in their DNA.¹⁷

The acute biological response to excessive UV exposure for Skh-1 mice is different from humans. In response to modest over-exposure to UV radiation (3-5 MED), the Skh-1 mouse generates mostly edema with very little erythema;¹⁸ humans generate almost exclusively erythema.

The antioxidant capacity of human skin is greater than that of mouse skin. The antioxidant capability is composed of several enzymatic systems, several lipophilic systems, and several hydrophilic systems. These systems are found in the epidermis and the dermis, although greater in the epidermis. In murine skin, enzymatic antioxidant systems were higher in epidermis by 49% to 74%; lipophilic and hydrophilic systems were higher in epidermis by 24% to 95%.¹⁹ In human skin, enzymatic antioxidant systems were higher in epidermis by 61% to 720%; lipophilic antioxidant systems, vitamin E and ubiquinol 10, were higher in epidermis by 90% and 900%, respectively; hydrophilic antioxidant systems, ascorbic acid and uric acid, were higher in epidermis by 425% and 488%, respectively.²⁰

Other natural defense mechanisms for protection against UV-induced damage are either attenuated or missing in Skh-1 and in Skh-2 mice. This makes them suspect as a model system for UV-induced damage in humans. Consequently, correlating UV responses in the Skh mouse to UV responses in humans is likely to be inappropriate.

¹ Yuspa SH. The pathogenesis of squamous cell cancer: Lessons learned from studies of skin carcinogenesis—Thirty-third G. H. A. Clowes Memorial Lecture Award. *Canc Res.* 1994; **54**:1178-1189.

² Kligman LH, Akin FJ, Kligman AM. Prevention of ultraviolet damage to the dermis of hairless mice by sunscreens. *J Invest. Dermatol.* 1982; **78**; 181-189.

³ Sterenborg HJCM, de Gruijl FR, van der Leun JC. UV-induced epidermal hyperplasia in hairless mice. *Photodermatology* 1986; **3**: 206-214.

⁴ Mitani H, Koshiichi I, Toyoda H, Toida T, Imanari T. Alterations of hairless mouse skin exposed to chronic UV irradiation and its prevention by hydrocortisone. *Photochem Photobiol.* 1999; **69**: 41-46.

⁵ Caswell M. The kinetics of the tanning response to tanning bed exposures. *Photodermatol Photoimmunol Photomed.* 2000; **16**: 10-14.

⁶ Lookingbill DP, Lookingbill GL, Leppard B. Actinic damage and skin cancer in albinos in northern Tanzania: Findings in 164 patients enrolled in an outreach skin care program. *J Amer Acad Dermatol.* 1995; **32**: 653-658.

⁷ Kligman L. personal communication.

⁸ De Gruijl FR, van der Leun JC. Development of skin tumors in hairless mice after discontinuation of ultraviolet radiation. *Canc Res.* 1991; **51**: 979-984.

⁹ Magnus K. The Nordic profile of skin cancer incidence. A comparative epidemiological study of the three main types of skin cancer. *Int J Canc.* 1991; **47**: 12-19.

¹⁰ Green A, Battistutta D. Incidence and determinants of skin cancer in a high-risk Australian population. *Int J Canc.* 1990; **46**: 356-361.

¹¹ Ko CB, Walton S, Keczes K, Bury HPR, Nicholson C. The emerging epidemic of skin cancer. *Br J Dermatol.* 1994; **130**: 269-272.

¹² Bajdik CD, Gallagher RP, Astrakianakis G, Hill GB, Fincham S, McLean XX. Non-solar ultraviolet radiation and the risk of basal and squamous cell skin cancer. *Br J Canc.* 1996; **73**: 1612-1614.

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- ¹³ Krickler A, Armstrong BK, English DR, Heenan PJ. Does intermittent sun exposure cause basal cell carcinoma? A case-control study in western Australia. *Int J Canc.* 1995; **60**: 489-494.
- ¹⁴ Rosso S, Zanetti R, Martinex C, Tormo MJ, Schraub S, Sanchogarnier H, Franceschi S, Gafa L, Perea E, Navarro C, Laurent R, Schrameck C, Talamini R, Tumino R, Wechsler J. The multicenter south European study helios. 2. Different sun exposure patterns in the aetiology of basal cell carcinoma and squamous cell carcinomas of the skin. *Br J Canc.* 1996; **73**: 1447-1454.
- ¹⁵ Berton TR, Mitchell DL, Fischer SM, Locniskar MF. Epidermal proliferation but not quantity of DNA photodamage is correlated with UV-induced mouse skin carcinogenesis. *J Invest Dermatol.* 1997; **109**: 340-347.
- ¹⁶ Qin XZ, Zhang SM, Zarkovic M, Nakatsuru Y, Shimizu S, Yamazaki Y, Oda H, Nikaido O, Ishikawa T. Detection of ultraviolet photoproducts in mouse skin exposed to natural sunlight. *Japan J Canc Res.* 1996; **87**: 685-690.
- ¹⁷ Young AR, Chadwick CA, Harrison GI, Hawk JLM, Nikaido O, Potten CS. The *in situ* repair kinetics of epidermal thymine dimers and 6-4 photoproducts in human skin types I and II. *J Invest Dermatol.* 1996; **106**: 1307-1313.
- ¹⁸ Cole CA, Davies RE, Forbes PD, D'Aloisio L. Comparison of action spectra for acute cutaneous responses to ultraviolet radiation: man vs albino mouse. *Photochem Photobiol.* 1983; **37**: 623-631.
- ¹⁹ Shindo Y, Witt E, Packer L. Antioxidant defense mechanisms in murine epidermis and dermis and their response to ultraviolet light. *J Invest Dermatol.* 1993; **100**: 260-265.
- ²⁰ Shindo Y, Witt E, Han D, Epstein W, Packer L. Enzymatic and non-enzymatic antioxidants in epidermis and dermis of human skin. *J Invest Dermatol.* 1994; **102**: 122-124.

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