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## INTRODUCTION

In recent years, there has been a dramatic increase by the medical community in awareness of the public health risks associated with sun exposure. Consumers are also becoming increasingly aware of these deleterious effects which include erythema, photoaging and skin cancer, as they receive considerable attention in the media. Furthermore, the role of ultraviolet radiation (UVR) in the induction and development of non-melanoma skin cancer has been established (Urbach, F., 1997 and English, D.R., et al. 1997).

Until the mid-1970's, the UVA region of the solar UV spectrum was thought to be of little or no concern to dermatologists. It was only as a result of treating other skin diseases that dermatologists and photobiologists began to understand the effects of UVA radiation. With an increasingly active outdoor lifestyle, the long-term consequences of UV exposure, particularly that of UVA, becomes increasingly important. M. Pathak (Pathak, M. in *Sunscreens – Development, Evaluation, and Regulatory Aspects*, eds. Lowe, N.J., Shaath, N.A., and M.A. Pathak, 59-79, 1997) specifically discusses UVA radiation and its known effects to date, which are listed below:

- UVA output by the sun is significantly greater than UVB output throughout the year;
- UVA radiation penetrates deeper into the skin than UVB;
- UVA radiation causes sunburn;
- UVA effects are additive to the effects of UVB radiation;
- UVA activates melanocytes and causes tanning of the skin;
- UVA causes DNA damage and distinct damage to cell membranes;
- UVA radiation causes phototoxic and photoallergic reactions;
- UVA radiation causes alterations in the immune system;
- UVA radiation is carcinogenic;
- UVA radiation causes photodamage or dermatoheliosis.

As the focus of on-going research shifts to the role of UVA in the etiology of various photodermatoses, recent investigations have implicated UVA exposure with photogenotoxicity, photoimmunosuppression and photoaging consequences (Marrot, L., et al. 1998). While sunscreen products traditionally have provided sufficient protection against erythema, caused primarily by UVB radiation, products often lack protection against the UVA portion of the solar spectrum.

To date, the only accepted parameter for assessing UV protection under the sunscreen monograph, is with respect to testing a product for its ability to prevent erythema *in vivo* by determining its sun protection factor (SPF). [The action spectrum for erythema covers mainly the UVB and UVA II portion of the UV spectrum.] The methodology is recognized as providing a "sunburn" protection factor or an actinic erythema protection factor yet it is assumed that this methodology also covers other biological effects induced by UVB radiation, such as pigmentation, skin cancer, etc. As specific markers for chronic UVA induced damage have not yet been fully elucidated, several biological

surrogate markers for UVA have been proposed and used in similar test methodologies including UVA erythema (PFA) (Cole, C. and R. Van Fossen, 1992; Cole, C., 1994), persistent pigment darkening (PPD) (Chardon, A., et al. 1997) and phototoxic erythema (8-MOP)(Lowe, N.J., et al. 1987).

The most deleterious effect of UV radiation to humans is certainly skin cancer. There are many lines of evidence suggesting that a primary biological target of UVA radiation involves DNA damage through various UVA-absorbing molecules i.e., chromophores, such as urocanic acid present in the skin. These changes may ultimately induce skin cancer, but are also responsible for short and long-term responses to UVA light such as sunburn, pigmentation and inflammation.

As such, effective photoprotection of DNA against the harmful effects of UVR exposure is a critical parameter to be considered when formulating sunscreen products, particularly those claiming to provide UVA and UVB 'broad spectrum' protection. Various *in vivo* and *in vitro* methods have been proposed for determining a sunscreen product's UVA protection factor, although there is currently no consensus in the scientific community as to the most appropriate method. The crux of the ensuing debate between L'ORÉAL and Procter & Gamble<sup>1</sup> revolves solely around the issue of quantification of a product's UVA protection level and consequently, identifying an appropriate method for making this assessment. An *in vitro* method, such as the Critical Wavelength Method ( $\lambda_c$ ) which only measures the breadth of a product's absorbance profile and does not account for the magnitude of the product's UVA protection level is deficient in providing this very important information to both the consumer and the medical profession.

In this submission, our primary objective is to highlight the biological relevance and subsequent necessity for quantifying the UVA protection efficacy of sunscreen products. Results of published investigations will disprove Procter & Gamble's contention that:

*"...presently there is no biologically relevant marker for evaluating longwave UV protection in vivo. Importantly, the primary if not singular function of UV filters is to reduce the dose of UV radiation and thus no biological response needs to be modeled."*<sup>2</sup>

**L'ORÉAL Research / L'ORÉAL USA Products, Inc. believes that the risk of overestimating a sunscreen's UVA protective properties due to the absence of definitive surrogates for UVA, is far outweighed by the significant health risk to consumers from products providing only minimal UVA protection.** This is particularly relevant for those individuals diagnosed with various forms of photodermatoses, those having a history of skin cancer or those who are at high risk for the development of melanoma, not to mention those individuals desiring minimal photodamage.

<sup>1</sup> The Procter & Gamble Company submission of May 2, 2000 to Docket 78N-0038

<sup>2</sup> Ibid, Executive Summary, page 2.

In the first part of this submission, we will:

- demonstrate the efficiency of sunscreen protection levels, with specific focus given to UVA efficiency using various *in vitro* and *in vivo* models, in order to substantiate our position that quantification of UVA protection levels is a critical assessment for accurate product characterization which cannot be overlooked.
- assess the effects of UVA exposure on photogenotoxicity, photoimmunosuppression and photoaging by comparing the protective properties of two different prototype sunscreen products, each having nearly identical SPF values and critical wavelength values that would qualify each product for 'broad spectrum' labeling, but having very different UVA protection factors.

The data presented herein will correct the misconception by Procter & Gamble that:

*"it is difficult to understand how the advancement of any current in vivo method to evaluate longwave UVA protection is being done for altruistic reasons or otherwise to benefit consumers. The outcome of any in vivo UVA test is a generation of a "protection factor" a misleading number..."*<sup>3</sup>

A UVA protection factor is not a 'misleading number' as stated above. It is an essential element of a product's overall protection efficacy which must be considered in tandem with a product's SPF value in order to ensure proportional protection across the entire UV spectrum. The investigations conducted by L'ORÉAL Research, Advanced Life Sciences Research Group and the Applied Research and Development Group, underscore our commitment to basic scientific research and conscientious product development and will demonstrate the biological basis for *in vivo* UVA quantification.

In the second part of this submission, we will:

- focus on the technical aspects of *in vivo* UVA methodologies criticized by Procter & Gamble in their May 2, 2000 submission;
- provide new data generated by a round-robin study sponsored by the CTFA and submitted by the Industry Association of Interested Parties,<sup>4</sup> which demonstrates, once again, the absence of any correlation of the critical wavelength to "...provide a complete description of a product's inherent photoprotective characteristics"<sup>5</sup> even when combined with the product's *in vivo* SPF value.

We trust that the data presented herein will graphically demonstrate the need to assess the quantitative parameter of a sunscreen product's UVA protection efficacy and remove any misconception that this can be achieved utilizing the Critical Wavelength Method.

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<sup>3</sup> Ibid.

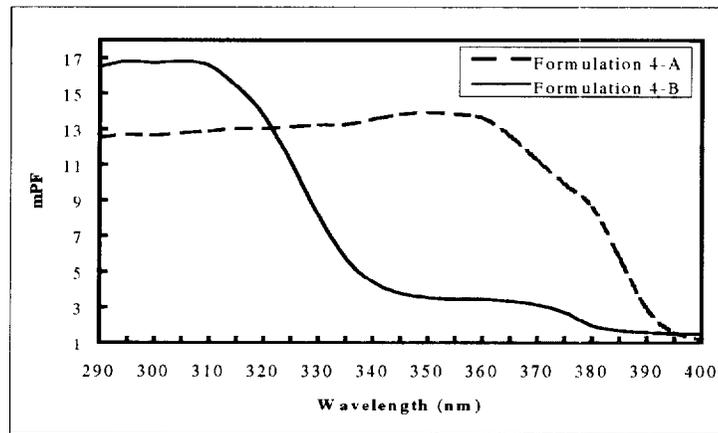
<sup>4</sup> Industry Association of Interested Parties submission of August 30, 2000 to Docket 78N-0038

<sup>5</sup> The Procter & Gamble Company submission of May 2, 2000 to Docket 78N-0038, page 27.

**PART 1: BIOLOGICAL RELEVANCE OF PHOTOPROTECTION:  
 Comparative Protective Effects of Two 'Broad Spectrum' Sunscreens  
 with Nearly the Same SPF and Critical Wavelength ( $\lambda_c$ ) Values, but  
 Different UVA Protection Factors.**

In the first part of this document, a set of *in vitro* and *in vivo* studies will be presented to demonstrate the photoprotective effects provided by two 'broad spectrum' sunscreens,<sup>6</sup> each having quite different UVA protection factors (UVA-PF) but with similar SPF and  $\lambda_c$  values. Three critical biological endpoints for photoprotection including photogenotoxicity, photoimmunosuppression and photoaging were assessed yielding results that demonstrate dramatic differences between sunscreen products for each of the effects studied.

In our submissions of May 15, 1998 and March 3, 2000 to Docket 78N-0038, we provided data to demonstrate the inadequacies of the Critical Wavelength Method in providing a distinction between products with very different UVA protection levels but having a critical wavelength value  $\geq 370$  nm, thereby qualifying for a 'broad spectrum' labeling designation. As we previously reported, it is evident from the shape of the spectral absorption curves (shown below), that the formulations do not display the same protection level, with product 4-A being much more protective in the UVA range. **For the purpose of this submission, these differences in UVA protection, as determined by the PPD method, will be confirmed in the biological models described below which vividly show the effects of UVA damage at a cellular level.**



**Monochromatic Protection Factor Curves of Two Sunscreen Products**

Product Code	UV Filters	SPF ( <i>in vivo</i> )	$\lambda_c$ (nm)	UVA-PF (by PPD)
4-A (408-312)	OCTO 7% +BMDM 3 %	7.4± 1.5	379	7.2± 1.8
4-B (408-320)	OMC 3.75 % + ZnO 7.5%	7.5± 1.6	372	2.8± 0.8

OCTO – Octocrylene, BMDM – Avobenzone, OMC – Octyl Methoxycinnamate, ZnO – Zinc Oxide

<sup>6</sup> The studies which are presented in Part 1 utilize the sunscreen products '4-A' and '4-B' shown in the figure above with exceptions where noted in which formulations having very similar absorbance curves but different UV filter combinations have been utilized.

## I. PHOTOGENOTOXICITY

### **Photogenotoxicity *in vitro*: Initial Investigations utilizing Human Keratinocytes and Fibroblasts**

Single cell gel electrophoresis, also known as the comet assay, is a simple technique for the detection of breaks in the DNA of an individual cell nucleus (Singh, N.P. 1996). These breaks in DNA can be produced by radicals, alkali-labile lesions or excision of damage such as pyrimidine dimers. The comet assay was used to visualize the DNA damage in the form of "comet", observed after UVA exposure, based on a recent publication (Alapetite, C. et al. 1996).

An initial investigation utilized the method as described by Alapetite, C., et al. (*Int J Radiation*, 69:359-369, 1996) to evaluate the two prototype sunscreen formulations described above as products 4-A and 4-B for their photogenotoxic protection properties. Two cell types were chosen: keratinocytes from the epidermis and fibroblasts from the dermis, both of which are susceptible to UVR damage. These cells were exposed to full spectrum UVR, i.e., conditions that can be described as realistic in comparison to actual sun exposure, with and without application of the prototype sunscreen products. Refer to Appendix I for a description of the material and methods utilized; a summary of the findings is presented below.

One hour after application of the sunscreen products, the spectral power distribution of UV transmitted was recorded by spectroradiometry (refer to Figure 1). The spectra clearly show a difference in the UVA filtration capacity between the two prototype sunscreen products, with a greater efficacy shown for product 4-A (408-312).

Results of this investigation demonstrated the following compelling differences between products 4-A and 4-B with respect to their photoprotective properties at a cellular level:

- When keratinocytes and fibroblasts are nonirradiated, DNA damage is not induced and their nuclei appear circular as shown in Figures 2a and 3a.
- When both keratinocytes and fibroblasts are irradiated with full spectrum UVR (produced by a solar simulator) but are not protected with sunscreen, a large number of lesions are produced. The UV radiation induces breaks in the DNA which generate fragments that migrate during electrophoresis to form the comet tails as seen in Figures 2b and 3b.
- When protected by product 4-A (408-312) which provides true broad spectrum filtration throughout the UVA spectrum, comet formation has nearly disappeared for the keratinocytes and is very significantly reduced for the fibroblasts, as shown in Figures 2c and 3c.
- When protected by product 4-B (408-320), which provides only partial filtration across the UVA spectrum, yet would qualify for 'broad spectrum' labeling as per the

criteria of the Critical Wavelength Method, i.e.  $\lambda_c \geq 370$  nm, comet formation is clearly visible, particularly for the fibroblasts (Figures 2d and 3d).

It is postulated that the difference in the extent of comet formation between the keratinocytes and fibroblasts could result from differences in UV sensitivity between these two cell types; keratinocytes are the first layer of cells exposed to UV radiation and have an inherent antioxidant defense mechanism against oxidative stress (Applegate, L.A., et al. 1995).

**This initial investigation demonstrates that UVA, even when partially filtered, can induce genetic alterations to the DNA of cultured human skin cells.** Utilizing the comet assay under irradiation conditions simulating exposure to outdoor sunlight, it was shown that the two sunscreen products, each with a similar SPF value and a critical wavelength value  $\geq 370$  nm, do not provide the same degree of protection against DNA damage in cultured human keratinocytes and fibroblasts. It is also shown by comparison, that the higher UVA protection factor, e.g, product 4-A (UVA-PF = 7.2 by *in vivo* PPD) is necessary to prevent DNA damage at a cellular level. The biological evidence presented above clearly supports the premise that requires an *in vivo* UVA protection factor determination in tandem with an *in vivo* SPF determination to provide comprehensive information concerning a sunscreen's efficacy in preventing damage from solar UVR.

