

## Active ingredients, mechanisms of action and efficacy tests of antipollution cosmetic and personal care products

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Urban population around the globe is direct exposed to the pollution caused by several sources (vehicles, industries, smokes *etc.*) and primary pollutants are divided in particulate matter and toxic gases. Current researches in populous countries indicated that exposure to pollution could affect sebum composition, stratum corneum quality and signs of skin aging. Hair and scalp are also affected by the excessive exposure to pollutants, resulting in a dull, dry and lifeless appearance. Cosmetics have been evolved conceptual and scientifically to achieve substantial effectiveness against pollution damaging on the cutaneous tissue, involving the development of innovative multipurpose active ingredients and efficacy tests, skilled to prove the protection and benefits of such personal care products. In this review, we highlighted the skin and hair/scalp damages provoked by the main environmental pollutants and the active substances used in antipollution cosmetics/personal care products with the respective mechanisms of action. Likewise, *in vitro* and *in vivo* efficacy tests were discussed concerning the antipollution claim substantiating.

**Keywords:** Active ingredients. Antipollution. Cosmetics. Efficacy tests.

### INTRODUCTION

The population living urban lifestyles continues to grow around the globe, and they are direct exposed to the pollution caused by vehicle fumes, industry, cigarette smoke and others. The primary pollutants are divided in two main groups: particulate matter (PM) that could also have polyaromatic hydrocarbons (PAHs), and toxic gases, like CO<sub>2</sub>, CO, SO<sub>2</sub>, NO, NO<sub>2</sub> and other nitrogen oxides (NO<sub>x</sub>) (Portugal-Cohen *et al.*, 2017). The skin, the outermost barrier of the body, is sensitive to these pollutants and it is frequently exposed to the environment. This is of major concern, since one of the skin main function is to protect the organism from such damaging substances. Pollution can cause dermatological hazard effects, like inflammation,

oxidative stress and metabolic impairments, and could also generate cancer, which might be amplified by the deleterious synergy of the sun, particularly UVA (Marrot, 2017).

Nowadays, the mechanisms understanding the cutaneous effects of pollutants continue unclear. However, its impact on the worldwide population has been reported in the last decade. Current research works in populous countries, as Mexico and China, indicated that exposure to pollution could affect sebum composition, stratum corneum quality and, also, intensify the signs of skin aging, such as pigmented spots and wrinkles. The skin is the main defense barrier against environmental agents and it is daily exposed to air pollutants that can be absorbed and cause toxic effects. The stratum corneum (SC) is the outer layer of the skin and represents the primary barrier to external agents. SC is composed of a compressed fusion of keratinized cells surrounded by an extracellular lipid matrix, arranged in a “brick and mortar” model. The “bricks” are composed of keratinocytes and “mortar” by the expelled contents of the lamellar

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granules, including lipids and proteins. Penetration/permeation through the skin is related to the integrity of the barrier function, anatomic place, age, cutaneous hydration properties and physicochemical characteristics of the contaminants. Consequently, children and patients with damaged skin barriers are often affected by dermal exposure considering the increased absorption of air pollution. Polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs) and particulate matter (PM) are significant air pollutants that can damage the skin. Simple daily activities can expose the skin to toxic pollutants. For example, products containing organic solvents, as paints and varnishes can generate VOCs. These agents increased the inflammatory reaction, inducing cancer lesions and atopic dermatitis in animal and *in vitro* models. Additionally, the action of this harmful agents can be amplified with the interaction of UV-light and cause oxidative skin damage (Kim, Cho, Park, 2016).

PAH is mainly produced by wood/oil burning and in all smoke emerging from the combustion of organic matter. Regarding the nature of PAHs to generate reactive oxygen species, it also can induce melanocyte proliferation. Furthermore, the association of UV-light and PAH provokes melanin synthesis in melanocyte, resulting in the formation of environment-induced lentiginos. Several studies showed that exposure to PAHs is associated with a development of skin cancer. Topical contact of mixtures of PAHs intensifies the frequency of skin cancers, such as papilloma, carcinoma, and squamous cell carcinoma. Daily a human can absorb up to 3.7 mg of PAH and interestingly the primary exposure source of PAH contamination in human is food (smoked meat) and diet (Marrot, 2017; Soeur *et al.*, 2017).

