PHOTOTOXICITY EVALUATION OF TWO PHOTOPROTECTORS ACTIVE COSMETIC INGREDIENTS AT A THREE-DIMENSIONAL HUMAN SKIN MODEL

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An *in vitro* full thickness three-dimensional human skin model represents the possibility of assess the photodamage and the photoprotection afforded by sunscreens, being able to overcome the need for animal models at least in the early stages of the tests of already marketed sunscreens but also the new formulations and other innovations in photoprotection. The setting of skin models are typically based on a de-epidermized dermis or collagen scaffold, supplanted with fibroblasts and keratinocytes. The effects of sunlight exposures on such a biological arrangement like the skin cells are well featured by sunburn and suntan, as well known short-term reactions and the more profound and serious long-term consequences like the photoaging and photocancers. The aim of this study was to determine the phototoxicity effect of two usual photoprotectors active cosmetic ingredients with different SPF using the direct citotoxicity approach in detecting the early signs of UV-induced cellular damage in a full thickness three-dimensional human skin model, comprising a differentiated epidermis and a living dermal equivalent. Typical markers of the sunburn reaction could be reproduced in that model as well. Both sunscreen agents used in the tests are widely recognized for their functionality by the pharmaceutical community and broadly employed as sunscreens worldwide. The Octyl Methoxycinnamate, also known as Octyl p-Methoxycinnamate, is currently the most frequently used sunscreen worldwide for protection of the UVB short wavelength radiation. The Avobenzone is the only agent that protects specifically of the UVA long wavelength radiation. This substance is very unstable and depending on the formulation, destabilizes itself with sunlight, placing its cell phototoxicity protection in question. The UV-induced cell damage was assessed immediately post irradiation using the *in vitro* colorimetric assay NR (3-amino-7-dimethylamino-2-methyl-phenazine hydrochloride), a water-soluble vital dye, to pass through the intact plasma membrane and become concentrated in lysosomes of viable cells. Since this is an *in vitro* assay based on human cells, this technique becomes non-invasive, representing a very plausible alternative way of substitution in animal models for testing of cyto and phototoxicity in general. Another advantage presented by cytotoxicity assay in three-dimensional human skin models is the often more reliable and trustworthy data acquisition than those originated from animal models. Furthermore, this kind of technique used for the tests are

reasonably rapid and reproducible as they generated toxicity profiles within hours of running the assays.

Is expected that the standardization of this technique can make the assessment of the new active sunscreen easier, regarding their phototoxicity at the cellular level having as future prospect, the ability to assess the photoprotection in both, innovative formulations as those already marketed.

Key words: Neutral Red, phototoxicity, photoprotectors, UV-induced cell damage, three-dimensional human skin model

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