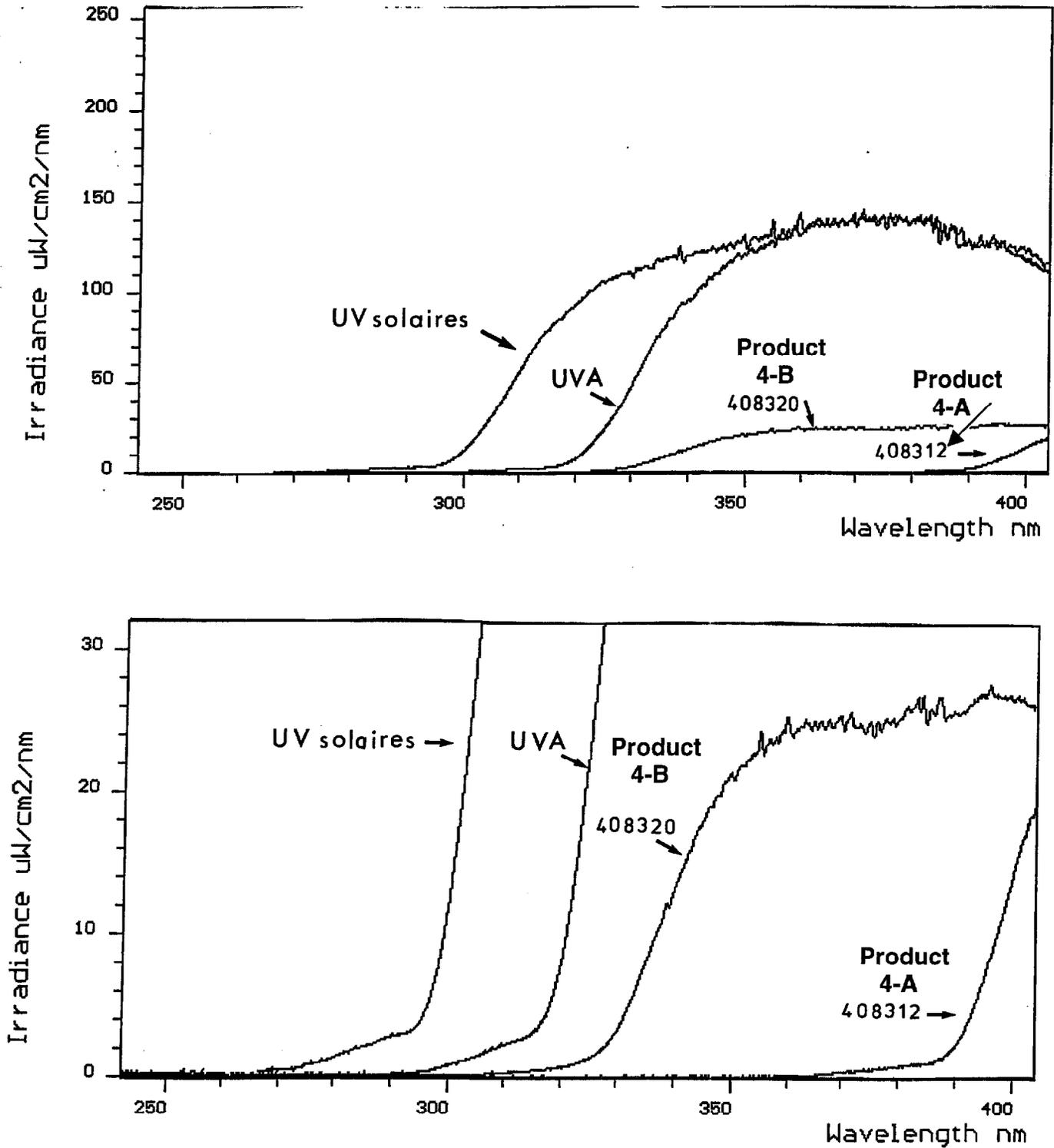
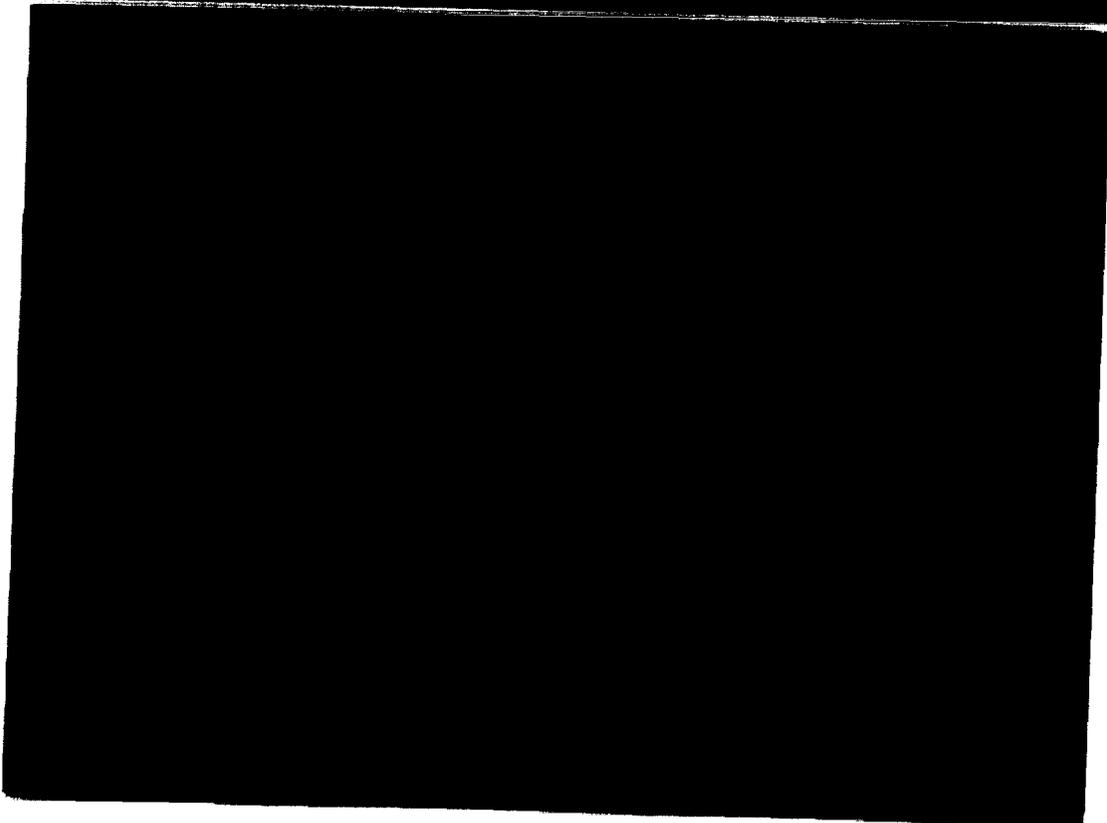
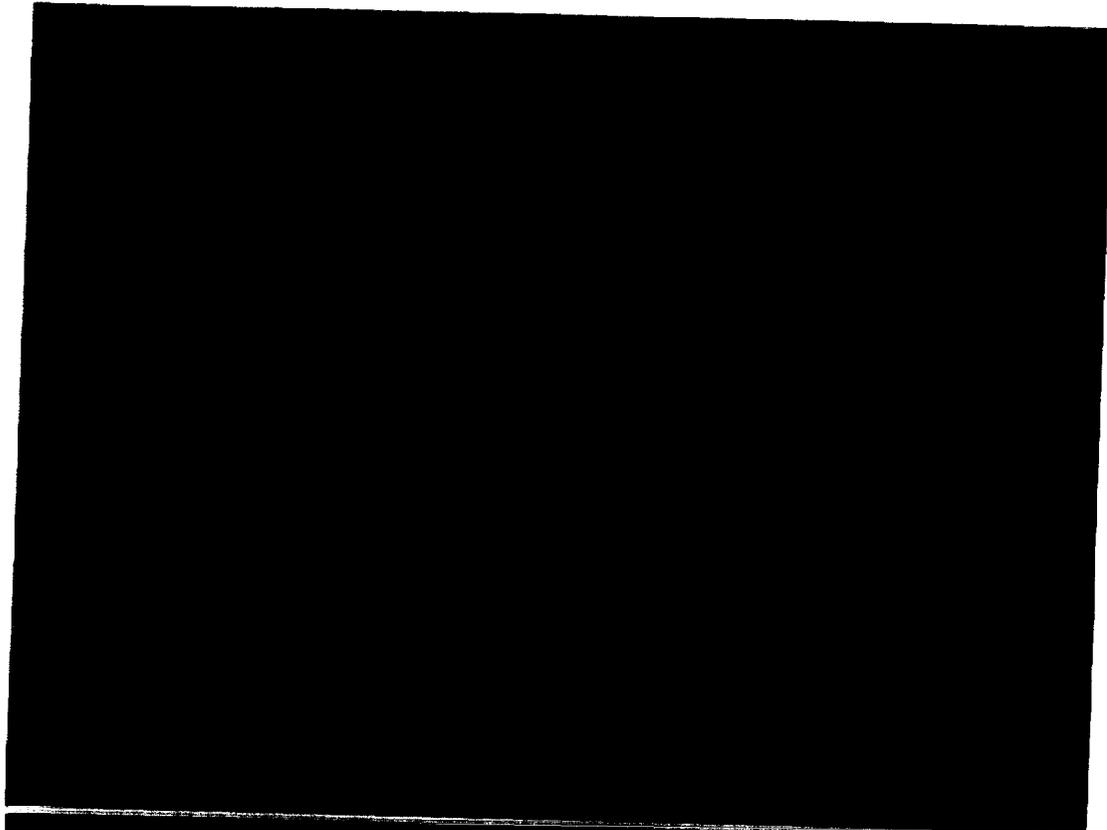


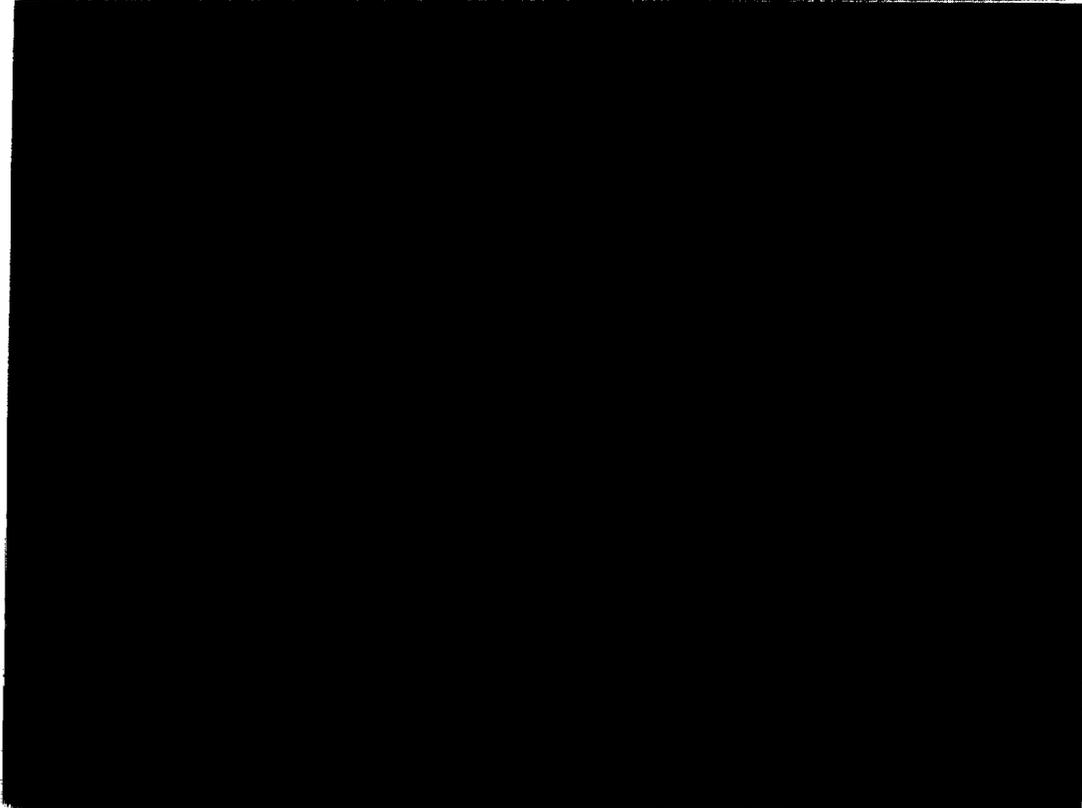
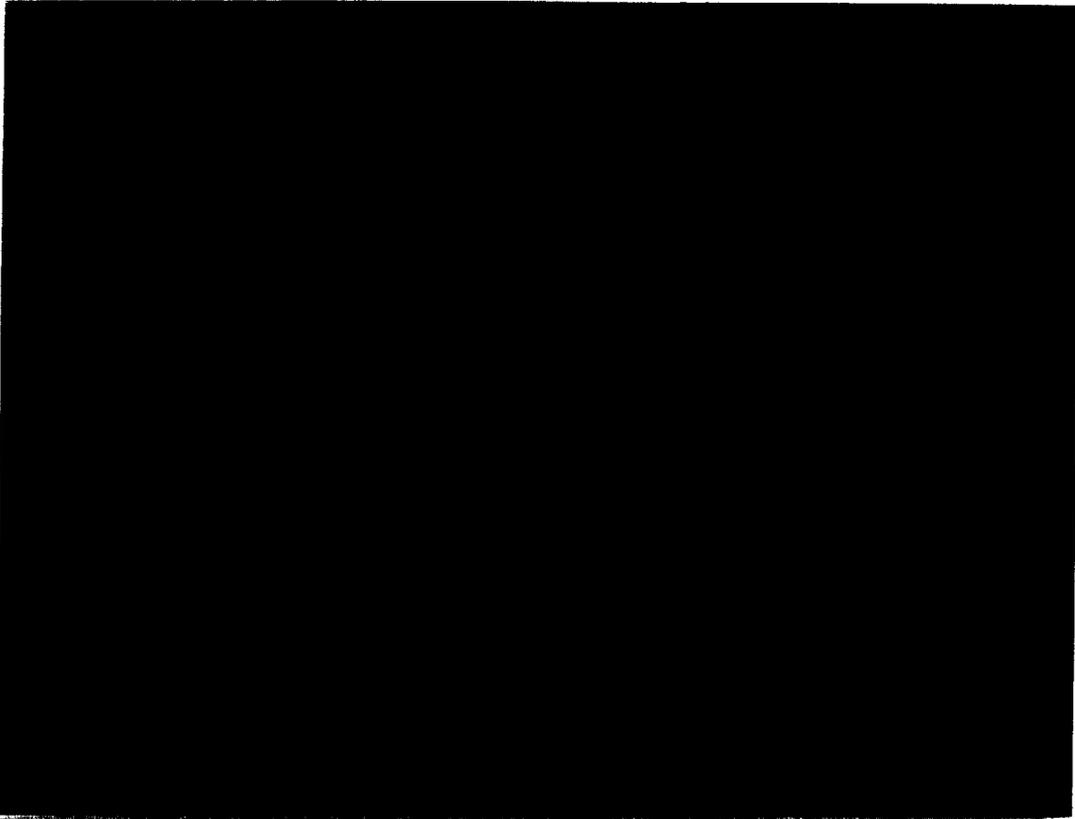
Figure 1: Spectra of incident UV solar rays and UVA as well as fractions of visible light transmitted through a 20  $\mu\text{m}$  of formulations 408312 and 408320. Sunscreen product 4-A (408312) and 4-B (408320)

**2-a Keratinocytes : non irradiated control**



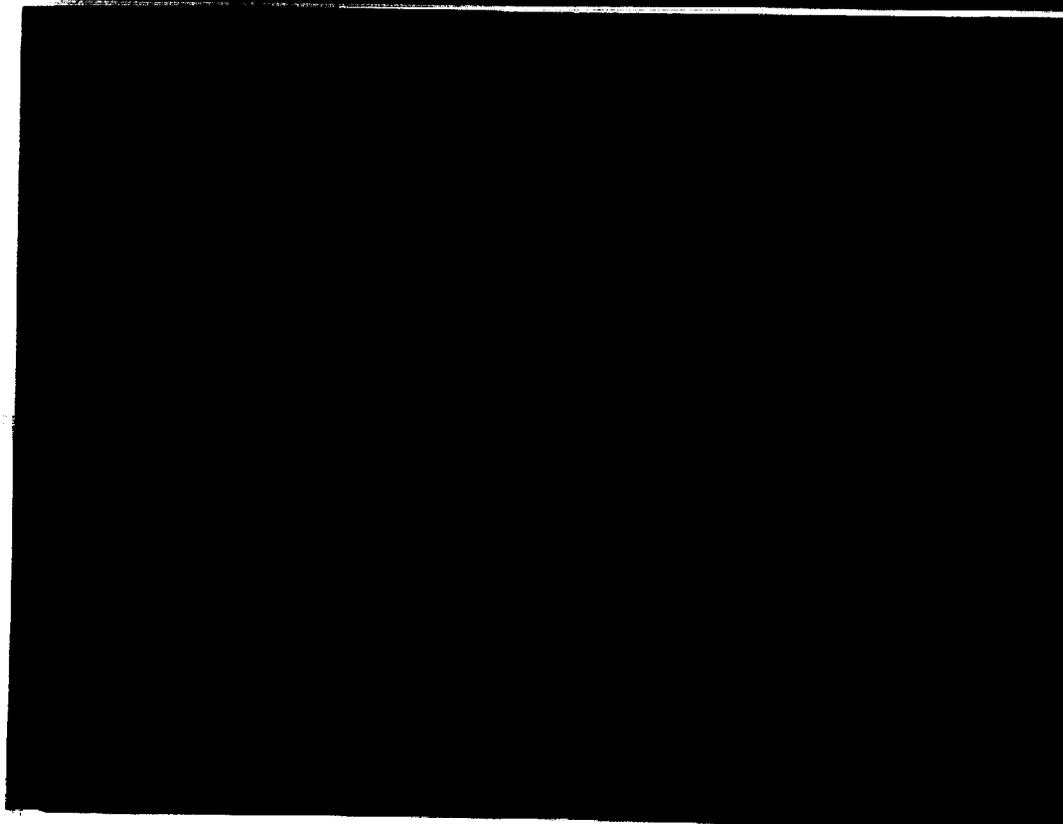
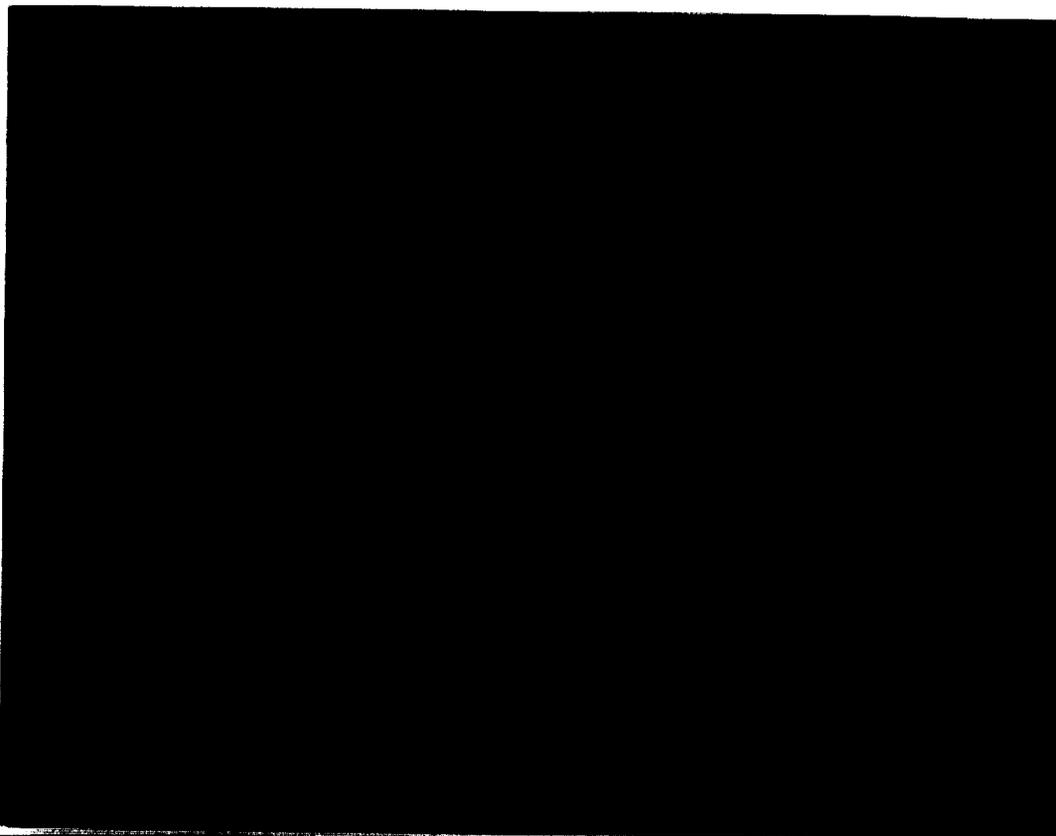
**2-b Keratinocytes : UV solar rays 45 minutes**

**2-c Keratinocytes : UV solar rays 45 minutes + Product 4-A (408-312)**



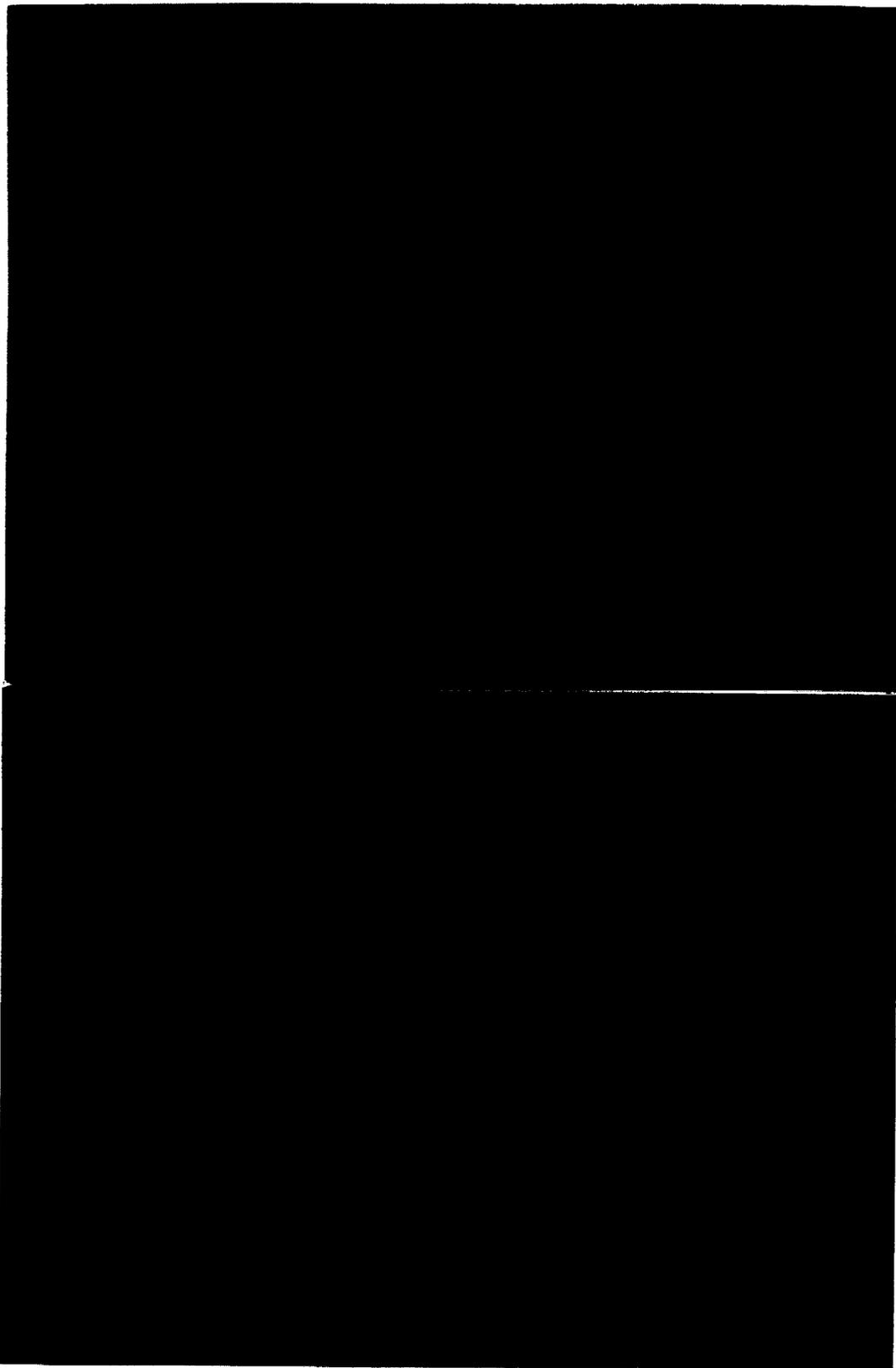
**2-d Keratinocytes : UV solar rays 45 minutes + Product 4-B (408-320)**