PM is the most harmful component of air pollution. It is generated by factories, power plants, incinerators, cars, construction industry, and fires. PM has a small size, few microns, and often includes mineral components, various metals which can generate harmful effects on the skin. However, the impact of PM on skin has not yet been characterized, its transcutaneous penetration has been recently described and may be limited, as recent studies rejected the bioavailability of particles larger than 20 nm. Additionally, the phototoxicity behavior of PM was studied *in vitro*. In a Keratinocyte model, the PM was phototoxic from 25 mg.mL<sup>-1</sup>. The UVA seemed to mostly contribute to this phototoxic process, probably by exciting few chemicals, particularly UVA-photo-reactive (Kim, Cho, Park, 2016; Soeur *et al.*, 2017).

Human hair has long been used as a biological marker for assessment of environmental pollutants, toxins, drug abuse and exposure to pesticides. Damages caused

by pollution nowadays are evidenced in the scalp and hair. Large suspended particle, small airborne particles, smoke and gaseous pollution settle on the scalp and hair causing irritation, dandruff, redness, faster exfoliation of outer layers of the scalp and more sensitivity leading psoriasis on prolonged exposure. In addition, damage like exposing inner layers of the fiber and dry frizzy hair (Bencko, 1995; Rajput, 2015).

Hair fiber may be divided in cuticles, cortex and medulla. The medulla is the innermost portion of hair shaft. The cortex gives the hair strength, flexibility, elasticity and color (due to natural and artificial pigments). The cortex is surrounded by a single layer of overlapping transparent, scalelike cells of cuticle, the external layer of the hair. Its core function is to protect the hair shaft against environmental and chemical damages. Adherence and the orientation of cuticle are responsible for surface properties such as brightness and resistance to combing (Dario, Baby, Velasco, 2015; Gama, Baby, Velasco, 2017).

The hair fiber nature attracts some substances found in the air pollution. Hair absorbs water under both liquid and steam form. Keratin may absorb up to 40% of its own weight in water. Hydration is favored by temperature increase, by pH changing and by all polar solvents, which break hydrogen bonds. Hydration changes the fiber elasticity. This absorption is followed by a swelling in the hair, with 15-10% increase in its thread diameter and 0.5-1.0% in its length. Both absorption and swelling essentially depend on the medium pH. Polar solvents have a similar effect on hair threads. Normally, the hair resistance to swelling is due to the existence of bonds maintaining the reticular integrity, which avoids the molecules penetration (Gama, Baby, Velasco, 2017).

This absorptive and swelling phenomenon may favor the penetration of certain organic molecules. When the hair fiber is not intact, the porosity can be affected. Some situations influence on porosity: alkaline pH, over 8; high temperature; chemical processes, as permanent waving, discoloration, straightening, dyeing and air relative humidity (Dario, Baby, Velasco, 2015; Gama, Baby, Velasco, 2017). Specific strategies can protect the hair from environmental damage, among them: avoiding exposure to air pollutants, frequent scalp wash with mild shampoos, use of special antioxidant shampoos and the protection of the fiber with substances that act by reducing the porosity.

### **Antipollution active ingredients and mechanisms of action**

Skin naturally protects itself from pollutants at some extent by pathways of the immune system cells,

however, prolonged and repetitive daily exposure to high levels of pollutants may impair the protective mechanisms. Some pollutants (e.g., PAHs) that can easily penetrate through the skin barrier still can induce damage even without percutaneous penetration to deeper skin layers via signal transduction mechanism (Valacchi *et al.*, 2002). For that reason, daily application of skincare products that work as an effective shield against polluting agents are required to maintain skin balance. Antipollution formulations comprise mainly film formers and antioxidants designed to improve skin barrier function by physically shielding the skin and scavenging the oxidative pollutants. Cosmetic ingredients may either prevent the contact between skin and pollutants or start biochemical processes to reduce the oxidative primary and secondary products. It is recommended to design a formulation to achieve both goals. The active molecules can play a preventive role when blocking pro-inflammatory membrane signaling pathways like Keap1-Nfr2 and IKB-NFKB (Tanaka *et al.*, 2010). It can immediately reduce short-term health damage caused by inflammation factors, such as COX and interleukins. Other signaling pathways can be up-regulated increasing cell differentiation like NOTCH1 and WNT (Wang *et al.*, 2011) or improving xenobiotics metabolism like AhR (Jux *et al.*, 2011). Multipurpose treatment strategies that combine biological and physical defense against contemporary pollution agents are more effective on both short and long-term skin damage.

