

Contact Dermatitis Caused by Sunless Tanning Treatment with Dihydroxyacetone in Hairless Descendants of Mexican Hairless Dogs

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ABSTRACT: Dihydroxyacetone (DHAT) is a color additive that is added to sunless tanning products to produce an artificial tan. Although this agent has been used extensively as safe sunless tanning, no published data are available to judge whether the abuse of DHAT causes a potential hazard to the human skin. The purpose of this study was to clarify whether frequent treatment with DHAT solutions had a deleterious effect on the wide skin surface of hairless descendants of Mexican hairless dogs. The skin reactions to the DHAT-treatment were investigated by daily clinical observations and histopathological examinations (21 and 42 days after the beginning of the DHAT-treatment). Clinical observations showed that skin color changes were apparent within 6 h after the first treatment with 5% DHAT solutions, with maximal darkening between 12 and 24 h. Twenty-one days after the beginning of the treatment with 5% DHAT solutions, the skin developed irritant dermatitis, and then the skin lesions gradually became severe during this study. Histopathological examinations showed entire epidermal thickening, 21 days after the beginning of the treatment with 5% DHAT solutions. Forty-two days after the beginning of the treatment with 5% DHAT solutions, the skin exhibited remarkable epidermal degeneration (hyperplastic and dyskeratotic changes) and moderate inflammatory reactions in the dermis. In severe dermatitic sites, I found focal epidermal necrosis or interepidermal blister formation beneath the thickened parakeatotic corneum. Throughout this study, there were no clinical and histopathological changes in the sites treated with vehicle alone. These results revealed that the skin coloring generated by frequent wide treatments with DHAT caused severe contact dermatitis which was associated with the damaged *stratum corneum*. © 2008 Wiley Periodicals, Inc. *Environ Toxicol* 24: 506–512, 2009.

Keywords: contact dermatitis; dihydroxyacetone; hairless dogs; *stratum corneum*; sunless tanning

INTRODUCTION

As many dermatologists have pointed out, traditional ultraviolet light (UVL) tanning methods have been linked to melanoma and nonmelanoma skin cancers. Sunless tanning methods using the chemicals represent an alternative to using UVL for tanning. Chemical-induced sunless tanning is believed to be a very safe method of changing skin color. Dihydroxyacetone (DHAT) is a color additive that is added

to sunless tanning products to produce an artificial tan. The mechanism of sunless tanning color that develops after dermal application is due to DHAT binding to amino acids in the *stratum corneum* (Kurz, 1994; Nguyen and Kochevar, 2003). DHAT bound to amino acids forms brown-black compounds called melanoidins (Johnson and Fusaro, 1994; Lloyd et al., 2001; Draelos, 2001; Benamar et al., 2004; Fu et al., 2004). This interaction is also known as the browning or Maillard reaction. The color that develops from staining the *stratum corneum* is temporary and is eventually sloughed off. DHAT-containing sunless products have been approved by the Food and Drug Administration (FDA) for external use since 1977 (Fu et al., 2004). DHAT has been

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typically used in over-the-counter (OTC) lotions and creams. DHAT is considered to be nontoxic and noncarcinogenic for humans and experimental animals (Akin and Marlowe, 1984; Levy, 1992, 2000; Kim, 2001).

Sunless tanning booths emerged in the market in 1999, and then the booths are replacing solar salons (Fu et al., 2004). However, recently available sunless tanning booths introduced an unusual technique in response to their rapidly expanding population (Meadows, 2003; Fu et al., 2004; Rogers, 2005; Sheehan and Leshner, 2005). The sunless tanning booths use sprays to apply an even coat of sunless tanning solution to the consumer's bare skin. Although full-body, sunless tanning is cosmetically acceptable for the youth, no published data are available to judge whether the abuse of DHAT causes a potential hazard to the human skin.

Recently, we have established a colony of hairless descendants of Mexican hairless dogs. Hairless dogs have been utilized for investigating delayed contact hypersensitivity with chemical substances such as dinitrochlorobenzene, agricultural chemicals, and hair dyes (Kimura and Doi, 1994, 1999; Kimura et al., 1998). In this colony, we have encountered spontaneously occurring contact hypersensitivity, which was resulting from constant contact with chromium metal (Kimura, 2007).

The purpose of this study was to clarify whether frequent treatment with DHAT solutions had a deleterious effect on the wide skin surface of hairless dogs.