**Figure 3 a : Fibroblasts : non irradiated control**



**Figure 3 b : Fibroblasts : UV solar rays 45 minutes**

**Figure 3 c : Fibroblasts : UV solar rays 45 minutes + Product 4-A (408-312)**



**Figure 3 d : Fibroblasts : UV solar rays 45 minutes + Product 4-B (408-320)**

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## PHOTOGENOTOXICITY (continued)

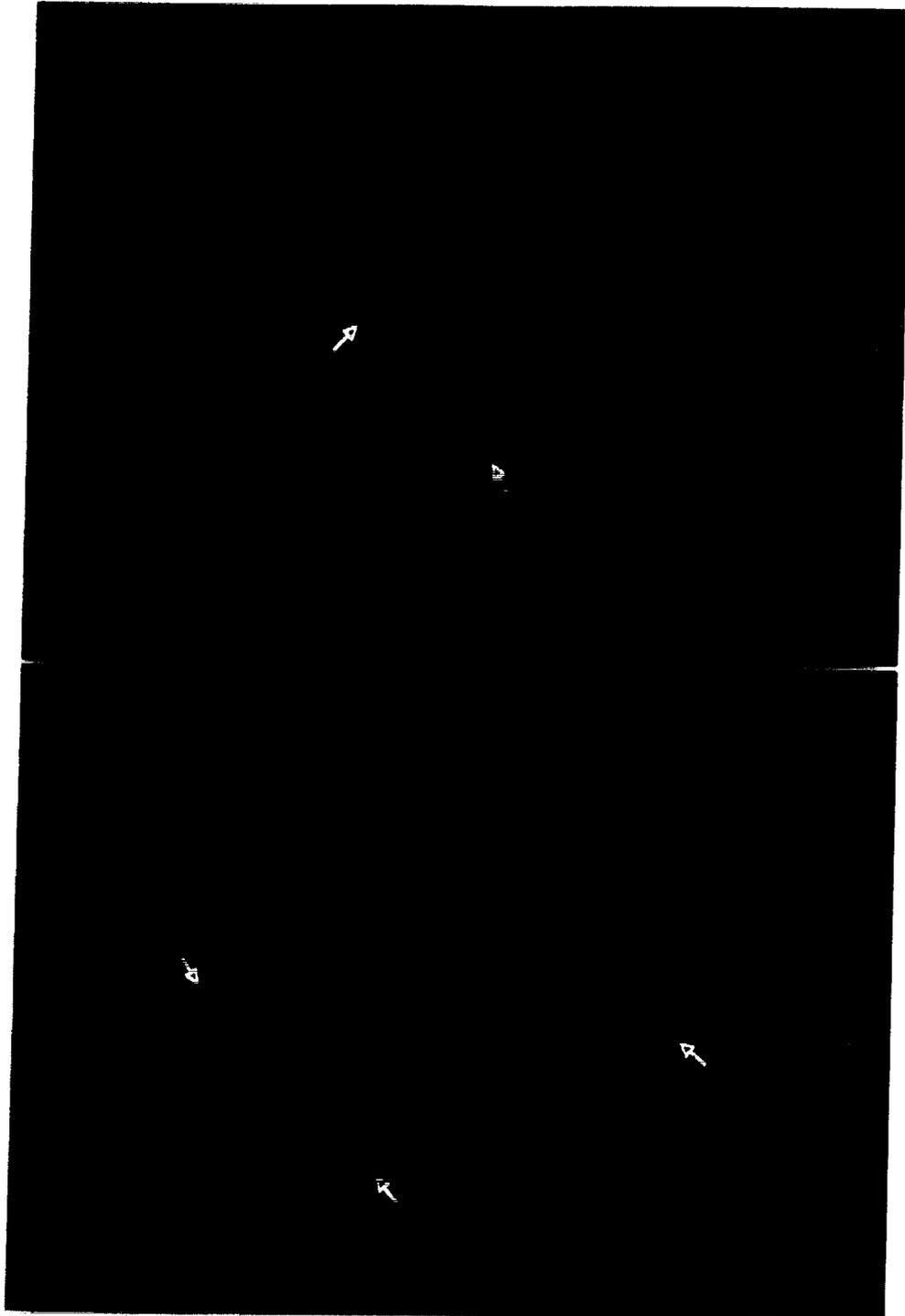
### **Photogenotoxicity *in vitro*: Published Investigations utilizing the Normal Human Melanocyte (NHEM)**

In contrast to non-melanoma skin cancer for which the role of UV radiation in the etiology is now well established (Urbach, F., 1997 and English, D.R., et al 1997), the relationship between melanoma incidence and sunlight exposure is still a matter of debate. Epidemiological studies show strong evidence for increased risk related to sunburn, but the connection with total sun exposure is unclear and experimental studies are limited by the lack of suitable animal models. Thus at the present time, there is an absence of a definitive surrogate for longwave UVA damage; however this fact cannot serve as the rationale to disregard the contribution of UVA exposure when considering the photoprotective properties of sunscreen products.

The initial experiments previously reported were extended to assess the effects of UVA radiation on another cell type, the human melanocyte. Melanocytes were chosen as they represent a visible target for UVR effects due to their role in the pigmentation process and for one of the most dangerous forms of skin cancer, i.e., malignant melanoma. The induction of DNA breaks by UVA in the nucleus of normal human melanocytes in culture was investigated, once again, using the comet assay. This work of L'ORÉAL Research, Advanced Life Sciences Research Group, entitled "*The Human Melanocyte as a Particular Target for UVA Radiation and an Endpoint for Photoprotection Assessment*" (L. Marrot et al. *Photochem. Photobiol.*, 69 (6):686-693, 1999) analyzed the photoprotection of products 4-A and 4-B against the genotoxic effects of UVR. Additional information complementary to this publication is presented below; materials and methods can be obtained directly from the above publication provided in Appendix I.

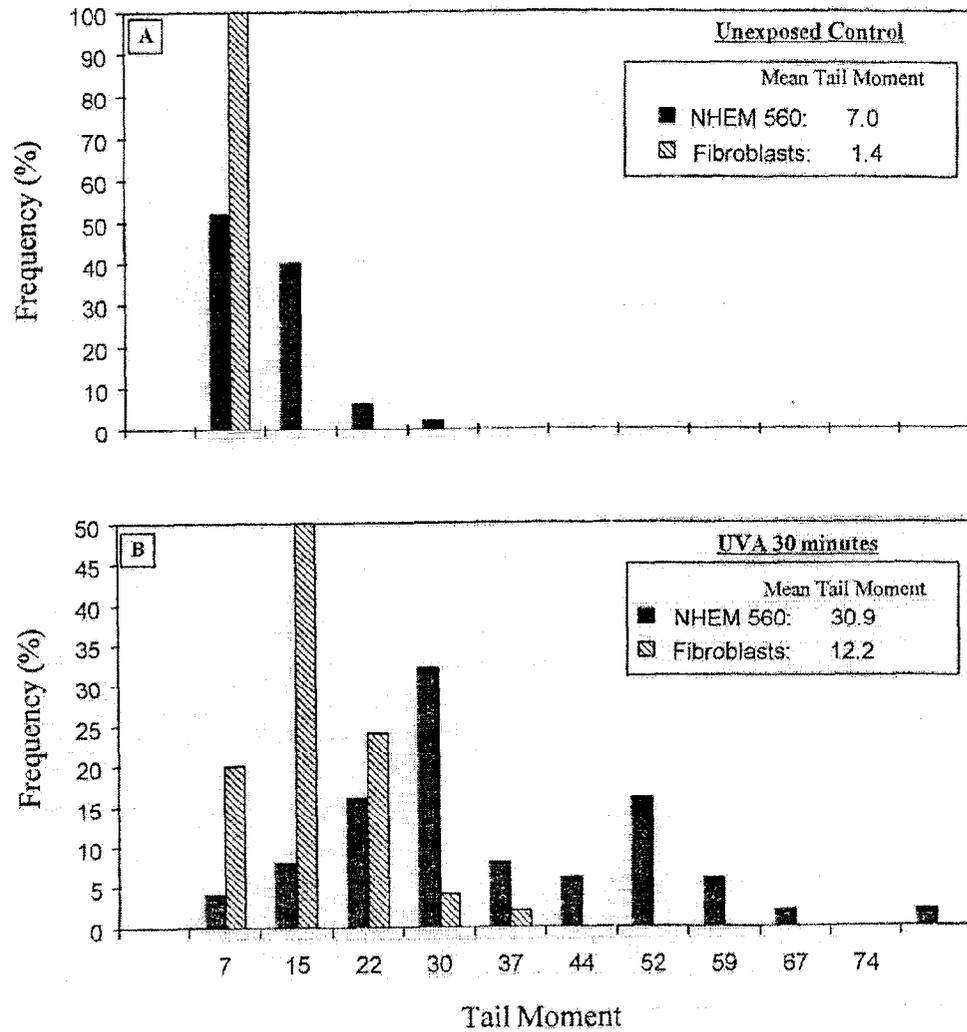
Even if the mechanisms involved in the initiating steps of melanoma are not completely clear, an involvement of DNA photodamage is highly probable, bringing into question the protective role of melanin-related molecules. In this investigation, endogenous pigment and/or melanin-related molecules were found to enhance UVA-induced DNA breakage (shown in Figure 4). Apoptotic-like comet formation was more intense in melanocytes than in fibroblasts (described in Figure 5), as well as in cells containing high melanin content, or after simulated melanogenesis was performed by supplying tyrosine in the culture medium.

## UVA - Induced Comets in Normal Human Melanocytes



→ : Apoptotic-like comets

Figure 4



**Figure 5:** Distribution of tail moments of comets from fibroblasts or melanocytes: nonirradiated (figure A); or irradiated 30 minutes with UVA (140kJ/m<sup>2</sup>) (figure B) and immediately lysed after exposure.

— Following exposure of untreated melanocytes to UVA doses resulting in the formation of intense, visible comets, neither cytotoxicity nor stimulation of tyrosinase activity were detected. However, the accumulation of p53 protein suggested that these cells suffered from genotoxic stress under these experimental conditions. [Note that the role of the p53 protein will be discussed in more detail in the next section.]

The same approach was used to compare the effect of UVA exposure on melanocytes treated with the two sunscreen products with nearly identical SPF values but very different UVA protection factors. **These products are designated as Product A (Note: this formulation is designated elsewhere in this document by the product code '4-B' and corresponds to formulation number 408-320) and Product B (Note: this formulation is designated elsewhere in this document by the product code '4-A' and corresponds to formulation number 408-312).**

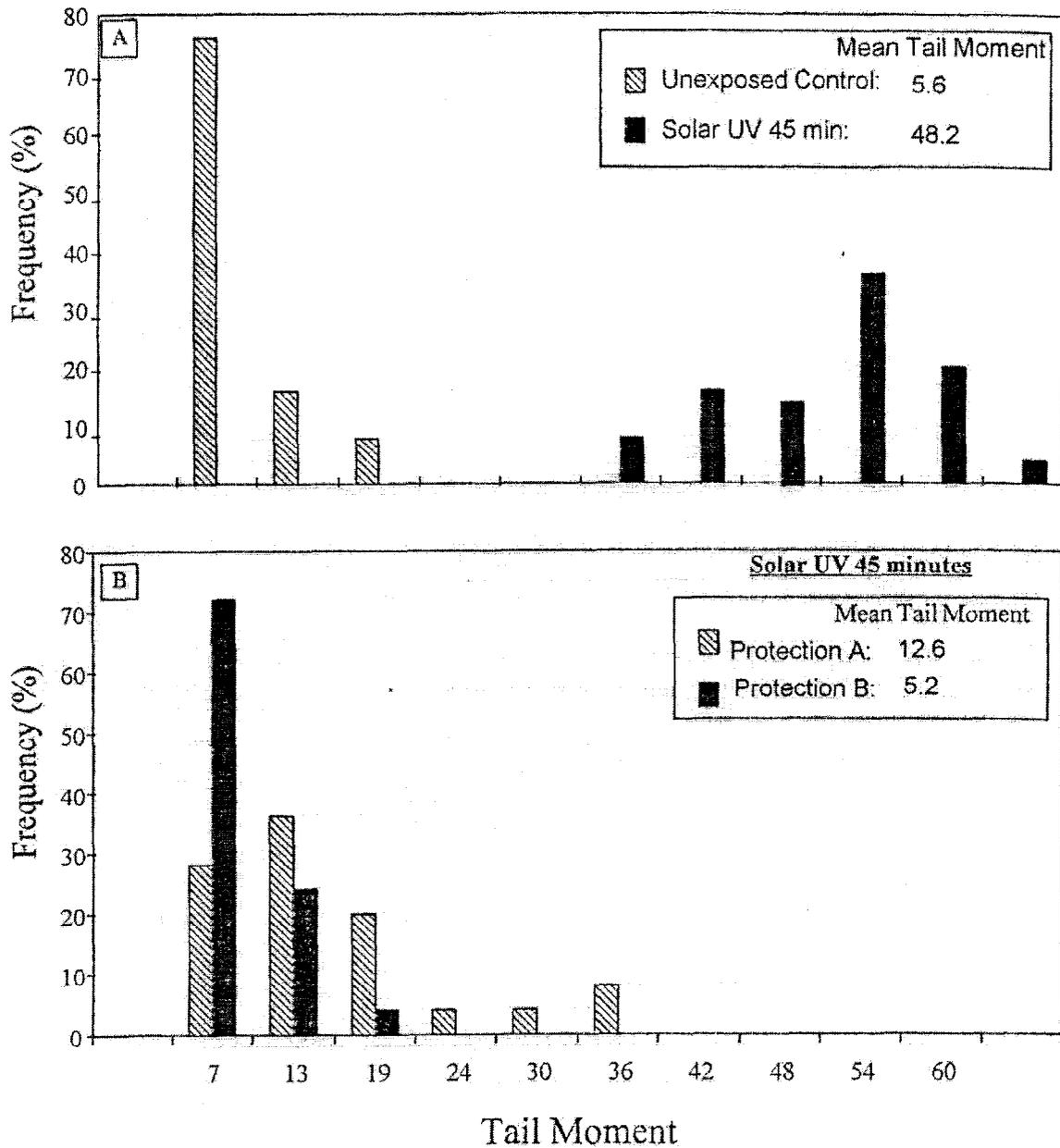
The results illustrated in Figures 6 and 7 show that both sunscreen formulations significantly reduced the extent of comet formation. However, a difference can be clearly detected between the two products, related to their different photoprotective properties particularly in the UVA range, emphasizing the fact that measuring the qualitative level of UVA protection is critical to providing adequate protection to the consumer. Here it is shown that Product A does not totally abrogate the induction of DNA breaks, and in this case, the mean tail moment\* remains higher than that of the nonirradiated control or of the sample protected by Product B.

In conclusion, these results show that UVR exposure hazards increase when pigmentation is starting, suggesting that skin undergoing tanning could be more susceptible to photogenotoxic stress. Additionally, these data provide useful information for elucidating the role of sunlight in the initiating steps of melanocyte transformation. These results also suggest that the human melanocyte may be used as a target cell to evidence broadspectrum photoprotection during the early stages of sunscreen product development.

Further to the purpose of this submission, these published results support the use of this assay as a means for assessing the photoprotective properties of a sunscreen as it can differentiate between products having different UVA-PF values as does the *in vivo* PPD method. These data also confirm the results of the initial investigations utilizing keratinocytes and fibroblasts as the target cells for DNA damage. Once again, it is shown here that there is a difference at the cellular level between the UVA protective properties of these two sunscreen products, 4-A and 4-B, which corresponds to the differences in their respective UVA-PF values as determined by the PPD method. SPF and critical wavelength values, even when considered together, do not do this.