Besides the use of specific antipollution treatment, there are general recommendation measures that can protect the skin against pollution-induced damage such as using rinse-off products (e.g. shower gel) to clean off pollution over the skin; sunscreens to absorb/scatter UV radiation and to prevent photo-reactive compounds responding on UV exposure; emollients to preserve and restore skin barrier function and the avoidance of over washing the skin, preventing the damage of the natural, cutaneous protective barrier (Krutmann *et al.*, 2014).

Hair and scalp are also affected by the excessive exposure to pollutants, resulting in a dull, dry and lifeless appearance. Several types of pollutants show an adhesive property to hair fibers through adsorption and it can be used as an indicator of human exposure to pollution (Nakao *et al.*, 2002). Antipollution hair cosmetics work through film forming and anti-adhesive effects. The film blocks pollutant particles adsorption and can be removed easily after rinsing (Blosl, Ott, Schacht, 2017). Table I shows an overview of the main active compounds and their mechanisms of action as antipollution agents.

## ***In vitro* antipollution efficacy tests**

To better understand the different mechanisms that the pollutants have on cutaneous tissue, numerous *in vitro* studies have been made, mainly with human cell culture. *In vitro* human cell models are a representation of various cell types, derived by its embryological origin or specific tissue or organ (Alfaro-Moreno *et al.*, 2008). The advantage is that these kind of cells are a representation of cell types that may be of interest and it is readily available for detailed mechanistic investigations, avoiding species-to-species extrapolations, since it is derivate from human cells (Jia, Wang, Liu, 2017). Thus, they have demonstrated susceptibility to the toxic effects generated by environmental air pollutants, mimicking the human skin, and so being an interesting model (Cotovio *et al.*, 2001).

Cotovio *et al.* (2001) exposed cultured immortalized human keratinocytes and a reconstructed human epidermis to 10 ppm ozone and monitored the oxidative stress. They employed fluorescence intensity as an indicator of the extent of oxidative stress. Their results proved that there was a positive correlation between these factors in both models exposed to ozone. These results led them to investigate the protective potential of products like vitamin C, a thiol derivative (N-acetyl cysteine) and a green tea extract. Reduced fluorescence levels measured in the keratinocytes and reconstructed epidermis compared to non-protected skin cells were observed; therefore, they were able to confirm that all samples were efficient in preventing ozone-induced cellular oxidative damage.

Duche, Cotovio, Catroux (2004) proposed the use of ellagic acid, a polyphenol, as a topical cosmetic antipollution agent. Using a monolayer culture of human keratinocytes, they proved that ellagic acid had cytoprotective and antioxidant effects against the atmospheric pollutant, ozone and combustion residues. Heavy metals and PAHs penetrate keratin proteins (like the hair and skin) and induce oxidative stress and cell apoptosis - not to mention the carcinogenic effects that PAHs have. They tested how well ellagic acid trapped PAHs and heavy metals with a series of formulations, containing distinct concentrations of ellagic acid. The outcomes of these tests were: following pretreatment with ellagic acid, binding of PAHs to keratinocytes decreased, cell viability after exposure to the heavy metal cadmium chloride increased, and ozone-induced stress decreased (measurement of fluorescence in the keratinocytes) (Duche, Cotovio, Catroux, 2004).

Biatry (2002) also discussed a compound that could be employed in a cosmetic product as an antipollution agent to protect keratin materials. Emulsions of phytantriol, an