MATERIALS AND METHODS

Dogs

Two 2-year-old male N₇ hairless hybrids (male N₆ hairless hybrids × female Beagles) and a 4-year-old female N₅ hairless hybrid (male N₄ hairless hybrids × female Beagles) were used. The dogs were housed individually in stainless steel cages (90 cm × 90 cm × 90 cm) and kept under standard laboratory animal conditions, which were maintained at 25 ± 2°C with 50 ± 10% relative humidity. The room air was ventilated 10 to 15 times per hour automatically and 12 h/12 h light-dark cycle (lighting 07:00–19:00) was imposed. The animals received a commercial dry dog food (TC-2, Aixia Corporation, Tokyo, Japan) and water *ad libitum*.

Chemical Solutions

5% DHAT solution was diluted in vehicle, which contained ethanol (Wako Pure Chemical Industries, Osaka, Japan): propylene glycol (Sigma Chemical Co., St. Louis, USA): distilled water, 2:1:2 (v/v) in ratio. Vehicle only was used as a control.

Test Procedures

4 μL/cm² of DHAT were applied to the test site (6 cm × 6 cm) at the left dorsal skin in each dog twice (on Monday

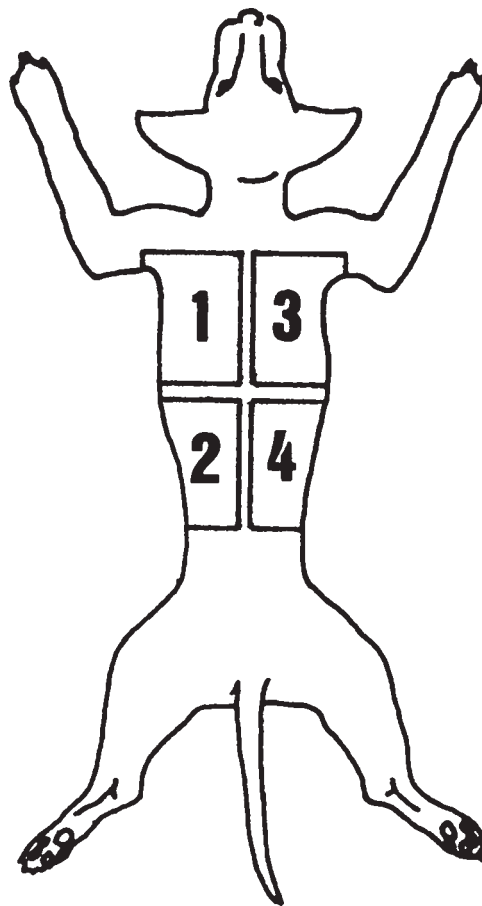


Fig. 1. Diagram of the skin sites examined on the dorsum. 1 and 2: 5% DHAT solution; 3 and 4: Vehicle only (control).

and Thursday) a week for 42 days (Fig. 1). This dosage was based on our previous experiments in the dermatological toxicity of chemical substances (Kimura and Doi, 1994, 1999; Kimura et al., 1998). The dogs were restrained for 15 min without pain to allow the DHAT to dry and to prevent them from spreading over to other skin areas. An Elizabethan collar (Tsubawa Yohkoh Co., Tokyo, Japan) was placed at the cervical area in each dog to protect from grooming. The right dorsal sites applying the vehicle were as controls. Clinical findings of the skin on the DHAT-applying sites was daily observed compared with the appearance on the control sites.

All procedures involving animals were approved by the Animal Use Committee of National Institutes of Natural Sciences (NINS) and followed the guidelines of animal care and experiments of the NINS.

Histopathology

Tissue specimens were obtained from the DHAT-treated and the control sites with a 6-mm biopsy punch (Nagatoishi Co., Tokyo, Japan) under a general anesthesia with



Fig. 2. Macroscopic photograph of skin color changes. The browning reactions are seen 12 h after the treatment with 5% DHAT solution. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

medetomidine (Domitol, Meiji Seika Co., Tokyo, Japan) at 1 day before this study and at 21 and 42 days of the DHAT application program. These tissue specimens were fixed in 10% phosphate buffered formalin (7.4), and 4- μ m paraffin embedded sections were stained with hematoxylin and eosin (HE), toluidine blue (TB), van Gieson's and Weigert's staining, and Fontana-Masson's staining (FM).