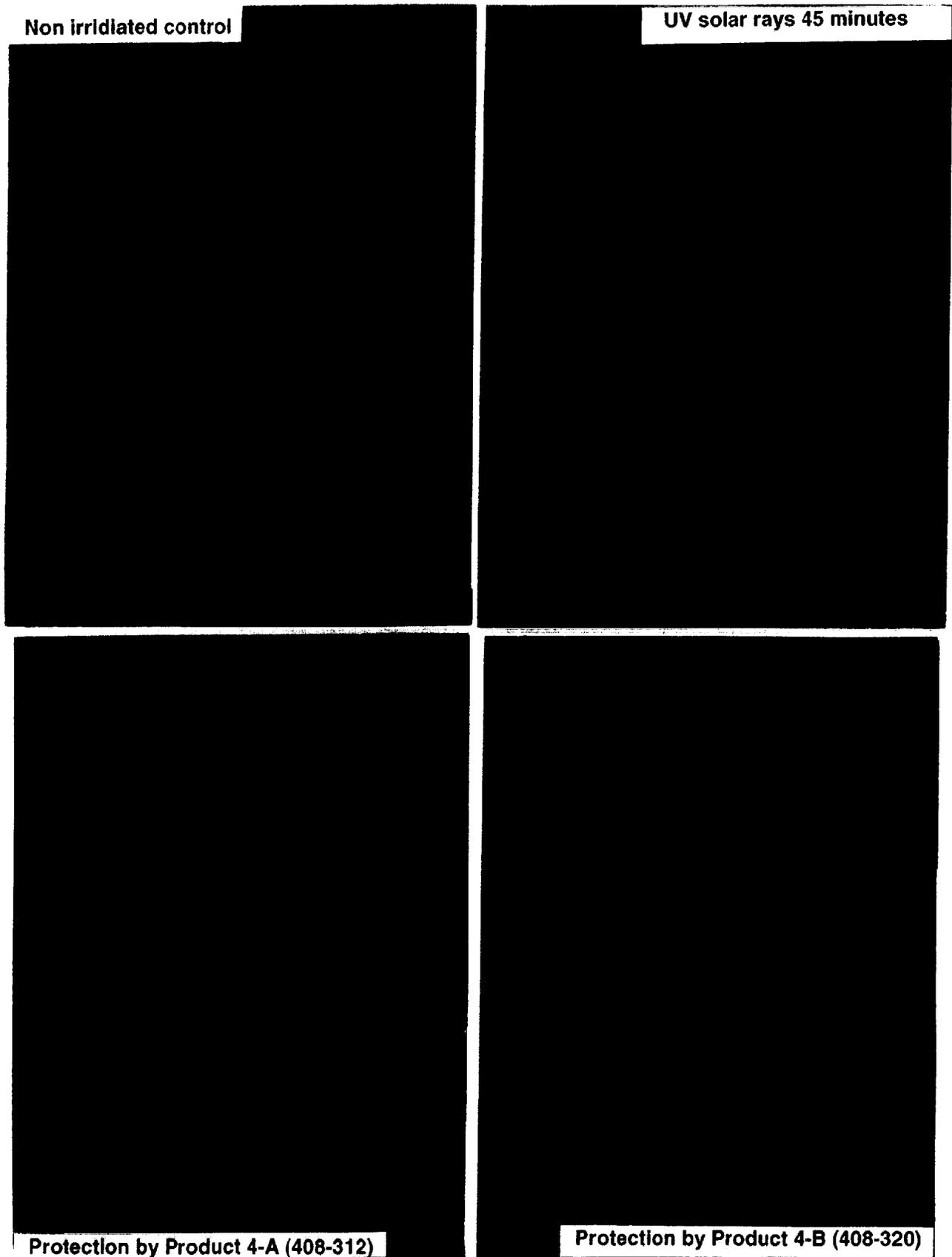
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\* The mean tail moment is a measure of the length of the comet tail and is a direct indicator of the extent of cellular damage and subsequent fragmentation of cellular content.



**Figure 6: Distribution of tail moments of comets from melanocytes (NHEM 4528): nonirradiated cells or cells irradiated for 45 minutes to solar UV without sunscreen protection (figure A); melanocytes protected by a 20  $\mu\text{m}$ -thick layer of sunscreens A or B after solar UV exposure (figure B).**

**Assessment of Photoprotection Provided by Sunscreens A and B Using Comet Assay**



**Figure 7**

## PHOTOGENOTOXICITY (continued)

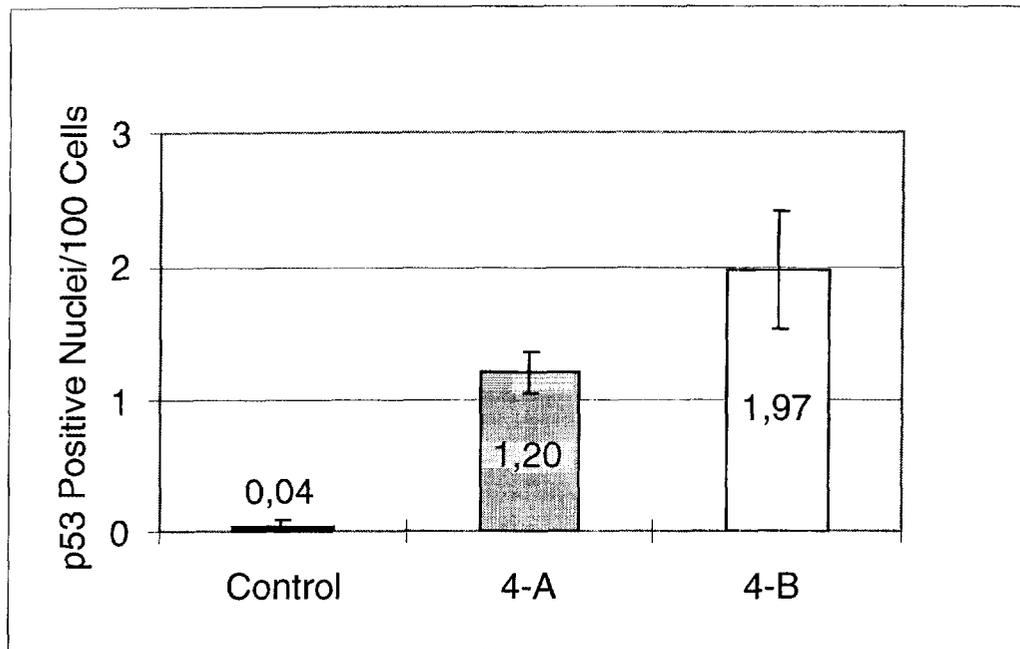
### Photogenotoxicity *in vitro*: p53 Accumulation in Epidermis

Utilizing a different experimental model, i.e., p53 expression as a hallmark for genotoxic stress, the photoprotective properties of the two sunscreen products 4-A and 4-B were once again compared, with particular focus on the UVA protective properties of these products.

Nuclear p53 expression levels associated with DNA damage have been shown to play a key role in cell cycle arrest, apoptosis and possibly with skin carcinogenesis. p53 expression has been used as well, to detect UV-induced skin damage and to evaluate sunscreen products. It is interesting that results of p53-positive nuclei do not correlate with erythema induction (Burren, R., et al. 1998).

Results of clinical investigations performed by L'ORÉAL Research, Advanced Life Sciences Research Group entitled "*Accumulated p53 Protein and UVA Protection Level of Sunscreens*" were recently published by Seité, S. et al (*Photodermatol. Photoimmunol. Photomed.*, 16:3-9, 2000). It was demonstrated that the photoprotection provided by the two products 4-A and 4-B, both labeled as '*broad spectrum*' yet having a significantly different UVA protection factor, is different based on nuclear p53 protein accumulation, but is consistent with their respective UVA protection factor as determined by the PPD method. This investigation is summarized below; the publication is provided in Appendix I.

The results show that both sunscreen products offer only partial protection against the increased expression of p53 protein induced by repetitive solar simulated radiation (SSR) exposures (5 individual MED determinations repeated 8 times over the course of two weeks) of healthy Caucasian volunteers of skin types II and III. However, a significantly lower level of p53-positive cells was found in areas protected with the sunscreen having the higher UVA protection factor (product 4-A) when compared to the other sunscreen (product 4-B) protected areas (Figure 8). Representative photographs (Figure 9) from one subject show the extent of p53 expression for each of the three sites (untreated control, treated with product 4-A prior to SSR and treated with product 4-B prior to SSR).



**Figure 8:** p53 protein induction with cumulative SSR exposure of human skin attenuated by sunscreen products 4-A and 4-B

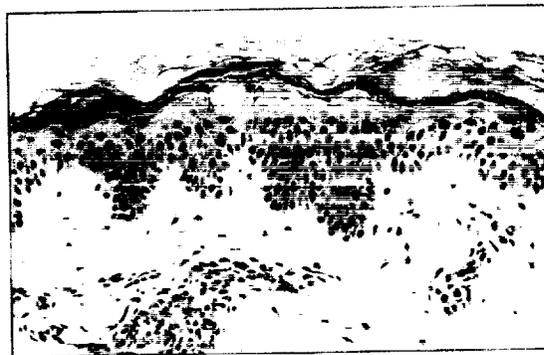
p53 expression in human skin expressed as the percentage of p53-positive nuclei [1000-2000 cells counted from distant tissue sections of each biopsy specimen of nonirradiated skin or skin exposed after application of either product 4-A (high UVA-PF) or product 4-B (low UVA-PF)]. Results are given as means  $\pm$  SEM. Product 4-A with higher UVA-PF (7.2) appears significantly more efficient than product 4-B with lower UVA-PF (2.8), although both products have nearly identical SPF values (SPF = 7.4 and 7.5, respectively).



Control



A



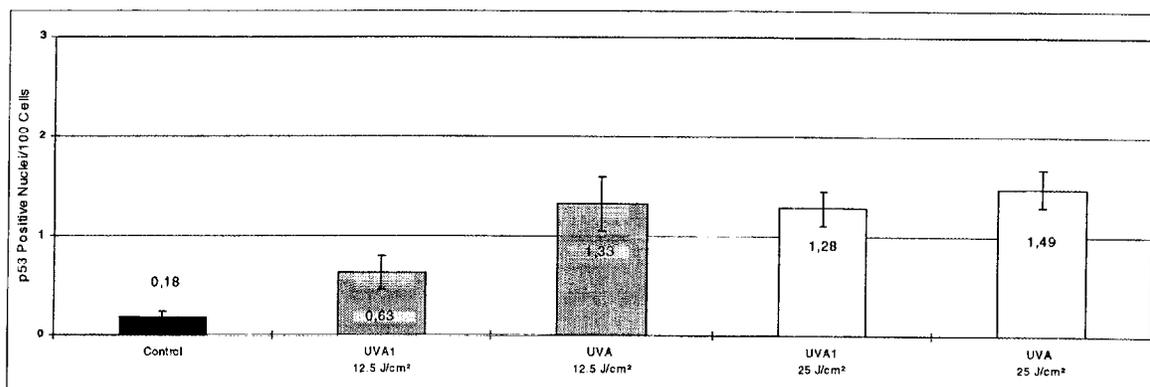
B

**Figure 9:** p53 immunoperoxidase detection in unexposed control skin or skin exposed after application of either sunscreen product 4-A (high UVA-PF) or 4-B (low UVA-PF).

Skin sections were stained with antibody against p53 protein. In unexposed control skin, the specimen shows essentially no reactivity in epidermal cells. (A)- In protected exposed skin, positive staining for p53 is indicated by the dense red nuclear coloration seen in basal as well as suprabasal epidermal cells. A clear scattered reactive pattern is observed in the picture of the area protected by Product 4-A with a strong reactive area (right part), an unreactive area (middle part) and a weak reactive area (left part). (B)- In the picture of the area protected by Product 4-B the staining pattern is more homogenous.

It is generally agreed that DNA damage is the initial step in UV-induced skin carcinogenesis and that the p53-mediated response is a response of skin epidermal cells to DNA damage (Ziegler, A., et al. *Nature*, 372:773-776, 1994). Moreover, Campbell, C., et al (*Cancer Res.* 53:2697-2699, 1993) showed that p53 expression in the epidermis was wavelength specific. Therefore, sunscreen product efficacy across the entire UVA range is a particularly important product characteristic.

In order to verify whether the difference in efficacy of these products was due to the difference in their respective UVA absorption capacity, the authors quantified epidermal p53 protein accumulation after 8 exposures to either UVA (320-400 nm) or UVA I (340-400 nm) (Figure 10). These results indicate that p53 expression was induced by repeated exposures to low doses of 12.5 and 25 J/cm<sup>2</sup> of UVA (320-400 nm) or UVA I (340-400 nm).



**Figure 10: p53 Protein induction after repeated UVA exposure.**

p53 expression in human skin expressed as the percentage of p53-positive nuclei (from 1000 -2000 cells counted from distant tissue sections of each biopsy specimen) as a function of dose of UVA or UVA I radiation. Results are given as mean  $\pm$  SEM

These low UVA doses were unable to induce p53 expression after a single exposure, confirming recent results reported by Burren, R., et al. (*Int. J. Cancer* 76:201-206, 1998). Also, as has been previously demonstrated by Lavker, R.M., et al. (*J. Am. Acad. Dermatol.* 32:53-62, 1995), the present study confirms the importance of protecting the skin against the entire UVA spectrum. In the present investigation, it was also shown that the protection provided by two sunscreen products with different UVA protection factors, as determined by the PPD method, is different based on nuclear p53 protein accumulation.

## **Photogenotoxicity *in vitro*: Summary of Key External Investigations**

Presented below is a selection of complementary papers which place the *in vitro* investigations of L'ORÉAL Research Advanced Life Science Group, presented above, in a global scientific context regarding the phototoxicity of UVA radiation.

- The implication of UVA radiation in photomutagenesis and photocarcinogenesis is questioned by Drobetsky, E.A., et al. (1995), Roberts, L.K., et al. (1996) and Ley, R.D. (1997) who highlight the possible role of UVA in the development of cutaneous melanoma. Such a hypothesis is now quite well established among photobiologists, especially if one considers other studies *in vivo*, e.g., the fish model developed by Setlow, R.B., et al. (1993) and epidemiological studies related to the role of UV exposure at different longitudinal locations and the corresponding incidences in skin cancer.
- Studies performed *in vitro* using cultured cells have shown that UVA can trigger molecular responses related to genotoxicity such as apoptosis (Godar, D.E., 1999) and induce DNA lesions as shown by the comet assay (Alapetite, C., et al., 1995, Lehmann J., et al., 1997, Emont-Picardi, N., et al., 1998).
- Finally, the L'ORÉAL Research data based on the comet assay showing that the normal human melanocyte is a particular target for UVA-induced damage are in agreement with those published by Wenczl, E., et al. (1997, 1998) in which strand breaks in the genome of melanocytes were detected using an immunological approach.

These select references underscore the relevance and importance of *in vitro* biological markers for assessment of UVA effects on the photoprotective properties of sunscreen products, particularly during the new product development phase.

### **PHOTOGENOTOXICITY: Conclusion**

**Despite the conclusion reached by Procter & Gamble that “A combination of *in vivo* SPF and critical wavelength provide a complete description of a product’s inherent photoprotective characteristics.”<sup>7</sup>, the investigations presented herein utilizing two different models for genotoxic stress, i.e., the comet assay, and p53 expression have shown this premise to be false.** These results demonstrate that SPF coupled with critical wavelength information is not adequate to evaluate the comprehensive photoprotection of a sunscreen product. Moreover, these results show that a precise assessment of the UVA protection factor is an additional but essential requirement to adequately characterize a sunscreen product’s UVA and UVB protective properties, particularly since important markers like the p53 expression do not correlate to erythema induction in an individual.

This raises a key question as to what constitutes adequate photoprotection. Sunscreens are generally evaluated by their ability to prevent sunburn and, as a consequence, the SPF

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<sup>7</sup> The Procter & Gamble Company submission of May 2, 2000 to Docket 78N-0038, page 27.

is essentially related to UVB effects on the skin. The experiments reported herein using two sunscreen products with comparable SPF and critical wavelength values but very different UVA protection factors, demonstrate that it is essential for sunscreen products labeled as '*broad spectrum*' to **effectively** cover the entire UV range of sunlight. This can only be achieved with adequately balanced formulations which utilize a proportionality approach to avoid a situation where high efficacy in the UVB region would subject an individual to longer exposure to unfiltered UVA. This conclusion further supports the position of the Industry Association of Interested Parties<sup>8</sup> which provides a proposal for the evaluation and labeling of UVA protection based on concepts of proportionality and a quantitative measurement of product efficacy.

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<sup>8</sup> Industry Association of Interested Parties submission of August 30, 2000 to Docket 78N-0038.

## II. PHOTOIMMUNOSUPPRESSION

### **Photoimmunosuppression: *trans-cis* Photoisomerization of Urocanic Acid in the Skin**

Urocanic acid (UCA), an epidermal chromophore present in the stratum corneum as *trans*-UCA, absorbs UV radiation and isomerizes to *cis*-UCA in a wavelength-dependent manner. Studies have shown the contribution of UVA radiation in the production of *cis*-UCA, which has been implicated as an important initiator of the immunosuppressive response to UV exposure. There has been a heightened interest in defining the function of UCA in human epidermis, particularly with respect to its utility as a model for the assessment of skin photodamage due to UV exposure. Recently, an investigation to further explore the nature and the origin of the wavelength dependent photoreactivity of *trans*-urocanic acid (*trans*-UCA) was conducted by Hanson, K.M. and J.D. Simon (*Urocanic Acid and Skin Photodamage, J. Cosmetic Science. 50:119-120, 1999*). The authors discuss how *trans*-UCA exhibits unique photochemical behavior, dependent on wavelength specific radiation, as UCA undergoes three specific wavelength-dependent transitions across the entire UV spectrum. From these data, the authors postulate on the role that *trans*-UCA may play in both photoimmunosuppression and photoaging of the skin.

In a different approach, the L'ORÉAL Applied Research and Development Group has utilized the photoisomerization of urocanic acid as a model to assess the protective efficacy of sunscreen products (Moyal D, and C. Mazilier *UVA Protection Efficacy: Urocanic Acid Photoisomerization in Human Skin. Poster 429. 57<sup>th</sup> Annual Meeting American Academy of Dermatology. New Orleans. March 19-24, 1999*) (refer to Appendix II). Quantification of the amount of UCA in irradiated and nonirradiated skin was used for comparative product efficacy as the amount of *cis*-UCA present in unexposed epidermis is usually very low (< 10%).

In support of our position concerning the need to quantify UVA protection efficacy of sunscreens, an investigation was undertaken to assess the UVA protection efficacy of the two sunscreen products 4-A and 4-B utilizing UCA photoisomerization. In this study, subjects were divided into two groups, with one group receiving full spectrum solar simulated radiation while the other group received only UVA radiation. Three test sites were delineated on the back of each subject: two "treated" sites (each site treated with either product 4-A or with product 4-B\*) and one untreated site (control). The amount of *cis*-UCA present in the epidermis both before and after exposure to UV radiation was quantified following six successive tape strippings of the test sites.

The results showed significantly higher levels of *cis*-UCA (> 50%) found even at the lowest exposure levels (0.125 MED or 10 J/cm<sup>2</sup>) at the control sites; however on the treated areas, significantly lower levels of *cis*-UCA were noted. Additionally,

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\* As presented earlier, Product 4-A is code number 408312, Product 4-B is code number 408320.

statistically significant differences ( $p < 0.05$ ) were found between products 4-A and 4-B in both exposure groups, with product 4-A showing a much higher efficiency in preventing *cis*-UCA formation. These results would also appear to support the use of urocanic acid as another biological marker for assessing the photoprotective properties of sunscreens.

In conclusion, utilizing this biological model, we have shown, but yet again, the different *in vivo* protection efficacies of the two sunscreen products (4-A and 4-B) with similar SPF and critical wavelength values and each qualifying for 'broad spectrum' labeling. The different UVA-PF values for these products, as determined by the PPD method, correspond to the differences shown herein utilizing *trans-cis* isomerization of urocanic acid.