**TABLE I** - Antipollution skin/hair treatments

Active compound	Mechanism	Potential antipollution effect	References
Dead Sea mineral-rich water	Reduce UVB irradiation induced cell apoptosis in the epidermis of skin organ cultures and reduce pro-inflammatory cytokine, tumor necrosis $\alpha$ (TNF $\alpha$ ) secretion	Reduce inflammation marker interleukin 1 $\alpha$ (IL-1 $\alpha$ ) when applied before heavy metal and atmospheric particulate matter to <i>in vitro</i> human skin models based on reconstructed epidermis	Wineman <i>et al.</i> , 2012; Portugal-Cohen <i>et al.</i> , 2017
High molecular polysaccharides	Shield the skin/hair by physical barrier to atmospheric pollution and UV radiation		Portugal-Cohen <i>et al.</i> , 2017; Wei <i>et al.</i> , 2009
Chelating molecules	Heavy metal-scavenging	Reduce heavy metals availability by forming complexes	Pelletier, Catroux, 2002; Duche, Cotovio, Catroux, 2004
Fermentation products (ectoin and hydroxiectoin)	Regulate osmotic and oxidative cell pathways	Reduce UVA damage and mitochondrial DNA mutation	Buenger, Driller, 2004; Graf <i>et al.</i> , 2008
Peptides	Play an important role in antioxidant cellular system and skin hydration	Reduce the levels of reactive oxygen species (ROS) and DNA, protein, and lipid damage in keratinocytes subjected to solar-simulating UV oxidative stress	Lintner, 2003; Catroux, Cotovio, Duche, 2004; Markova <i>et al.</i> , 2009
Plant extracts	Flavonoids and anthocyanins play an important role in antioxidant pathways, saponins support skin autophagy	Reduce ROS levels and promote cell autophagy when damaged	Hocquaux, Loing, Bedos, 2015; Lintner, 2003; Sawa <i>et al.</i> , 1999
Skin/hair conditioning agents	Improve skin/hair hydration	Improve skin/hair barrier	Biatry, 2002; Mausner, 1996
UVA and UVB filters	Absorb/scatter UV radiation and prevent the formation of photoreactive compounds	Reduce photoactivation of pollutants and ROS formation	Mausner, 1996; Fu <i>et al.</i> , 2012

alcohol commonly found in cosmetics as a humectant, were applied to reconstructed-skin epidermal samples in order to assess *in vitro* its efficacy as an anti-pollutant. Combustion residue particles were C-14 radiolabeled and also applied to the samples. After washing and then quantifying the amount of any remaining particles, it was revealed that phytantriol limited the penetration of combustion residues in skin cells.

Portugal-Cohen *et al.* (2017), using *in vitro* human skin models based on reconstructed epidermis, which exhibited normal barrier functions (presence of well-differentiated SC), evaluated the protection of two products applied alone or in combination, Dead Sea minerals (Dead Sea mineral-rich water - DSW) and anionic polysaccharide (PS), against mixture of

pollutants (MOP) containing heavy metals / atmospheric particulate matter and ozone. The authors tested the levels of prostaglandin E2 (PGE2) and IL-1 $\alpha$ , two inflammation markers. Their results found that, when DSW and PS were combined, they had the potential to inhibit PGE2 and IL-1 $\alpha$ , being a model candidate for urban pollution protection

A research performed by Qiao *et al.* (2017) evaluated the hazard effects of SRM1649b, a standard reference material of urban air pollution particulate matters which contains a wide variety of PAHs, using human keratinocytes (HaCaT) and normal human dermal fibroblasts (NHDF) cultures. The authors found that SRM1649b induced cell aging through growth inhibition, apoptosis and cell cycle G1 phase arrest.

Soeur *et al.*, (2017) using normal human epidermal keratinocytes (NHEK) and reconstructed skin model, measured the biological impact of pollutants (PM, PAH and PM extract) exposed to or daily UV (d-UV) wavelengths range (300–400 nm), which contained UVB radiation and the entire UVA band (UVA1 + UVA2), or UVA1 radiation (350–400 nm). The UVA1 had a phototoxic influence on the samples and it was equal or greater than the effect produced by d-UV. PAHs were phototoxic at nanomolar concentrations, concluding that only traces of PAH were enough to produce a high cellular stress, if combined with UVA1 exposure (Soeur *et al.*, 2017).

### Antipollution efficacy assessment by *in vivo* methods

Currently, mostly due to scarcity of scientific evidence, there are no established guidelines currently available for protecting the skin against air pollution (Mancebo, Wang, 2015). Nevertheless, cosmetic products presently available in the market tackle the effect of pollution in one or several of the following manners (Valacchi *et al.*, 2012):

- Boost skin barrier structure and function.
- Provide topical antioxidants and decrease oxidative stress and inflammation.
- Decrease deposition of pollutants on skin by cleansing or exfoliation.
- Protect skin from harmful UV radiation, since it exacerbates the effect of the other environmental pollutants.
- Regulate melanogenesis.
- Promote synthesis of fibrous proteins in the dermis.