The severity of epidermal and dermal findings added estimation of pigmentation was graded as follows: negative (-), slight (\pm), mild (+), moderate (++), marked (+++).

RESULTS

Clinical Findings

Skin color changes were apparent within 6 h after the first treatment with 5% DHAT solutions, with maximal darkening between 12 and 24 h (Fig. 2). The skin showed a brown color that darkened with additional treatments with 5% DHAT solutions. The browning reactions were maintained with repeat treatments every 3 or 4 days.

21 days after the beginning of the treatment with 5% DHAT solutions, the skin developed irritant dermatitis, indicating swelling, edema, erythematous papules, exudation, scale and crust (Fig. 3). The skin lesions gradually became severe during the following period. By 42 days after the beginning of the treatment with 5% DHAT solutions, continued exposure to 5% DHAT solutions caused exfoliation of the epidermal layers in the skin lesions (Fig. 4).

Throughout this study, there were no clinical changes in the sites treated with vehicle alone.

Histopathological Findings

Histopathological findings were summarized in Table I. Twenty-one days after the beginning of the treatment with 5% DHAT solutions, the epidermis became entirely thick

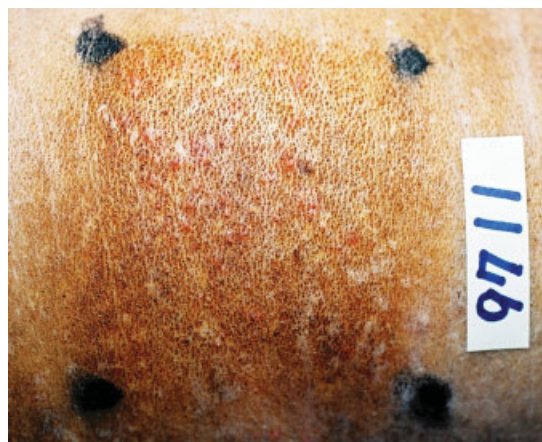


Fig. 3. Macroscopic photograph in the skin 21 days after the beginning of the treatment with 5% DHAT solutions. Irritant dermatitis (swelling, edema, erythematous papules, exudation, scale and crust) is seen. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

with disarrangement of component cells that had degenerative changes. Although the brown color complexes (DHAT-pigment masses) distributed irregularly in the *stratum corneum*, there were no increases in melanin granules in the epidermis (Fig. 5). In the dermis, the vessels were dilated and filled with blood. There was an infiltration of inflammatory mononuclear cells with dermal edema (Fig. 6).

Forty-two days after the beginning of the treatment with 5% DHAT solutions, the skin exhibited a remarkable increase in epidermal thickness, which increased by more than ten-fold to twenty-fold above the untreated sites. The



Fig. 4. Macroscopic photograph in the skin 42 days after the beginning of the treatment with 5% DHAT solutions. Exfoliation is seen. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I. Histopathological findings in the skin of hairless dogs

Changes	5% DHAT Solutions		Vehicle (Control)
	21 Days	42 Days	
Epidermis			
Browning	+ to ++ (4/6) ^a (2/6)	– (6/6)	– (6/6)
Parakeratosis	– to ± (3/6) (3/6)	++ to +++ (2/6) (4/6)	– (6/6)
Thickening	++ to +++ (2/6) (4/6)	+++ (6/6)	– (6/6)
Hyperplasia	++ to +++ (3/6) (3/6)	+++ (6/6)	– (6/6)
Degeneration	+ (6/6)	+++ (6/6)	– (6/6)
Intercellular edema	+ to ++ (1/6) (5/6)	++ to +++ (1/6) (5/6)	– (6/6)
Intracellular edema	– to ± (2/6) (4/6)	++ to +++ (2/6) (4/6)	– (6/6)
Necrosis	– (6/6)	–, ±, +, and ++ (1/6) (2/6) (2/6) (1/6)	– (6/6)
Blister formation	– (6/6)	–, ±, +, and ++ (1/6) (1/6) (2/6) (2/6)	– (6/6)
Melanin granules	± to + (3/6) (3/6)	– (6/6)	+ (6/6)
Dermis			
Cell infiltration	+ to ++ (2/6) (4/6)	++ to +++ (2/6) (4/6)	– (6/6)
Vasodilation	+ (6/6)	+ to ++ (1/6) (5/6)	– (6/6)
Edema	+ (6/6)	+ to ++ (1/6) (5/6)	– (6/6)

The degree of histopathological changes: negative (–), slight (±), mild (+), moderate (++), marked (+++).