### **Photoimmunosuppression *in vivo*: The Comparative Immunoprotective Efficacy of Two 'Broad Spectrum' Sunscreens - I**

This L'ORÉAL Advanced Life Sciences Research Group has also performed other studies to assess the effects of UV radiation on immunosuppression. One such study entitled "*Immunosuppression Induced by Chronic Ultraviolet Irradiation in Humans and its Prevention by Sunscreens*" (Moyal, D., *Eur J Dermatol*, 1998) examined the effect of UV exposure on the delayed-type hypersensitivity skin response (DTH) in humans and the corresponding efficacy of sunscreens in preventing these biological responses.

In the first part of this investigation, the effect of UVR exposure on human skin, i.e., UVB + UVA radiation or UVA radiation alone on the DTH skin response without sunscreen protection was studied. Subjects were divided into four groups: control (no UV exposure), full spectrum solar simulated UV, UVA I and II, and longwave UVA I only. In the second part of this investigation, the efficacy of two sunscreen products was compared in the prevention of immunosuppression induced by UVB + UVA exposure. It is noted that the two sunscreen formulations A and B tested in this study had identical SPF values of 9 but each having a very different absorption spectra (note that the absorption spectra of these products is very similar to the prototype sunscreen products 4-A and 4-B used in the other investigations). Sunscreen formulation A contained two UVB filters (octocrylene, 9% and phenylbenzimidazole sulfonic acid, 2%) as well as two UVA filters (MEXORYL<sup>®</sup>SX (ecamsule) 0.7% and avobenzone 2%). Sunscreen formulation B contained only the two UVB filters mentioned above whose concentrations were adjusted to yield the same SPF value. Despite their identical SPF values, formulations A and B had significantly different UVA-PF values, i.e., 9 and 2 respectively, as determined by the PPD method.

Two groups of subjects were treated with two sunscreen formulations A and B and the DTH skin response was measured using seven different recall antigens (Multitest Kit, Pasteur/Mérieux). Additional details of the investigation are provided in the publication located in Appendix II.

In summary, results from the first part of the study showed that in the absence of sunscreen protection, the response to the DTH tests was significantly reduced by not only exposure to full spectrum solar simulated radiation, but also by exposure to UVA I + UVA II and to longwave UVA I radiation. In all cases the immunosuppression was induced locally and in a distant non-exposed site. In the second part of this study, the results indicated that test sites, which received sunscreen B, formulated to protect mostly against erythema, failed to provide immune protection as compared to sunscreen formulation A, having an absorption spectrum covering the entire UV range with a flat profile. This investigation confirms that UVA exposure significantly contributes to the induction of photoimmunosuppression and that only sunscreen products having a high level of UVA protection, as determined by an *in vivo* method such as the PPD, can significantly prevent modification of the cutaneous immune response induced by full spectrum UV radiation.

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**Photoimmunosuppression *in vivo*: The Comparative Immunoprotective Efficacy of Two 'Broad Spectrum' Sunscreens - II**

The L'ORÉAL affiliate, Galderma Research & Development, France, in collaboration with the Department of Environmental Dermatology of St Thomas Hospital in London, conducted a subsequent investigation relative to hypersensitivity entitled "*Improved Protection Against Solar-Simulated Radiation - Induced Immunosuppression by a Sunscreen with Enhanced Ultraviolet A Protection*" (Fourtanier, A., et al. *J. of Invest. Dermatol*, 114:620-627, 2000) (Appendix II).

As in the previous investigations cited in this section, this publication stresses the importance of photoimmunological responses. Ultraviolet radiation-induced immunosuppression is thought to play a part in skin cancer. Several studies have indicated that sunscreens that are designed to protect against erythema failed to give comparable protection against UVR-induced immunosuppression. One possible explanation for this discrepancy is related to an inadequate level of UVA protection.

In order to answer some of the questions raised by earlier studies, the authors compared the immunoprotective efficacy of two broad spectrum sunscreen products,\* designated as 5-A and 5-B, each with similar SPF values (7.1 and 8.2 respectively), but with quite different UVA protection factors (7.8 and 3.1 respectively) as determined by the PPD method.

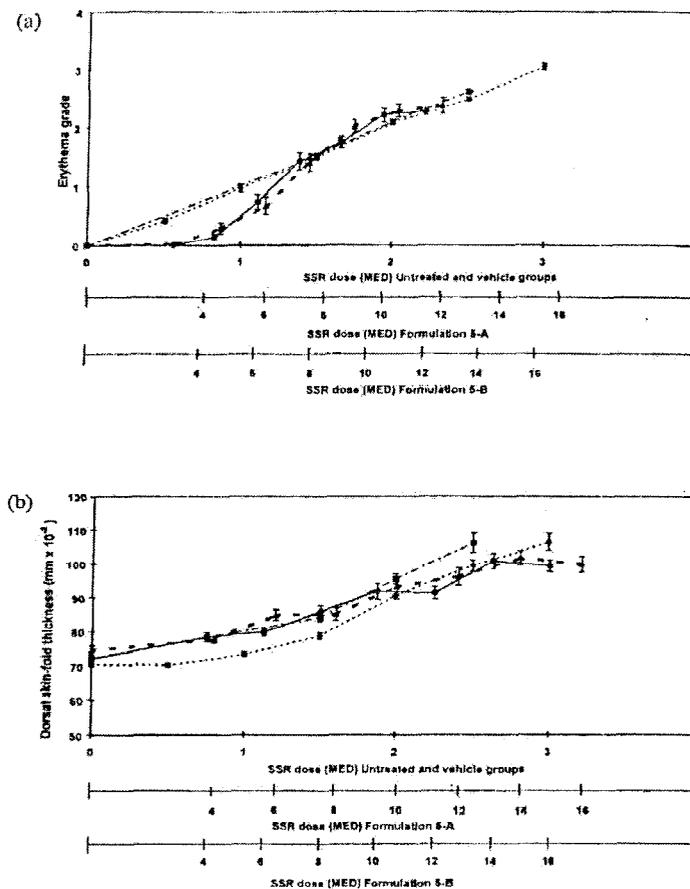
UVR-induced suppression of cell-mediated immunity can be evaluated *in vivo* by measuring the impairment of the contact hypersensitivity response (CHS) to chemical haptens in the mouse and in humans. In this investigation, UVR induced immunosuppression was assessed in hairless mice by the inhibition of the systemic CHS response to dinitrofluorobenzene (DNFB) after a single exposure to solar-simulated radiation (SSR). In addition, SSR response curves for erythema and edema were generated (representing inflammation markers) and these three methods were used to derive protection factors for each endpoint.

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\* In this investigation, product 5-A (containing octocrylene 7% and avobenzone 3%) is identical to product 4-A (408312) mentioned earlier in this document. Product 5-B (containing 10% octocrylene and avobenzone 0.5%) however is different from product 4-B used in most of the other investigations reported herein.

The results obtained using the three different techniques for determining immunoprotective factors show:

- that two broad-spectrum sunscreens (SPF 7-8) afford comparable protection from erythema and edema over the SSR dose range tested (4-16 MED); [Fig. 11, (a) and (b)]



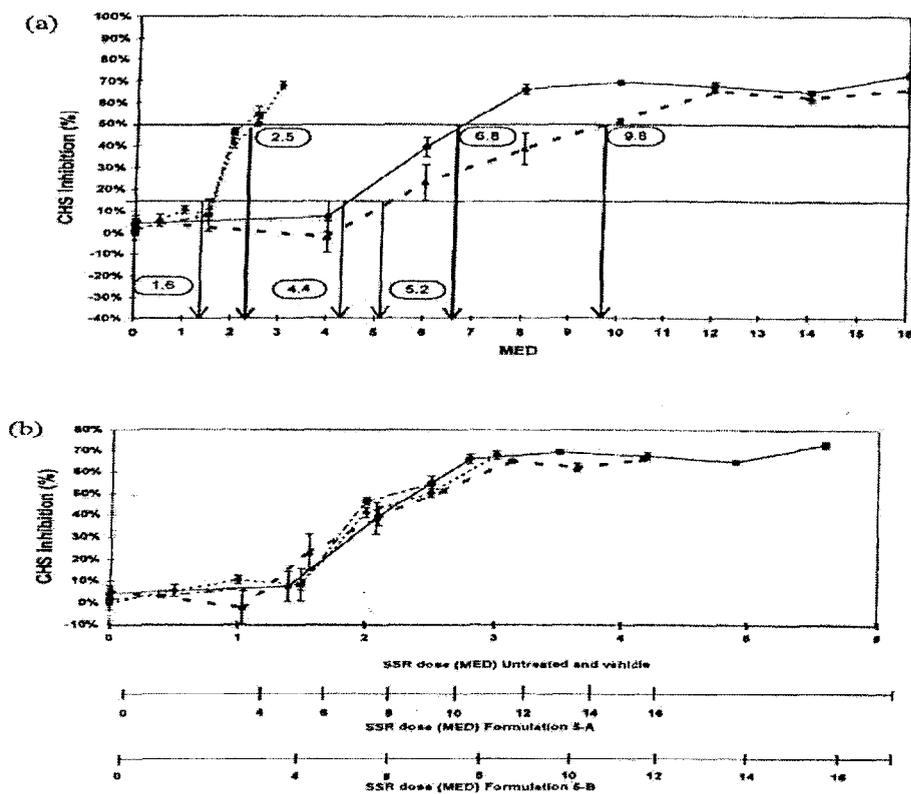
**Figure 11: Significant ( $p < 0.05$ ) SSR dose-dependent increases in the two inflammation endpoints, i.e. erythema and dorsal skin-fold thickness.**

- (a) For erythema, the dose-response curves in the different experimental groups are superimposed when Erythema Protection Factor is 6.9 for sunscreen 5-A and 7.2 for sunscreen 5-B.
- (b) For edema, the dose-response curves are superimposed when the Edema Protection Factor is 5.0 for sunscreen 5-A and 5.3 for sunscreen 5-B.

The vehicle had no effect on erythema or edema when compared with the untreated groups. No pretreatment (...), vehicle (-.-.-), sunscreen 5-A (- - -), and sunscreen 5-B(-). Data are given as mean  $\pm$  SEM.

Additionally it was shown that:

- both sunscreens protected against suppression of contact hypersensitivity but the product with the higher UVA-PF (product 5-A) showed significantly greater protection [Figure 12 (a)]
- the Immune Protection Factors (IPF) are lower than SPF and that higher IPF is obtained with the sunscreen having the higher UVA-PF [Figure 12 (b)]



**Figure 12: The sunscreen with the higher UVA-PF affords better immune protection as determined by the *in vivo* PPD method.**

- (a) The immune protection afforded by product 5-A was significantly higher ( $p < 0.01$ ) than obtained with product 5-B. The Immune Protection Factor (IPF) and IPF<sub>50</sub> were obtained with the estimated Minimal Immunosuppressive dose (MISD) or IPF<sub>50</sub>.
- (b) The dose-response curves for the inhibition of contact hypersensitivity response (CHS) in the different experimental groups with and without sunscreens are superimposed when IPF is 3.9 for sunscreen 5-A and 2.9 for sunscreen 5-B.

SSR dose-response for inhibition of CHS are shown: no pretreatment (....) vehicle (-.-.-.-), sunscreen 5-A (- - -) and sunscreen 5-B (-.-). Data are given as mean  $\pm$  SEM.

In conclusion, these data demonstrate that all three different methods of assessing IPF (erythema, edema and CHS) yielded very comparable results. They provide indirect evidence that UVA radiation plays a part of unknown biologic significance in SSR-induced immunosuppression as the product with the higher UVA-PF provides significantly greater protection.

These data also suggest that the level of immunosuppression afforded by sunscreen products labeled '*broad spectrum*' cannot necessarily be predicted by SPF or by the level of protection from edema. This concept is extremely important in the context of sunscreen protection, especially when effects, such as suppression of CHS in human skin, are seen after single suberythral SSR exposures (Kelly D.A. et al., 2000).

### **Photoimmunosuppression: Summary of Key External Investigations**

Presented below is a selection of complementary investigations implicating UVA in immune suppression, supporting the finding of L'ORÉAL Research presented in this document.

- Under some experimental conditions UVA decreases the number of Langerhans cells in the skin (Alcaly, J. and M. Kripke, 1989; Lavker, R., et al. 1995; Grabbe, J., et al. 1996; Levee, G.J., et al. 1997) and their antigen presenting function (Clement-Lacroix, P., et al. 1996).
- In humans, UVA radiation can suppress natural killer cell function (Hersey, P., et al. 1988; Hersey, P., et al. 1991).
- In humans, UVA II exposure was shown to induce the appearance of a novel population of dendritic antigen presenting cells and increases the number of macrophages (LeVee, G.J., et al. 1997; Hersey, P., et al. 1991).
- UVA has been shown to up-regulate mRNA and protein expression of various cytokines like interleukin: IL1 $\alpha$ , IL6, IL8 and TNF $\alpha$  (Morita, A., et al. 1997).
- UVA has been shown to induce *trans* to *cis* isomerization of urocanic acid, a chromophore involved in the photoimmunosuppression response (Webber, L.J., et al. 1997).
- In animals and in humans, the delayed type hypersensitivity (DTH) and the contact hypersensitivity (CHS) reactions to chemical allergens, infectious agents or viruses are suppressed by acute or repeated exposures to UVA (Bestak, R. and G.H. Halliday 1996; Damian, D.L., et al. 1997; LeVee, G.J., et al. 1997; Nghiem, D., et al. 1999; Iwai, I., et al. 1999).

While several studies have looked at sunscreen efficacy against the different endpoints of immune suppression, only a select few have evaluated broad spectrum sunscreen products (Bestak, R., et al. 1995; Damian, D.L., et al. 1997; Guéniche, A., et al. 1997; Moyal, D., et al. 1998; Gil, E.M. and T.H. Kim, 2000). In all cases, sunscreen products which absorb UVA were more effective than products that provide only UVB protection.

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**PHOTOIMMUNOSUPPRESSION: Conclusion**

The investigations presented in this section utilized two different models to evaluate the immune response induced by UV exposure and the comparative protective properties of 'broad spectrum' sunscreen products having very different *in vivo* UVA-PF values. Assessments of product performance against UVA induced immunosuppression by the *in vivo* contact hypersensitivity response (CHS) to chemical haptens and *trans-cis* photoisomerization of urocanic acid, have shown significant differences in the protection levels afforded by each product, consistent with the product's UVA-PF value as determined by the *in vivo* PPD method. In this series of investigations, it was shown once again, that a product's protective properties against mainly UVB radiation as determined by the SPF value taken together with a product's critical wavelength value, provides insufficient information to accurately evaluate the product's protective properties against UVA radiation.

### III. PHOTOAGING

#### **Photoaging *in vitro*: The Evaluation of Photoprotection on Reconstructed Skin**

Clinical studies of the photoaging process observed after chronic UVR exposure have demonstrated the occurrence of cutaneous modifications such as hyperpigmentation, sallowness, wrinkling, etc. One of these symptoms is solar elastosis, an accumulation of abnormal elastic tissue in the dermis. These changes are attributed to UVA radiation since it penetrates deeper in the skin as compared to UVB radiation. Thus, the development of *in vitro* reconstructed "full" skin models (epidermis plus dermis) has enabled the L'ORÉAL Research Advanced Life Sciences Research Group to identify and study biological markers correlated with photoaging.

The most relevant biological markers can be distinguished with regards to the specific UV wavelengths applied to the reconstructed skin. UVB exposure induces mainly epidermal damage in the DNA such as pyrimidine dimers and the induction of sunburn cells (Bernerd, F. and D. Asselineau, *Dev. Biol.* 183:123-138, 1997) while UVA exposure induces damage in the dermis resulting in the death of fibroblasts by apoptosis 48 hours after exposure (Bernerd, F. and D. Asselineau, *Cell Death and Differentiation* 5:792-802, 1998).

These biological endpoints are currently used by L'ORÉAL Research for the assessment of the photoprotection efficiency of sunscreen formulations. In particular, using these markers, an investigation conducted by F. Bernerd, et al., has shown a difference in the photoprotective properties between two prototype sunscreen formulations: one containing a predominantly UVB filter (2-ethylhexyl-*p*-methoxycinnamate) and a second formulation composed of a broad spectrum UVA filter, i.e., Mexoryl®SX (ecamsule). This investigation has been recently published and summarized below (Bernerd, F., Vioux C., and D. Asselineau, *Photochem. Photobiol.* 2000) (Appendix III).

The evaluation of photoprotection on skin reconstructed *in vitro* demonstrated good efficacy for both formulations in preventing UVB-induced damage, as shown by sunburn cell counts and pyrimidine dimer immunostaining. By contrast, only the formulation containing Mexoryl®SX was able to efficiently prevent UVA-specific damage such as dermal fibroblast disappearance. This investigation further supported the fact that 'full skin', reconstructed *in vitro*, is a reliable system to evaluate the photoprotection provided by different sunscreen products against specific UVB and UVA biological damage.

A subsequent investigation evaluated the two 'broad spectrum' sunscreens, products 4-A and 4-B utilized throughout the series of investigations presented in this document, (same SPF values and  $\lambda_c \geq 370$  but different UVA-PF) under full spectrum solar simulated light and UVA only, exposures. The results obtained show that these two products can be distinguished with regard to their respective ability to protect against dermal damage under UVA irradiation as well as SSR. Product 4-A, providing higher protection in the UVA range, was shown to protect more efficiently against the dermal damage characteristic of photoaging than product 4-B, having a lower protection efficacy in the

UVA range as determined by the PPD method. These results confirm, yet again, the inability of the Critical Wavelength Method to effectively evaluate the UVA protective properties of a sunscreen product and underscore the need for the *in vivo* determination of a sunscreen product's UVA protection factor. These results will be submitted for publication in the coming weeks.

**Photoaging *in vivo*: An Evaluation of a 'Broad Spectrum' Daily-Use Product**

L'ORÉAL Advanced Life Sciences Research Group conducted another investigation to evaluate in humans, the protection afforded by a daily use cosmetic product containing a photostable combination of UVA and UVB filters. This study entitled, "A *full-UV Spectrum Absorbing Daily Use Cream Protects Human Skin Against Biological Changes Occurring in Photoaging*" was recently published (Seité, et al., *Photodermatol. Photoimmunol. Photomed* 18:147-155, 2000) and is provided in Appendix III

A daily-use cosmetic product formulated with sunscreen protection was evaluated for its photoprotective efficacy against solar-UV-induced skin damage. The product contained a photostable combination of octocrylene, avobenzone and Mexoryl®SX with a SPF value of 8 and UVA-PF value of 7.4 as determined by the *in vivo* PPD method. Subjects received one minimal erythema dose (MED) of solar simulated radiation per exposure for a six-week period (5 irradiations per week). A comparison of irradiated and nonirradiated skin was made for endpoints indicative of photodamage including: erythema, pigmentation, skin hydration, skin microtopography, histology and immunochemistry, and collagen and metalloproteinase (MMP) mRNA levels.