Skin barrier function is most commonly assessed *in vivo* through measurements of transepidermal water loss (TEWL) (Lotte *et al.*, 1987; Pinnagoda *et al.*, 1990). Nevertheless, poor correlations between damage inflicted to the skin and changes in TEWL values have often been reported in the literature (Bashir *et al.*, 2001; Chilcott *et al.*, 2002; Chilcott *et al.*, 2007; Rosado, Pinto, Rodrigues, 2009), and “dynamic” strategies using TEWL data have been developed to improve the sensitivity of the methodology (Berardesca *et al.*, 1990; Berardesca, Herbst, Maibach, 1993; Piérard-Franchimont *et al.*, 1995; Rosado, Pinto, Rodrigues, 2005; Pinto *et al.*, 2011). The impact of urban particulate pollution on skin barrier function and the subsequent drug absorption was assessed *in vivo* using the pig model (Pan *et al.*, 2015). In this study, heavy metals (1648a) and PAHs (1649b) were used as pollutant models to treat the skin, and it was found that the latter

significantly disrupted the TEWL by 2-fold compared to a PBS control.

The efficacy of antioxidants is more often assessed *in vitro*, for instance by DPPH assay, although a few *in vivo* methodologies have been developed (Ratz-Lyko, Arct, Pytkowska, 2012). EPR spectroscopy and imaging (EPRI) of spin labels allow measurement of the kinetics and the mapping of the spatial distribution of free radicals in biological systems, thus allowing indirect evaluation of cosmetics antioxidant capacity (He *et al.*, 2004). An interesting approach to efficacy testing of antioxidants was developed using the methyl nicotinate micro-inflammatory model (Vertuani *et al.*, 2003). In the experiment, the skin was exposed to this vasodilator, and then it was deposited on a preparation containing antioxidants (vitamin C and E). Protective effects of the antioxidant substance were determined using the DermAnalyzer system, and the results of measurements were validated using standard skin measuring instruments: chromameter (assessing the color of the skin and the erythema through its a\* parameter), laser Doppler (for measuring perfusion) and TEWL. The methodology was further developed in another study assessing the antioxidant capacity of UV filters containing rutin (Oliveira *et al.*, 2016).

A research group has advocated the oxidization of squalene, a human skin lipid, as a marker of environmental pollution studies (Pham *et al.*, 2015). The squalene (per) oxidization process generates molecules that negatively impact the skin, thus, preventing such events can be a goal of topically applied antipollution preparations, mainly those containing antioxidants. An *in vivo* procedure to assess efficacy of such formulations was suggested, where, after application of the products, the forehead (or back) of subjects is exposed to low doses of UVA–visible rays, at the end of which sebum is collected and quantification of the possible different amount of (per)oxidized products present is conducted.

In a recent study, a pollution model to mimic particulate matter trapped in sebum and oils as a surrogate for dirty, polluted skin was developed, aiming to evaluate the cleansing efficacy/protective action of two different procedures against particulate pollution trapped in human sebum: a sonic brush vs. manual cleansing (Peterson *et al.*, 2017). The pollution model consists of atmospheric particulate matter/pollution combined with grease/oils typical of human sebum. This mixture was applied to the cheek of human volunteers and the efficacy of the two cleaning protocols was compared using photography and imaging analysis.

Finally, researchers should take into account, as in any other *in vivo* efficacy study, that the panel of volunteers that

is gathered to establish efficacy of antipollution cosmetics has to be judiciously selected. It has been suggested that the adverse effects of pollution particles are most prominent in susceptible subjects, particularly the elderly and patients with cardiopulmonary diseases (Huang, Ghio, 2009). Researchers have found that young and old mice exposed to either O<sub>3</sub> or cigarette smoke have different oxidative stress and inflammatory marker responses (Lim *et al.*, 2006; Fortino *et al.*, 2007). In humans, it is known that elderly individuals have higher levels of both oxidative stress and inflammatory markers (Harman, 2009).

## CONCLUSIONS

Considering the cutaneous tissue the largest organ of the human body and its direct and usual exposition to environmental pollutants (UV radiation, PAHs, nitrogen oxides, ozone and cigarette smoke, amongst others), the cosmetic and personal care products claiming antipollution action are of utmost relevance to be deeply investigated, achieving safety profile and notorious efficacy. According to a multipurpose/multifunction characteristic of these active ingredients and finished products, complementing methods (efficacy tests) are required to entirely determine the protection and benefits from antipollution cosmetics. Yet, there is still much to be done to establish methodologies concerning the *in vivo* assessment of skin protection against pollution, even though a few interesting studies in this area can already be found in the literature.

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