^aThe fractions in parentheses represent the incidence of the histopathological changes.

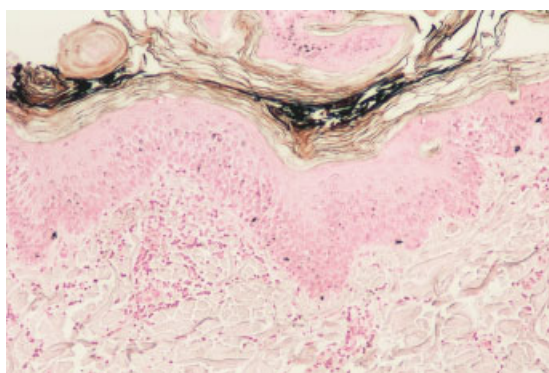


Fig. 5. Microscopic photograph in the skin 21 days after the beginning of the treatment with 5% DHAT solutions. Epidermal thickening is seen. Distribution of the brown color complexes (DHAT-pigment masses) is found in the *stratum corneum*. ×200, FM. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

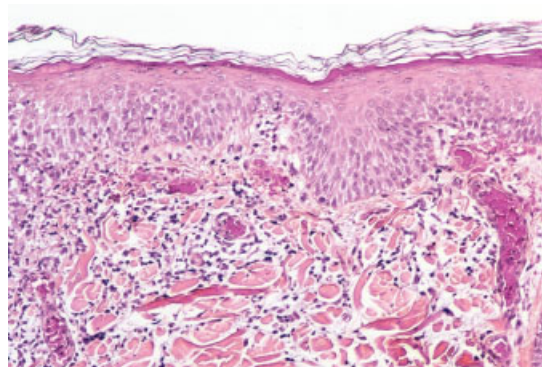


Fig. 6. Microscopic photograph in the skin 21 days after the beginning of the treatment with 5% DHAT solutions. Vasodilation and infiltration of inflammatory mononuclear cells with dermal edema are seen. ×200, HE. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

epidermis became hyperplastic with an increase in number and size of component cells. The dense *stratum corneum* consisted of nucleated keratinocytes, indicating notable dyskeratotic changes (parakeratosis). The intercellular spaces were wider and more prominent. The epidermal ingrowths were well developed and their morphological features were finger-like projections extending into the dermis (prominent acanthosis) (Fig. 7). Although the dermis showed moderate infiltration of inflammatory cells, the number of mast cells were unaffected by the continued exposure to 5% DHAT solutions.

In some portions, there was focal necrosis in the dermal-epidermis junction, accompanied by severe epidermal

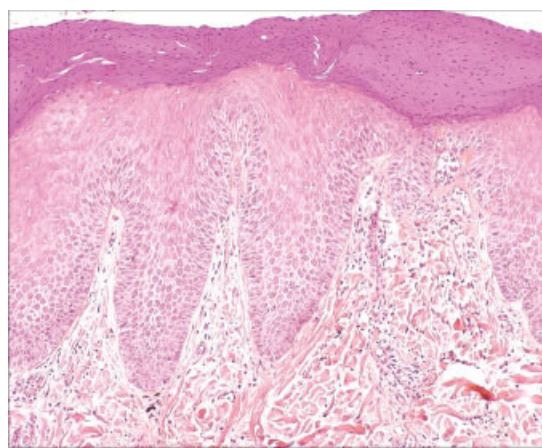


Fig. 7. Microscopic photograph in the skin 42 days after the beginning of the treatment with 5% DHAT solutions. Epidermal hyperplasia, parakeratosis, and epidermal ingrowths are seen. ×100, HE. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