Results showed significant changes in irradiated, unprotected skin sites including melanization and changes in the skin hydration and microtopography. The epidermis revealed a significant increase in stratum corneum and stratum granulosum thickness. In the dermis, an enhanced expression of tenascin and a reduced expression of type I procollagen were seen just below the dermal and epidermal junction. Types I and II collagen mRNA were slightly increased and a significant enhancement of MMP-2 mRNA level was observed. By comparison, the 'broad spectrum' product was shown to prevent all of these biological changes thereby demonstrating its efficacy against solar-UV-induced skin damage.

Clearly this study shows the importance of sunscreen products that offer true 'broad spectrum' protection which can only be measured accurately, using *in vivo* methods such as the PPD.

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## Photoaging: Summary of Key External Investigations

Photoaging is a concern to many consumers throughout the world as evidenced by the million dollar sales of daily use cosmetics products containing sunscreens. The selection of key publications given below clearly implicate UVA in this role and as additional investigations are needed to fully elucidate this phenomenon, the use of human reconstructed skin *in vitro* can be a suitable model for sunscreen product evaluations.

- UV exposure of skin is known to lead to short term responses as well as long term effects such as UV-induced skin cancer and photoaging, affecting both the epidermis and the dermis. Recent publications reported that UVA radiation can produce various biological damages such as DNA lesions (Berg, R. J., et al. 1995; Freeman, S. E., et al. 1987), and mutagenic lesions (Drobetsky, E. A., et al. 1995; Robert, C., et al. 1996; Sage, E., et al. 1996), as well as skin tumors in animal models (Van Weelden, H., et al. 1988). The role of UVA in the photoaging process has been suspected for several years because of its penetration properties (Gilchrest, B. A. 1989), and the fact that photoaged skin revealed major changes in the dermis including degradation of the connective tissue, decreases in collagen content and accumulation of degenerative elastic fibers i.e. 'solar or actinic elastosis'.
- Studies on hairless mice chronically exposed to UV radiation clearly showed the induction of dermal actinic damages not only after UVB irradiation but also after UVA exposures (Fourtanier, A., et al. 1992; Kligman, L. 1995; Kligman, L., et al. 1985; Schwartz, E. 1988). Indirect evidences of the UVA contribution to the photoaging process arose from studies on cell cultures irradiated with UVA. UVA radiation has been shown to generate oxidative species (Tyrrell, R. M. and Keyse, S. M. 1990), related to several indirect effects such as the production of extracellular degrading enzymes (stromelysin 1 and interstitial collagenase) (Petersen, M., et al. 1995; Sawamura, D., et al. 1996; Scharffetter, K., et al. 1991), which are thought to play a crucial role in photoaging (Fisher, G. J., et al. 1996).
- The identification of early cellular markers of the UVA effects on skin may provide insights into the long-term process of photoaging. Recent studies in human volunteers revealed that several days of UVA exposure induced slight dermal changes such as an increase in lysosome deposition on elastic fibers (Lavker, R. M., et al. 1995a; Lavker, R. M., et al. 1995b; Lowe, N. J., et al. 1995).
- In the photoaged hairless mouse model chronically exposed to UV, comparison of different sunscreen products showed that a better efficiency was obtained when both the UVB and UVA portions of the spectrum were covered (Fourtanier, A., et al. 1992; Kligman L. and Zheng P. 1994; Kligman L., et al. 1996). Studies on human volunteers also revealed similar results with regard to early dermal damages obtained after several UVA exposures as reported earlier (Seite, S., et al. 1998).

Despite the abundance of research in the area of photoaging, additional studies are required to more fully assess the role of sunscreens in preventing this type of skin

damage. However such research is limited in that classical animal models raise several ethical issues and *in vitro* classical cell cultures do not adequately reflect physiological conditions and therefore cannot account for the dynamics of the interaction of UV through the skin.

- Human skin reconstructed *in vitro* can therefore be a useful tool. In this three-dimensional skin system including a dermal equivalent (human fibroblasts in a collagen gel matrix), fibroblasts have physiological properties closer to *in vivo* conditions compared to classical cultures, and a full thickness epidermis can be reconstructed (Asselineau, D., et al. 1985).
- The organotypic models provided a means for the assessment of the biological effects induced by UV light (Harriger, M. D. and Hull B. E. 1994; Haake, A. R. and Polakowska, R. R. 1995; Bernerd, F. and Asselineau, D. 1997; Bernerd F. and Asselineau, D. 1998). UVB or UVA irradiation of human skin reconstructed *in vitro* induced wavelength and tissue specific damages (Bernerd, F. and Asselineau, D. 1997; Bernerd F. and Asselineau, D., 1998). Epidermal keratinocytes were preferentially targeted by UVB, while UVA induced major alterations in the dermal compartment (fibroblasts alterations and production of interstitial collagenase), which have been related to the photoaging process, and which illustrate the assessment of the penetration properties of UVA radiation.
- Utilizing such three-dimensional models, the *in vitro* evaluation of the photoprotective properties of sunscreens becomes feasible. However, to date, only a few studies have been reported with respect to sunscreen evaluation. Some were performed on human reconstructed epidermis, therefore not addressing dermal damage and other studies focused on general parameters related to cytotoxicity (Reece, B. T., et al. 1992; Augustin, C., et al. 1997).

As of today, it is only the work of L'ORÉAL Research (Bernerd F., et al. 2000) reported above that has evaluated the differential efficiency of two sunscreen products having the same SPF to prevent dermal damages induced by UVA.

## **PHOTOAGING: Conclusion**

There is overwhelming evidence in the published literature that exposure of human skin to UV radiation leads to the development of cutaneous photoaging.

Our *in vitro* investigations utilizing reconstructed skin compared the photoprotective efficacy of the sunscreen products providing 'broad spectrum' protection. It was shown that:

- sunscreen products can effectively protect against UVB-induced damage for all the different products compared;
- only sunscreen products providing complete protection throughout the UV spectrum can effectively prevent mainly UVA damage such as dermal fibroblast disappearance;
- a sunscreen product's protection efficacy against UVA-induced damage can be correlated to the product's UVA-PF value as determined by an *in vivo* method such as PPD.

Utilizing the various biological endpoints indicative of cutaneous photoaging, sunscreen products 4-A and 4-B were compared. Results were similar to those obtained with biological markers of photogenotoxicity and photoimmunosuppression. Product 4-A, having a higher *in vivo* UVA-PF factor was shown to protect more efficiently against the dermal damage characteristic of photoaging than was product 4-B, despite both products having similar SPF and critical wavelength values. Once again this highlights the inadequacy of the Critical Wavelength Method, even when considered together with *in vivo* SPF data, to adequately predict a sunscreen product's true protection against UVA-induced damage.

## PART 1: OVERALL CONCLUSIONS

The biological endpoints considered, i.e., photogenotoxicity, photoimmunosuppression and photoaging, are of paramount importance in evaluating the impact of UVA radiation on the skin and have been particularly useful in comparing the efficacy of two 'broad spectrum' sunscreen products. Throughout the investigations of L'ORÉAL Research, products 4-A and 4-B, were utilized (unless otherwise noted), to demonstrate the inadequacy of the Critical Wavelength Method to discriminate between the protection efficacies of these products against the various biological markers considered. These products were formulated to qualify for 'broad spectrum' labeling as per the Critical Wavelength Method, have nearly identical SPF and critical wavelength values, but have very different *in vivo* UVA-PF values, as determined by the PPD method.

Despite the contention by Procter & Gamble<sup>9</sup> that "...presently there is no biologically relevant marker for evaluating longwave UV protection *in vivo*" the data obtained in the investigations conducted by L'ORÉAL Research have emphasized the ability to use several different biological markers to differentiate the protective properties of sunscreen products. Utilizing the various markers for genotoxicity, photoimmunosuppression and photoaging, we were able to demonstrate repeatedly, the inferiority of product 4-B with regards to its UVA protective properties as compared to product 4-A, despite both products having similar SPF and critical wavelength values as follows:

- Utilizing single cell gel electrophoresis, fibroblast cell culture protected with product 4-B was more susceptible to DNA damage as evidenced by distinct apoptotic comet formations (as compared to a control) and as compared to Product 4-A; product 4-A showed virtually no comet formation for keratinocyte cell cultures and significantly reduced comet formation for the fibroblast cells.
- Utilizing the comet assay with melanocyte cell cultures, it was shown using a quantitative measure (mean tail moment) that product "A" (4-B) does not abrogate the induction of DNA breaks as evidenced by distinct comet formations following full spectrum UV exposure; comet formation was significantly diminished in melanocyte cultures protected with product "B" (4-A), thus demonstrating the superiority of product "B" (4-A) in providing more UVA protection.
- P53 expression was used as a marker for assessing genotoxic stress. A significantly lower level of p-53 positive cells was found in areas protected with product 4-A as compared to product 4-B, again demonstrating the superiority of product 4-A in providing more UVA protection and consistent with UVA-PF values obtained by the *in vivo* PPD method.

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<sup>9</sup> The Procter & Gamble Company submission of May 2, 2000 to Docket 78N-0038, Executive Summary page 2

- *Trans-cis* photoisomerization of urocanic acid in the skin showed statistically significant differences between products 4-A and 4-B, with product 4-A demonstrating a much higher efficiency in preventing *cis*-UCA formation. UVA exposure of skin protected with product 4-B increased the production of *cis*-UCA which has been implicated as an important initiator of the immunosuppressive response to UV exposure.
- The comparative immunoprotective efficacy of two '*broad spectrum*' sunscreens (having absorption spectra very similar to prototype products 4-A and 4-B) have shown that a product formulated to protect mostly against erythema (due to the absence of longwave UVA I filters in the formulation and a corresponding UVA-PF value of 2) failed to provide immune protection as compared to the product formulated with proportional protection across the entire UV spectrum (UVA-PF = 9). Exposure to full spectrum UV, UVA I + UVA II and to only UVA I only confirmed that UVA exposure significantly contributes to the induction of photoimmunosuppression and that only products having a high level of UVA protection can prevent modification of the cutaneous immune response induced by full spectrum UV radiation.
- UVR-induced immunosuppression was assessed in hairless mice by inhibition of the systemic CHS response to DNFB after a single exposure to full spectrum UV radiation. Here two '*broad spectrum*' sunscreens afforded comparable protection from erythema and edema as expected, due to having very similar SPF values but displaying significantly different protection against suppression of contact hypersensitivity. The differences seen in protection against the three markers (erythema, edema and CHS) were consistent with differences in the *in vivo* UVA-PF values for each product.
- Utilizing human skin reconstructed *in vitro*, the comparative efficacy of product 4-A was shown to provide more effective photoprotection against dermal damage characteristic of photoaging as compared to product 4-B.

In the absence of definitive surrogates for longwave UVA protection, we have shown utilizing all of the above markers that product 4-A, having a higher UVA protection efficacy, as determined by the *in vivo* PPD method provided superior protection over product 4-B. However, using the criteria of UVA protection efficacy of the Critical Wavelength Method, both products would be labeled identically, i.e., '*broad spectrum*' despite huge differences in their respective UVA protective properties. Throughout our investigations, comparative product efficacy against UVA damage was assessed using two sunscreen products each having similar SPF values,  $\lambda_c \geq 370$  nm, but with very different UVA-PF values as determined by the PPD method. In each investigation, significant differences were shown between products with respect to their UVA protection efficacy.

**In summary:**

- **The Critical Wavelength Method, based on an arbitrary non-biological criterion, fails to provide an accurate measure of a sunscreen product's protection efficacy.**
- **Clearly these data demonstrate that the most significant failure of the Critical Wavelength Method is its inherent inability to differentiate UVA protection levels of sunscreen products which are of biological importance.**
- **The absence of absolute biological surrogates for long-term UVA damage fails to provide a sufficient rationale to ignore the compelling evidence presented herein concerning the necessity of quantifying UVA protection levels of sunscreen products.**

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## **PART 2: COMMENTS ON THE VALIDITY OF *IN VIVO* UVA METHODS**

The data presented in Part 1 of this document establish the biological significance of UVA exposure in the context of sunscreen products, particularly the magnitude of protection against such damage as afforded by different sunscreen formulations. In the second part of this submission, we would like to respond to the concerns raised regarding *in vivo* UVA test methods by Procter and Gamble, a proponent of the Critical Wavelength Method, in their letter of May 2, 2000 to this Docket. Furthermore, we will provide new data obtained from the CTFA round-robin study designed to test the reproducibility of two *in vivo* methods, persistent pigment darkening (PPD) and UVA erythema-pigmentation (PFA) and use these data to dispel some of the propositions put forth by Procter and Gamble concerning *in vivo* UVA methods. Most importantly, these data will be used to correct the representation by Procter and Gamble in their cover letter which stated:

*“ ...the Critical Wavelength is independent of SPF yet ensures UVA protection commensurate with SPF so that as the SPF increases, so too must the UVA protection to maintain the same Critical Wavelength.”*

This statement is incorrect because a strict proportionality\* between UVB and UVA protection cannot be assured using the Critical Wavelength Method. Therefore the concerns expressed by the Agency in recent meetings and correspondence with industry, together with those expressed by the American Academy of Dermatology (AAD) simply cannot be addressed by use of the Critical Wavelength Method to assess UVA protection of sunscreen products.

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\* in terms of wavelength covered and magnitude of protection

### **Suitability of *in vivo* UVA Test Methods**

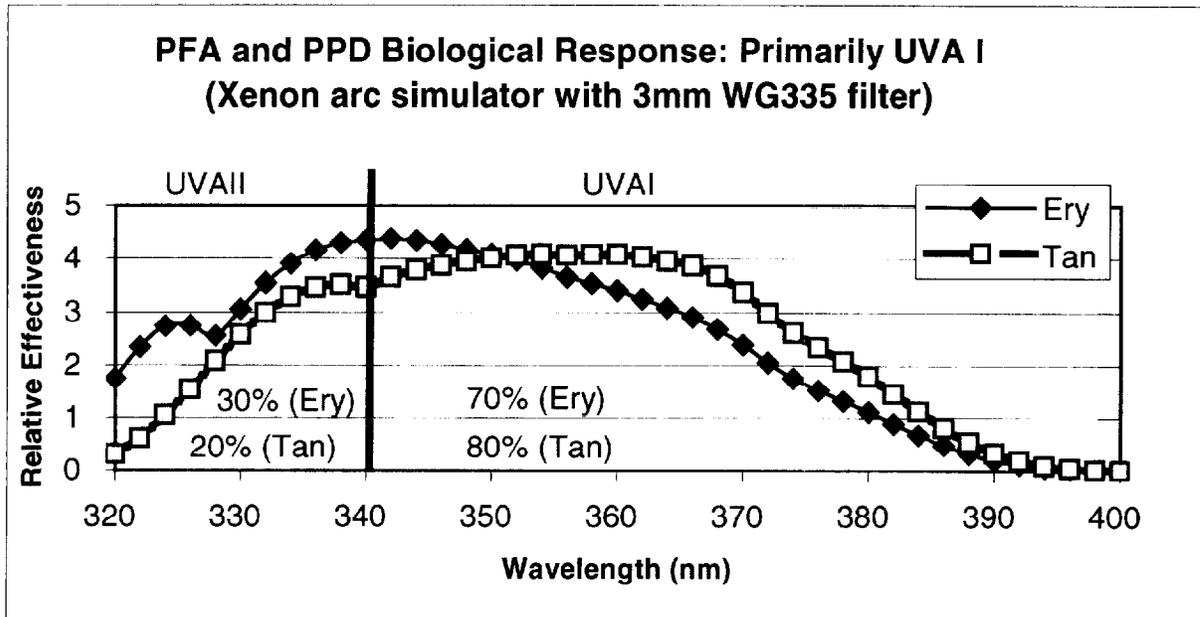
*In vivo* UVA test methods have been extensively criticized, as stated in the following comment of Procter and Gamble (page 8 of their May 2, 2000 letter):

*“ the fundamental limitation of all proposed human studies of UVA photoprotection is the absence of an endpoint measure that is a true surrogate marker for UVA-induced skin damage, especially the multifaceted endpoint carcinogenesis and photoaging. This essential defect undermines the meaning of any ‘protection factor’ derived from the current in vivo tests such as the ‘L’Oréal method (PPD) and the ‘J&J method (PFA)’.”*

This point seems to be a mainstay throughout the criticism offered by Procter and Gamble. While the absence of true and generally accepted biological surrogates for longwave UVA damage is true at this given point in time, the compelling evidence from various L'ORÉAL Research peer-reviewed publications (presented in the first part of this document) utilizing scientifically accepted biological markers for UV damage cannot be ignored. From a public health perspective, the Agency must weigh the risk of ignoring data from investigations that assess the comparative UVA protection properties of two sunscreen formulations utilizing the available **scientifically accepted biological markers for UVA damage** while waiting for the availability of definitive biological surrogate markers. It would seem that the consequences of the latter approach, as suggested by Procter and Gamble by substituting a method that does not measure any magnitude of UVA protection, would have a significant impact on the availability of safe and truly protective sunscreen products for the consumer. This in itself bears the risk of conveying misleading information, since the consumer could believe that a ‘broad-spectrum’ sunscreen offers broad **and effective** protection to all biological damages.

**The data presented in this document, underscore the absolute necessity for *in vivo* quantification of UVA protection of sunscreen products utilizing either of two *in vivo* methods, the PPD method or the PFA method, both of which have biological responses with defined action spectra covering the entire UVA range as shown in Figure 13a.**

Additionally, the same argument concerning the lack of biological endpoints is certainly true with regard to the Critical Wavelength Method. In the development and evaluation of any new drug, *in vivo* tests are essential to obtain data on the drug's specific activity in the target organ. The Critical Wavelength Method, based on a measure of a product's absorbance profile is, in effect, a surrogate marker for a desired *in vivo* effect, i.e., photoprotection. A critical wavelength value, in itself, has no direct correlation to the biological endpoints of photodamage, photogenotoxicity or photoimmunosuppression.



**Figure 13a: The Action Spectra for Erythema (Ery ♦) (CIE) and for Persistent Pigment Darkening (Tan □)**

These action spectra, when cross multiplied with the WG335 3mm filtered xenon arc solar simulator, clearly show that the predominant biological response is due to the UVA I portion of the spectrum (340-400nm). This illustrates that the proposed *in vivo* test methods, i.e., PPD and PFA, do account for longwave UVA damage and do not solely test UVA II and therefore are not "redundant" with SPF test results.

A second incorrect assumption put forth by Procter and Gamble throughout their May 2, 2000 commentary on *in vivo* UVA test methods is the statement (on page 2) that:

*"...the existing methods utilize endpoints that are: 1. Redundant with SPF testing, i.e. erythema/pigmentation, and 2. Oxygen and, by necessity, UV dose-rate dependent..."*

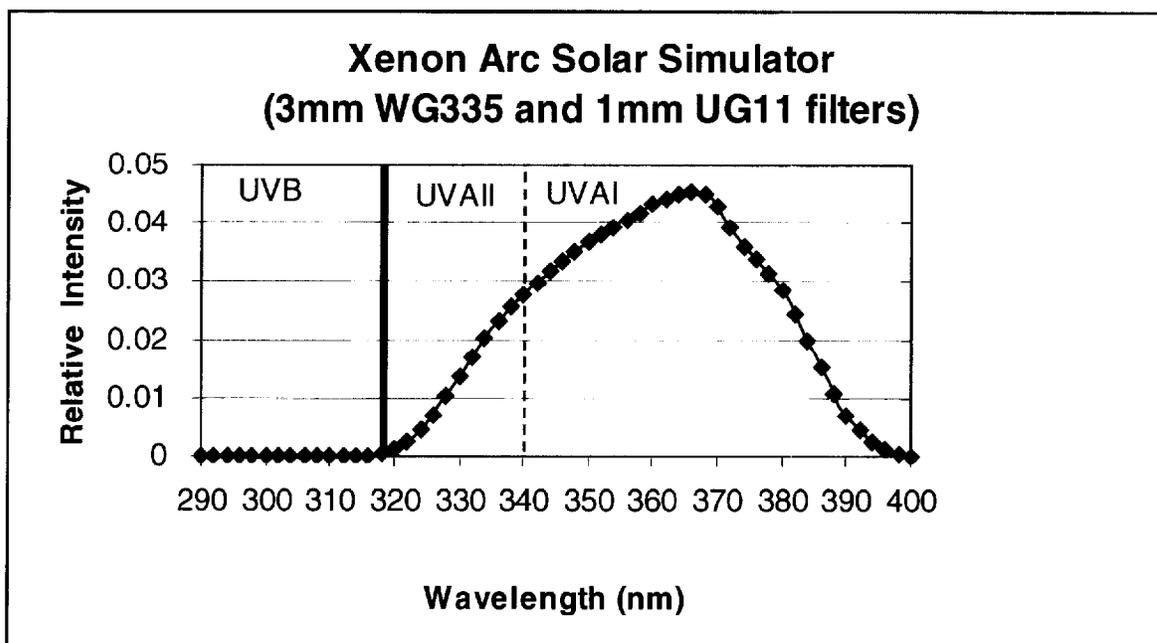
In considering the first point, we have shown throughout Part 1 of this document that the investigations conducted utilizing the two sunscreen products having identical SPF values and very different UVA-PF values as determined by the PPD method, provide drastically different protection against the selected biological markers. In particular, the investigation conducted by Fourtanier, A., et al. entitled *Improved Protection Against Solar-Simulated Radiation - Induced Immunosuppression by a Sunscreen with Enhanced Ultraviolet A Protection* (*J. of Invest. Dermatol.*, 114:620-627, 2000) demonstrated that two sunscreen products having essentially the same SPF values but formulated to provide either predominantly UVB protection or full spectrum UV protection, had considerably different UVA protective properties against three different endpoints of immune

suppression but consistent with the UVA-PF value for each product as determined by the *in vivo* PPD method.

Furthermore, as illustrated in Figure 13a, the action spectra for the pigmentation or erythema response to UVA clearly show that the predominant biological response is due to the UVA I portion of the spectrum (340-400nm). Figure 13b (below) shows that less than 2% of the biological response results from UVB radiation contained in the source.

Collectively, these data illustrate that the proposed *in vivo* test methods, i.e., PPD and PFA:

- DO account for long-wave UVA damage;
- DO NOT solely assess the effects of UVA II radiation;
- are NOT redundant with SPF results.

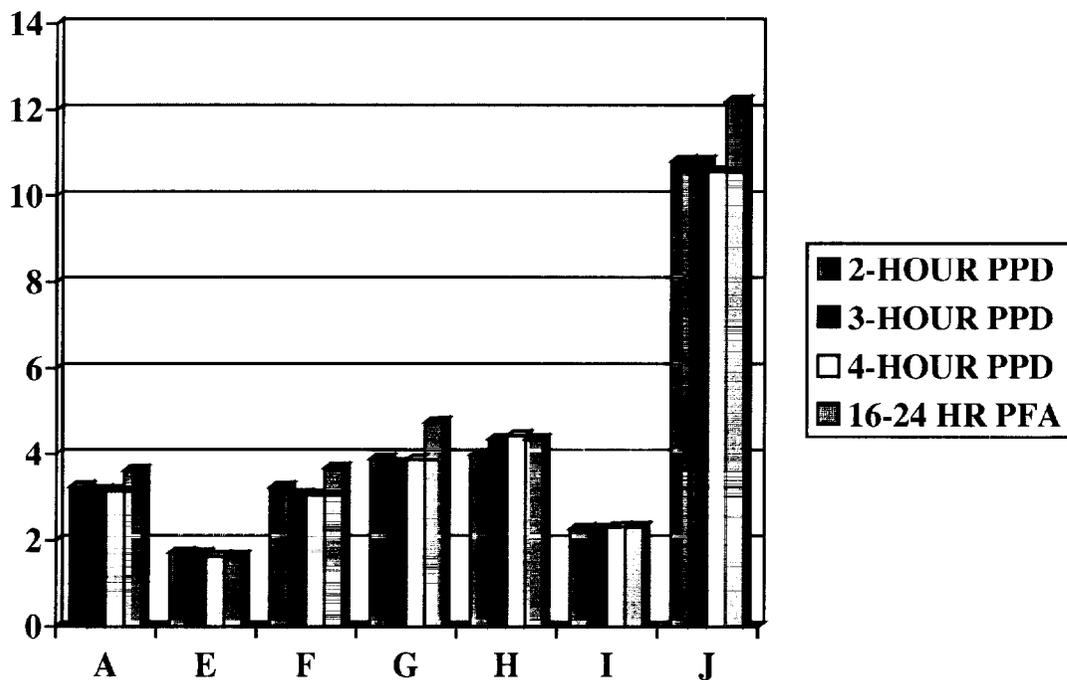


**Figure 13b:** Spectral distribution of the UVA source used in both the PFA and PPD test methods. Less than 2% of the biological response results from the UVB contained in the source.

As for the second point made by Procter and Gamble:

*"...the existing methods utilize endpoints that are...2. Oxygen and, by necessity, UV dose-rate dependent..."*

**This statement is incorrect** for either the PPD or PFA method because the endpoint response is stable (assessed 2 - 24 hours post-irradiation), as shown graphically below in Figure 14, and therefore not susceptible to variations caused by oxygenation of the skin. The stability of this *in vivo* endpoint is further supported by the CTFA round-robin study data, which will be discussed in more detail in the next section. In this study, PPD and PFA measurements were taken at 2, 3, 4, and 16-24 hours after irradiation. This data clearly demonstrates the stability of these methods in assessing UVA protection levels. Furthermore, both methods comply with the reciprocity law and therefore are not dose-rate dependent.



**Figure 14: Comparison of Mean PPD and PFA Values From One Laboratory**

### **CTFA Round-Robin Data**

In 1996, the Critical Wavelength Method was proposed to FDA by industry via the Cosmetics, Toiletries, and Fragrances Association (CTFA) as an *in vitro* UVA test method<sup>10</sup>. Subsequently, in a submission to FDA dated May 15, 1998, L'ORÉAL withdrew its support for the Critical Wavelength Method because of this method's inherent inability to accurately characterize the UVA protection level of sunscreen products. L'ORÉAL stated its preference for *in vivo* methods that would quantify the level of UVA protection. In the context of global harmonization, on March 3, 2000, L'ORÉAL submitted data to support the acceptance of the *in vivo* Persistent Pigment Darkening Method (PPD), a method for assessment of UVA efficacy based on a stable pigmentation response. The PPD method has been selected by the Japan Cosmetic Industry Association (JCIA) for the assessment of UVA protection efficacy of sunscreen products since 1996. It is also important to note that CTFA is currently taking no position concerning UVA test methodology.

The importance of assessing the quantity of UVA protection provided by sunscreen products was recently acknowledged by the Agency in correspondence relating to Citizen Petition 8, docket 78N-0038<sup>11,12</sup>. In that correspondence, the Agency asked Proctor and Gamble for additional UVA protection data beyond Critical Wavelength to support the UVA efficacy of select combinations of sunscreen active ingredients with avobenzone. It was evident from this correspondence that the Agency understood the need to assess not only the breadth of a sunscreen products' absorbance, but also the magnitude of UVA protection and the importance of using an *in vivo* method. The data that the Agency requested was to be based on the *in vivo* erythema-pigmentation (PFA) method (Cole, C., 1994).

As a result of the differences of opinion amongst industry concerning the selection of a UVA test method, the CTFA sponsored a round-robin test utilizing seven prototype sunscreen products manufactured by member companies of its Sunscreen Task Force. An objective of this study was to determine if there was a correlation between *in vivo* (PPD and PFA) and *in vitro* ( $\lambda_c$ ) methods in the assessment of UVA protection efficacies of the sunscreen products.

These products, representing a wide variety of sunscreen formulation vehicles and active ingredients, were used to determine the *in vivo* SPF, PFA, and PPD values in a typical clinical setting using three different independent laboratories. These seven prototype products were also evaluated to determine if they met the sunscreen monograph criterion of absorbing to or above 360 nm. The *in vitro* method used to assess the broadness of absorbance was the method previously submitted to the FDA in RPT 9<sup>10</sup> by the CTFA.

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<sup>10</sup> CTFA/NDMA taskforce report on Critical Wavelength determination for the evaluation of the UVA efficacy of sunscreen products. April 9, 1996. Docket 78N-0038, RPT 9.

<sup>11</sup> Letter 167, Docket 78N-0038 to T.S. Elliott, April 8, 1999.

<sup>12</sup> Letter 169, Docket 78N-0038 to T.S. Elliott, November 2, 1999.

An additional objective of this study was to compare the interchangeability of the PFA and PPD methods for determining UVA protection *in vivo*. The outcome of this comparison would confirm the charge by Proctor and Gamble that<sup>13</sup>:

*“...since introducing this method in the early 90s, the only significant change has been the acknowledgement that the ‘L’Oréal method’ (i.e., PPD) and the ‘J&J method’ (i.e., PFA) are for all practical considerations identical.”*

Two laboratories conducted both the PFA and PPD test procedures utilizing different solar simulator types, i.e., the multiple port and single port, to determine the *in vivo* UVA protection provided by each of the seven prototype formulations. Two laboratories also assessed the broadness of absorbance using the Critical Wavelength method as submitted by the CTFA in 1996. The round-robin data is presented in Table 1 below; the study protocols, prototype sunscreen information, and results are provided in Appendix IV.

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<sup>13</sup> The Proctor and Gamble Company submission of May 2, 2000 to Docket 78N-0038.

**Table 1**

<b>CTFA Round-Robin Data</b>			
<b>Product Code</b>	<b>Labeled SPF *</b>	<b>In vivo PPD*</b>	<b>Critical Wavelength**</b>
J	29	12.44	380
A	15	3.21	361
I	12	2.23	372
G	12	4.27	378
F	9	3.09	361
E	8	1.65	346
H	4	3.78	381

\* - mean of 2 values from 2 different laboratories

\*\* - mean of 2 values from 2 different laboratories, 1/3 SPF pre-irradiation.

In a previous submission to this Docket<sup>14</sup>, L'ORÉAL introduced 14 different products commercially available in the US and in Europe. This original data, along with the UVA-PF values (as determined by the PPD method), is presented in Table 2 below.

**Table 2**

<b>European and US Commercial Products (1997 sun season)</b>				
<b>Product</b>	<b>Code</b>	<b>Labeled SPF</b>	<b>In vivo PPD</b>	<b>Critical Wavelength</b>
Coppertone SPF 4	3D	4	1.6	351
Coppertone SPF 8	3E	8	1.9	356
Coppertone SPF 15	3F	15	3.3	356
Coppertone SPF 30	3G	30	2.9	357
Coppertone SPF 45	3H	45	2.8	356
Ambre Solaire SPF 4	3I	4	2.8	376
Ambre Solaire SPF 15	3J	15	7.2	378
Ambre Solaire SPF 30	3K	30	10.9	379
Ambre Solaire SPF 25	3L	25	9.2	378
Soltan SPF 25	3M	25	6.2	379
Nivea Sun SPF 30	3N	30	3.2	379
408312 SPF 7 (4-A)*	4A	7	7.2	379
408320 SPF 7 (4-B)*	4B	7	2.8	372
Shade UVAGuard SPF 15	4C	15	4.5	377
Avon Age Block SPF 15	4D	15	3.7	378
Le Mirador SPF 15	4E	15	2.8	377

\* - Experimental formulations also presented in Part I of this document.

A plot (SPF vs. Critical Wavelength) of the combined data contained in Tables 1 and 2 is presented as Figure 15.

<sup>14</sup> L'ORÉAL Research /Cosmair Cosmetics Corp. Comment C545, Docket 78N-0038, May 15, 1998.

### Critical Wavelength vs SPF

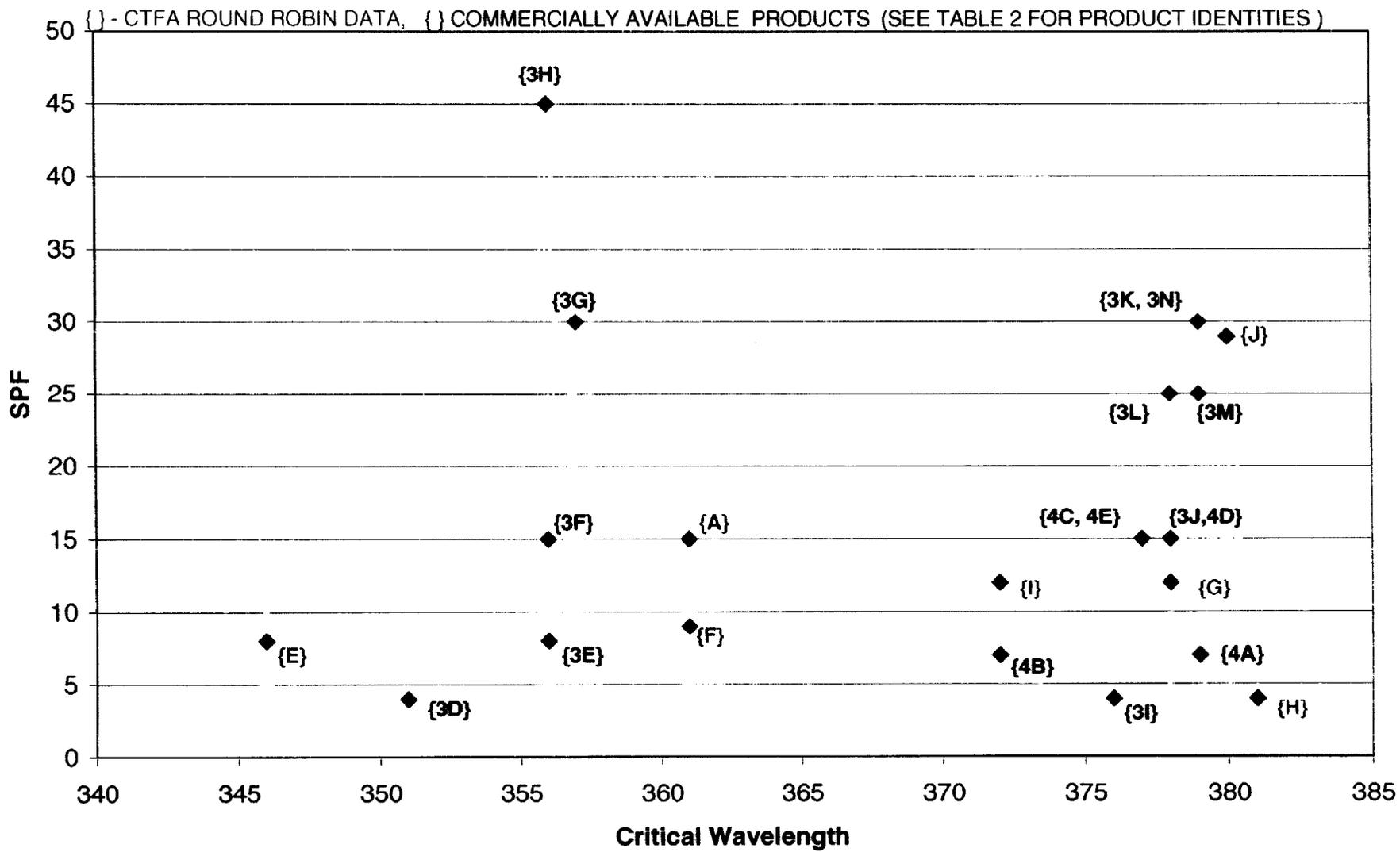


FIGURE 15

It becomes immediately apparent that this data cumulatively, represents a true cross-section of the various types of sunscreen products formulated within the industry. It is also clear that these products represent two distinct populations; those products that absorb over 370 nm, and those products that do not. For those products that do exhibit a  $\lambda_c \geq 370$  nm, there are a number of products whose SPF and  $\lambda_c$  values are nearly identical; specifically products 3J, 4D, 4C, 4E (for SPF 15); 3L, 3M (for SPF 25) and products 3K, 3N (for SPF 30).