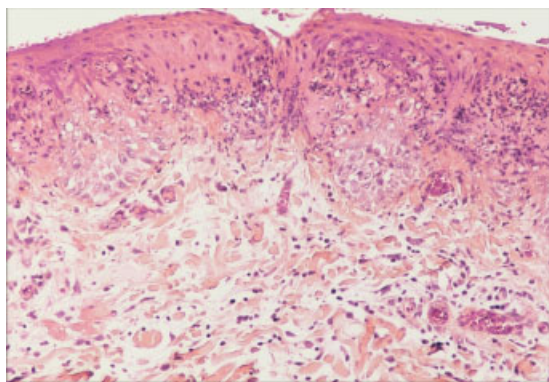


Fig. 8. Microscopic photograph in the skin 42 days after the beginning of the treatment with 5% DHAT solutions. Focal necrosis is seen in the dermal-epidermis junction. Infiltration of mononuclear cells, extravasation, and edema are also seen in the dermis. $\times 200$, HE. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

degeneration. The dermis showed prominent inflammatory changes including infiltration of mononuclear cells, extravasation and dermal edema (Fig. 8). In severe dermatitis sites, interepidermal blister formation with intracellular and/or intercellular edema was observed beneath the thickened parakeatotic corneum (Fig. 9). A reduction in epidermal pigmentation occurred in the sites treated with 5% DHAT solutions. Melanin granules disappeared throughout the degenerative epidermis (Fig. 10).

Throughout this study period, there were no histological changes in the control sites treated with vehicle alone. The distribution of melanocytes with melanin granules were not affected by the frequent topical application of vehicle alone.

DISCUSSION

After the treatment with DHTA solutions, the development of the browning reactions in the skin of hairless dogs was similar to the course of DHAT-induced skin pigmentation in humans (Levy, 2000). The previous study reported that the chronic administration (once each week for 80 weeks) of DHTA to mice induced no changes in gross physical appearances, behaviors, or vital signs (Akin and Marlowe, 1984). This coloring agent is considered to be generally well tolerated and DHAT has been approved by the FDA in 1973 as a color additive for drugs and cosmetics. However, its use is restricted to external application (Meadows, 2003).

Although DHAT is believed to be a nontoxic ingredient, both for ingestion and topical application, there have been a few clinical reports on adverse effects such as allergic and irritant dermatitis from frequent DHAT-application

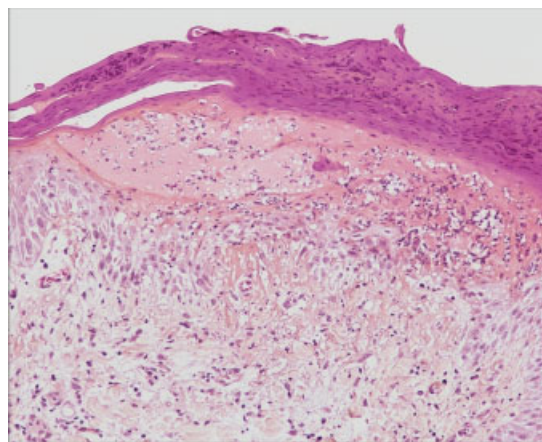


Fig. 9. Microscopic photograph in the skin 42 days after the beginning of the treatment with 5% DHAT solutions. Interepidermal blister formation is seen beneath the thickened parakeatotic corneum. $\times 100$, HE. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

(Morren et al., 1991; Draelos, 2000; Draelos, 2002). In these reports, patch tests demonstrated that DHAT was the sensitizer in the self-tanning products. The DHTA-treated sites in the hairless dogs clinically resembled the erythematous lesions reported in human patients.