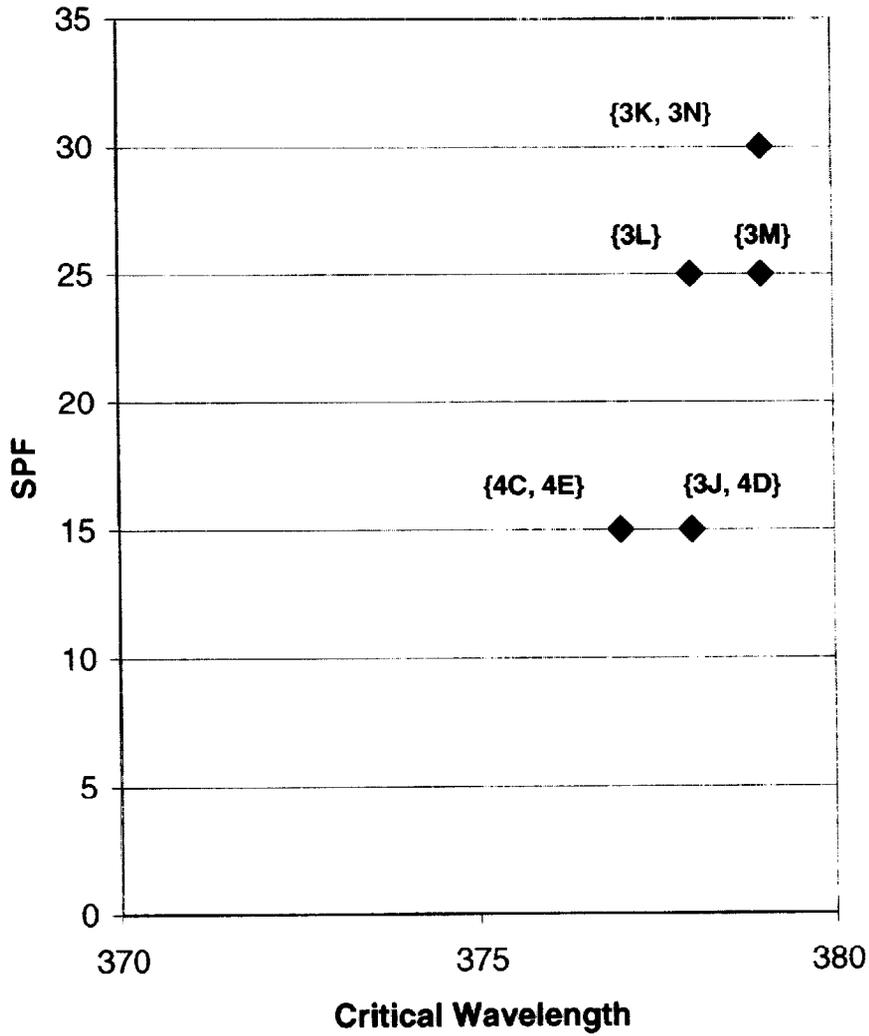
When these data are presented graphically, it is assumed that for any given SPF and  $\lambda_c$ , products that occupy the same point on the graph, for instance in the case of products 3K and 3N, would provide the same level of protection to the consumer. However when an *in vivo* method such as PPD is utilized to assess the UVA protection afforded by these products, the graphic depiction is quite different as shown in Figure 16. For the purposes of clarity Figure 16A displays only products having the same SPF and similar critical wavelength values. However, in Figure 16B, a plot of critical wavelength vs. PPD values shows very different picture of UVA protection for these same products. Here it is shown that previously 'identical' products as characterized by SPF and critical wavelength values, actually provide very different levels of *in vivo* UVA protection.

The SPF and  $\lambda_c$  values for products 3K and 3N are identical: SPF = 30 and  $\lambda_c = 379$ . However the *in vivo* UVA-PF values are 10.9 and 3.2 respectively, demonstrating a **three-fold** difference in the UVA protection levels which is not visible from the *in vitro* data obtained utilizing the Critical Wavelength Method, despite the consideration of the product's respective SPF value. A further comparison of the other products depicted in Figures 16 A & B shows the absence of a correlation between the broadness of absorbance and the magnitude of protection as both the SPF and Critical Wavelength values increase. These data unequivocally disprove the Procter and Gamble contention on page 27 that:

*"A combination of in vivo SPF and critical wavelength provide a complete description of a product's inherent photoprotective characteristics. A sunscreen product's critical wavelength value must always be considered in conjunction with its corresponding in vivo SPF. If two products (A and B) share the same critical wavelength but exhibit differing in vivo SPF values (15 and 30, respectively), then according to the critical wavelength calculation, Product B must have been formulated with significantly more long wavelength UVA protection than Product A (i.e. commensurate with SPF). SPF describes the amplitude of protection (at a given application rate) and critical wavelength provides a measure of the breadth of a product's spectral absorption capability."*

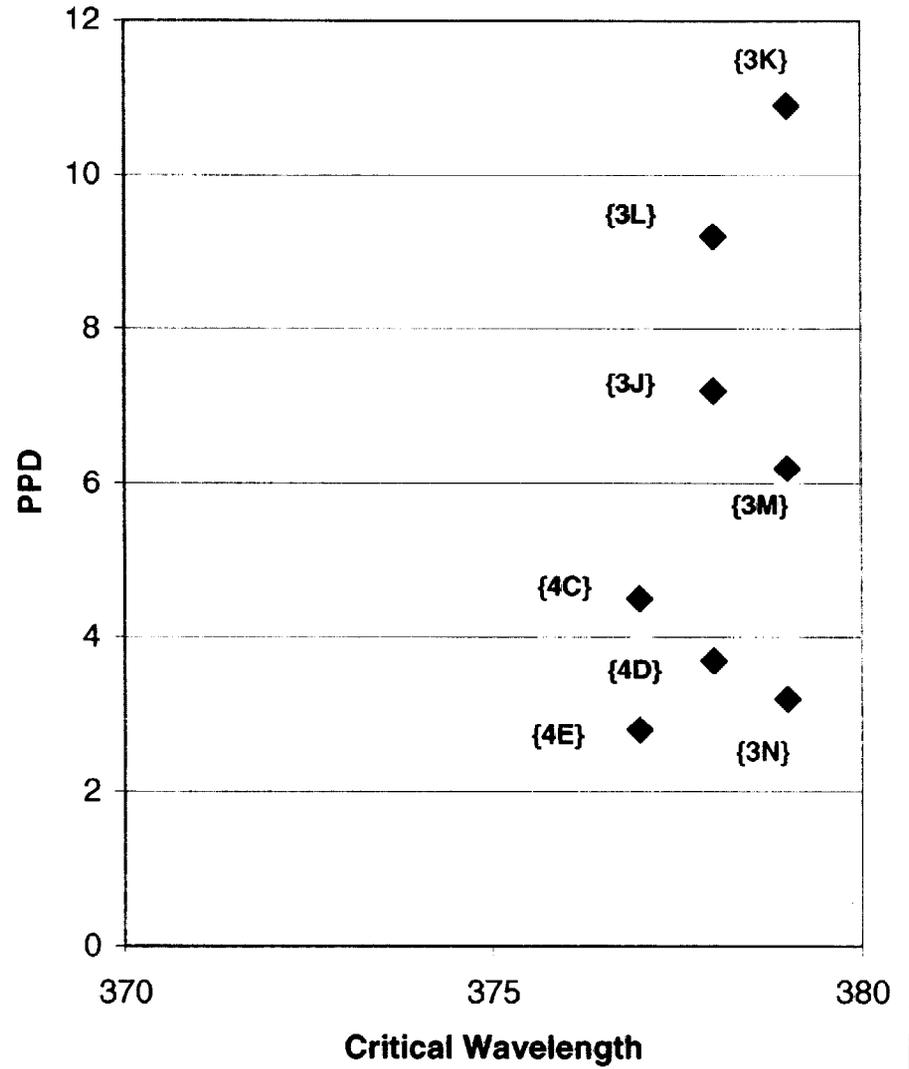
A careful examination of products 3J (*in vivo* SPF = 15) and 3N (*in vivo* SPF = 30) each with virtually identical  $\lambda_c$  values of 378 and 379 respectively, have very different *in vivo* UVA-PF values of 7.2 and 3.2 respectively with the lower UVA-PF value for the product having the higher SPF. These data are in complete contradiction to the example given above by Procter and Gamble and disprove the contention that UVA protection must increase commensurately with increasing SPF. There is no respect for UVA/UVB proportionality shown in this example.

**Critical Wavelength vs. SPF**



**FIGURE 16A**

**Critical Wavelength vs. PPD**



**FIGURE 16B**

— Additionally, this data also appears to contradict Proctor and Gamble's proposition that:

*"...use of erythema and pigmentation is more responsive to short wavelengths of UV and therefore redundant with SPF".*

If the assertion that the SPF and PPD/PFA measurements are "*redundant*" were true, the plots presented in Figure 16 would look identical. The fact that the data presented in these plots are so different, this further supports the L'ORÉAL contention that an *in vivo* assessment of UVA protection is a necessary parameter for accurate and comprehensive photoprotection assessment of a sunscreen product. These data are further proof that *in vivo* UVA-PF methods are not '*redundant*' with a product's SPF determination.

### **UVB/UVA Proportionality**

The importance of proportionality was raised at the October 26, 1999 feedback meeting between CTFA and the Agency. At that meeting, FDA asked industry to comment on the requirement for proportionality between the SPF and UVA protection. The request for information on this point was made again in the FDA letter to the CTFA of March 20, 2000. The importance of the proportionality of UVA to UVB was also addressed by the American Academy of Dermatology (AAD)<sup>15</sup> in their April 26, 2000 press statement on UVA:

*"...the AAD recommends that an increase in the SPF of a sunscreen must be accompanied by a proportional increase in the UVA protection value. These "proportional" values should be determined jointly by the FDA and the industry."*

— To accomplish this task, the UVA protection level should increase proportionally with higher SPF levels, which can be assured through the use of defined ratios of SPF to PFA or PPD. However, it is not possible to predict the level of UVA protection from the SPF alone, or to predict the quantity of UVA protection solely from the broadness of the protection (again, please refer to Figure 16). The lack of correlation between PPD values and critical wavelength values is also apparent when considering the proportionality concept as a basis for formulating products that provide balanced UVA/UVB photoprotection. This is once again shown in Figure 17 from a plot of the ratio of SPF/PPD vs. critical wavelength values for all products tested (CTFA round-robin data and L'ORÉAL data). These data confirm the absence of any correlation between critical wavelength values and *in vivo* UVA-PF values. **Thus the Critical Wavelength Method is an unsuitable *in vitro* surrogate for *in vivo* UVA photoprotection.**

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<sup>15</sup> American Academy of Dermatology, Press Release April 26, 2000: available at "<http://www.add.org/PressReleases/futuresunscreen.html>"

### SPF/PPD Ratio vs. Critical Wavelength

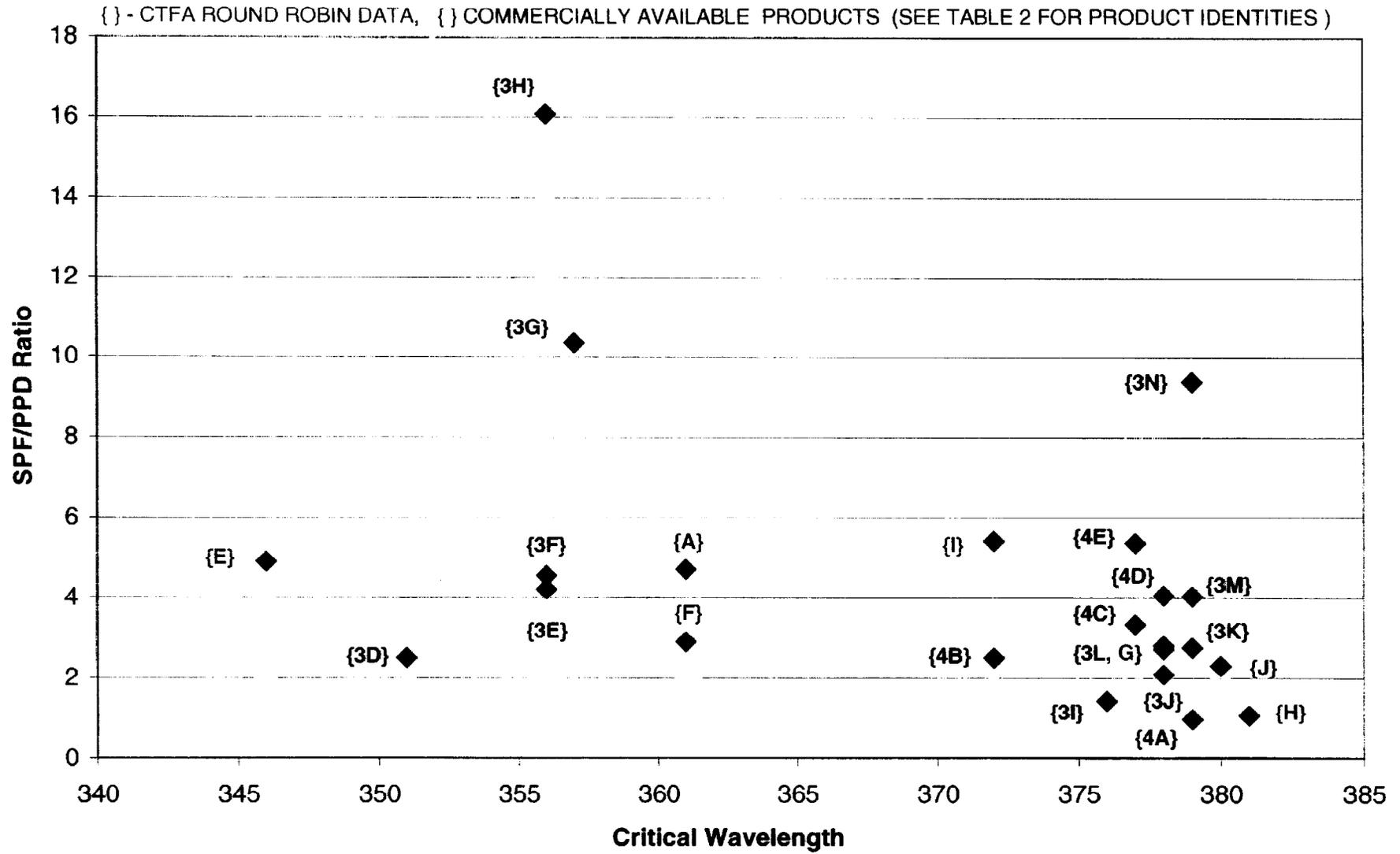


FIGURE 17

### Photo-Instability

Important benefits of an *in vivo* method for assessing UVA protection are its intrinsic ability to account for the biological responses of the skin as well as for product photostability. Procter and Gamble specifically proclaimed the “unique” ability of the Critical Wavelength Method to account for the photo-instability of sunscreen filters during exposure to solar simulated radiation (SSR) as one of its benefits. Procter and Gamble specifically addressed this topic as follows:<sup>16</sup>

*“Lastly, we found that the procedure can uniquely and readily account for photo-instability changes through UV pre-irradiation. Due to known photochemical processes, this accounting for potential photo-instability can only be appropriately and reliably accomplished through full-spectrum UV (290-400 nm) product irradiation which is a unique characteristic of this approach versus procedures that irradiate with UVA only.”*

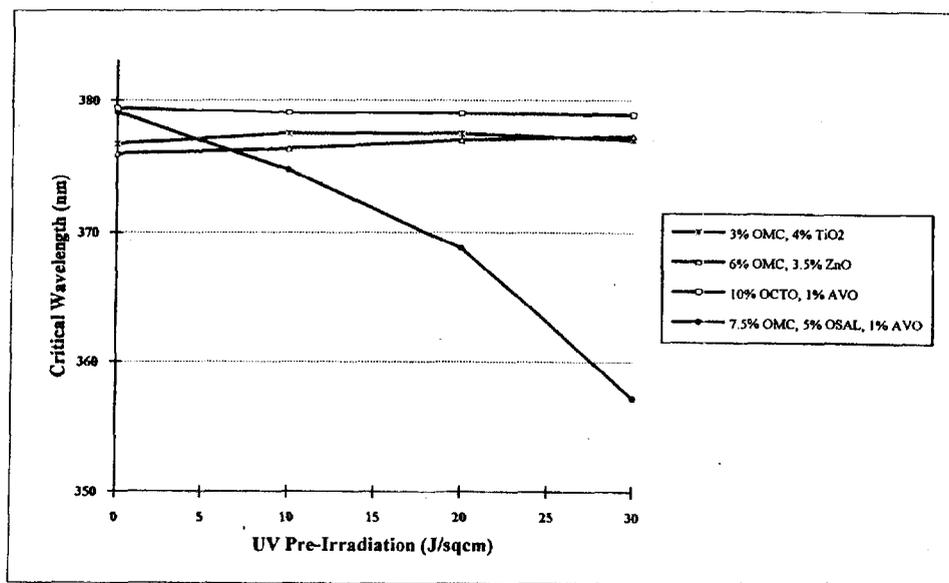
Procter and Gamble illustrated this point graphically with a plot that shows the photostability of three formulations and the photo-instability of one formulation (containing avobenzene without a stabilizer) with increasing doses of UV irradiation (0-30 J/cm<sup>2</sup>). This plot has been reproduced below as Figure 18.

In the CTFA round-robin test, the Critical Wavelength Method was employed in two external testing laboratories for the assessment of the seven prototype sunscreen products. However, results of these assessments did not support the Procter and Gamble finding of the same “unique characteristic of this approach”, i.e., photo-instability. From these data, presented in Table 3, it can be seen that when two different laboratories assessed product “G” utilizing the Critical Wavelength Method at pre-irradiation levels of almost twice that used in Procter and Gamble’s original submission (4, 20, 50 J/cm<sup>2</sup> and 10, 20, 30 J/cm<sup>2</sup> respectively), **no** photo-instability was noted by either laboratory. Interestingly enough, when a formulation consisting of a UVB filter only (product “E” - 7% octyl methoxycinnamate) was pre-irradiated as part of the same CTFA study, the critical wavelength increased from an initial value of  $\lambda_c = 346$  nm at 4 J/cm<sup>2</sup> to  $\lambda_c = 365$  nm at 50 J/cm<sup>2</sup>. As a definitive pre-irradiation dose has not been established, it is possible that this UVB only formulation, at a higher pre-irradiation dose, could qualify for UVA ‘*broad spectrum*’ labeling by the Critical Wavelength Method based on the upward data trend shown.

This round-robin data sheds an entirely new light on the data previously submitted to this Docket by Procter and Gamble and appears to confirm the limitations of the Critical Wavelength Method to provide *in vitro* data with any clinical significance. Additionally, it becomes obvious that product pre-irradiation does not always account for photo-instability and appears to be very filter specific.

<sup>16</sup> The Procter and Gamble Company submission of August 8, 1997 to Docket 78N-0038 page 9.

**FIGURE 18**  
**Procedure's Ability To Account For Photostability**



**Table 3**

Pre-irradiation data for Critical Wavelength Method							
Source	Composition	0 J/cm <sup>2</sup>	4 J/cm <sup>2</sup> *	10 J/cm <sup>2</sup>	20 J/cm <sup>2</sup>	30 J/cm <sup>2</sup>	50 J/cm <sup>2</sup>
P&G data ** Aug. 8, 1997	7.5% OMC, 5% OSAL, 1% AVO	379 nm		375 nm	369 nm	357 nm	
CTFA data *** Product G	7.0% OMC, 3% AVO		378 nm		377.4 nm		376.3 nm
CTFA data *** Product E	7.0% OMC,		346 nm		354.5 nm		364.6 nm

\* - based on 1/3 SPF, product labeled SPF= 12

\*\* - approximate values taken from Table 5, pg 16 of the August 8, 1997 submission by P&G

\*\*\* - mean of 2 values from 2 different laboratories

AVO = avobenzone, OMC = octyl methoxycinnamate, OSAL = octyl salicylate

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