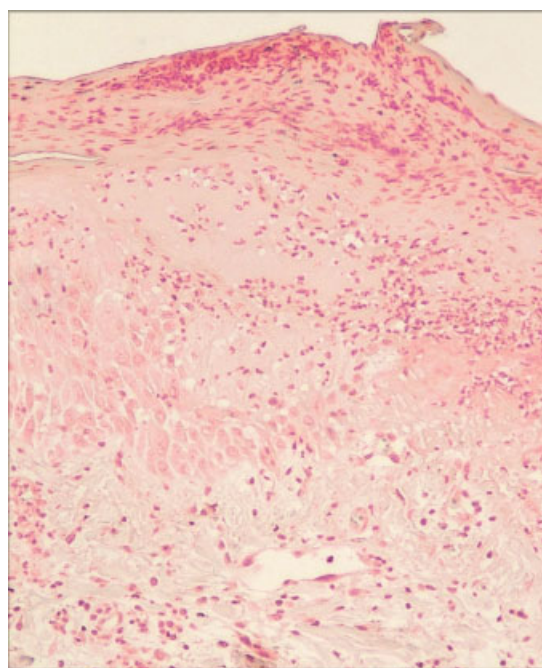


Fig. 10. Microscopic photograph in the skin 42 days after the beginning of the treatment with 5% DHAT solutions. Melanin granules disappear throughout the degenerative epidermis. $\times 200$, FM. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Our macroscopic observations revealed that repeated treatment with DHAT solutions caused severe dermatitis 21 days after desquamation of the DHAT-stained corneum. The onset of this adverse effect of the treatment with DHAT solutions agreed with the turnover (regrowth) time of the canine epidermis (22 days, Baker et al., 1973). Our results provided the following potential for irritant dermatitis for this agent: DHAT penetrates through the *stratum corneum*, reaching viable cells in which sensitization and systemic reactions can occur. Subsequently, DHAT may have some deleterious effects on its treated site.

To my knowledge, histopathological changes of contact dermatitis due to DHAT have previously been not examined in both humans and laboratory animals. Twenty-one days after the beginning of the treatment with 5% DHAT solutions, DHAT sufficiently produced skin pigment that was distributed well into the *stratum corneum*. This findings accorded with the fact that DHAT reacted with skin surface proteins to produce a durable brown color.

The histopathological findings reflected the visible changes in the DHAT-treated sites. Although the dermis showed nonspecific inflammatory reactions, the epidermis exhibited the onset of some structural alternations associated with hyperproliferation of component cells.

Forty-two days after the beginning of the treatment with 5% DHAT solutions, the skin specimens showed severe contact dermatitis accompanied by notable epidermal damage. The histopathological findings in the DHAT-treated sites resembled those observed in contact dermatitis which was experimentally induced by the oxidative hair colorings. Petersen et al. (2004) demonstrated that DHAT induced DNA damage, cell-cycle block and apoptosis in HaCaT cultured keratinocytes. They described that the main mechanism of the DNA damage was through direct redox toxicity of DHAT, with formation of reactive oxygen species that could react with DNA resulting in single strand breaks. The low molecular weight of DHAT (151.1 Da) enables its penetration through the epidermis of the hairless dogs. It is likely that the remarkable changes found in the DHAT-treated sites result from direct redox effects of DHAT. In addition, there were no increases in the number of mast cells in the DHAT-treated skin. This finding suggested that repeated topical treatment with DHTA should provoke cumulative irritant contact dermatitis in hairless dogs.

Pham et al. (1979) concluded that DHAT were mutagenic and caused primary DNA damage. They estimated that because of the high correlation between mutagenicity and carcinogenicity, DHAT containing tanning products were a potential human health hazard. Petersen et al. (2004) reported decreases in viability of cultured keratinocytes and inhibition of clonal cell growth were time- and dose-dependent after treatment with DHAT. The DHAT-skin complex is usually an integral part of the skin surface and its substantivity is only limited by natural desquamation of the skin or by physical removal of the *stratum corneum*. The

histopathological findings in the DHAT-treated sites showed that penetration of this browning ingredient through the *stratum corneum* caused severe inflammatory reactions in both the epidermis and dermis. Our results provided evidence that frequent treatment with DHAT solutions had some potential toxic effects on the wide skin surface undergoing spray-on sunless tanning.

Very recently, Jung et al. (2008) showed that the higher is the self-tanning agent concentration, the higher is the radical induction. The Maillard reaction begins to take place within the very first minutes, and then the coloring effects of DHAT appear only after 12 h of its application. Forty minutes after the treatment with 20% DHAT solution on the skin, more than 180% of additional free radicals are generated during UV irradiation as compared with untreated skin. Therefore, DHAT causes free radical formation and enhance the free radical injury during UV irradiation. From their results and our findings, sun exposure should be avoided after the treatment of self-tanning products including DHAT.

The skin damage caused by DHTA solutions seems to deteriorate, depending on the following conditions (frequency of application, applied concentration, skin type and environmental factors such as heat, light and UV irradiation). In contrast, DHAT-induced skin reactions may be attenuated by the use of UV filters, sunscreens, antioxidants and anti-irritants.

These results revealed that the skin coloring generated by frequent wide treatments with DHAT in moderate concentrations caused severe dermatitis in the skin of hairless dogs. The skin lesions were associated with the damaged *stratum corneum*. Hairless dogs are useful for the investigations of dermatotoxicity of DHAT involving its full-body sprays in sunless tanning booths.

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