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## ► To cite this version:

Cécile Guillon, Meriam Meziani, Soraya Abdelli, Dominique Sigaudou Roussel, Christelle Bonod, et al.. The Aryl hydrocarbon receptor pathway plays a central role in the cutaneous response to pollutants. European Journal of Dermatology, 2022, 32 (3), pp.305-311. 10.1684/ejd.2022.4262 . hal-03839908

**HAL Id: hal-03839908**

**<https://hal.science/hal-03839908>**

Submitted on 4 Nov 2022

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**Title: The Aryl hydrocarbon receptor pathway plays a central role in the cutaneous response to pollutants**

**Short title: Skin response to pollutants and the AhR**

Cécile Guillon<sup>1, 2, 3</sup>, Meriam Meziani<sup>2</sup>, Soraya Abdelli<sup>2</sup>, Dominique Sigaudou Roussel<sup>1, 2</sup>,  
Christelle Bonod<sup>1, 2</sup>, and Audrey Nosbaum<sup>4, 5</sup>

**Author affiliations:**

<sup>1</sup> CNRS UMR5305 LBTI, 7 passage du vercors 69367 Lyon cedex 7 France

<sup>2</sup> University Claude Bernard Lyon 1, 43 Boulevard du 11 Novembre 1918, 69100  
Villeurbanne, France

<sup>3</sup> URGO Research Innovation and Development, 42 Rue de Longvic, 21300 Chenôte, France

<sup>4</sup> CIRI (International Center for Infectiology Research) INSERM U1111, Ecole Normale  
Supérieure de Lyon, University Claude Bernard Lyon 1, CNRS UMR5308, 21 avenue Tony  
Garnier, 69007 Lyon, France

<sup>5</sup> Allergy and Clinical Immunology Department, Lyon Sud University Hospital, 165 chemin  
Grand Revoyet, 69495 Pierre Bénite cedex, France

**Corresponding author: Cécile Guillon, [c.guillon@fr.urgo.com](mailto:c.guillon@fr.urgo.com), +33 6 14 63 95 18.**

**Word count: 4159**

**Number of figures: 2**

**Number of references: 55**

**Abstract: 169 words (250 max)**

Short- and long-term exposure to atmospheric pollution has significant health effects. The skin is the organ directly in contact with pollutants and is responsible for protection of the organism. Particulate matter (PM) such as polycyclic aromatic hydrocarbons (PAHs) are the basis of certain pulmonary as well as dermatological complications. Pollution exacerbates certain illnesses such as atopic dermatitis and cancer, and it may also participate in delaying wound healing and in the occurrence of chronic ailments such as diabetes. The aryl hydrocarbon receptor (AhR) transcription factor at the core of these responses to pollutants is expressed by all cells of the skin. The AhR is subject to tight regulation that depends on its ligand. Pollutants act in a deleterious manner via the AhR on the behavior of keratinocytes as well as fibroblasts. Natural ligands, on the other hand, allow the noxious effects of pollution to be countered. Modulation of AhR appears to be an excellent therapeutic target to improve or stop the cutaneous problems linked to pollution.

**Keywords:** atopic dermatitis, pollution, skin, wound healing

## **Introduction**

It is nowadays recognized that pollution is a major public health problem caused by an increase in the prevalence of numerous cardiovascular, pulmonary, and dermatological illnesses (1, 2). Atmospheric pollution is due to air contamination by different natural gases derived from emissions by the vegetation as well as by soil erosion and of anthropomorphic origin produced by human activities. These pollutants are classified into two distinct families: primary pollutants and secondary pollutants, the latter undergoing modification by the primary pollutants. Atmospheric pollution comprises gases such as carbon dioxide and sulfur dioxide, as well as particulate matter (PM) emitted by various human activities. FPs contain heavy metals and polycyclic aromatic hydrocarbons (PAHs). These components are produced by road and air traffic as well as by cigarette smoke (3). Dioxins such as TCDD (2,3,7,8-tétrachlorodibenzo-*p*-dioxin) are derived mainly from wood stoves and forest fires; they are part of the family of PAHs. Exposure to pollutants particularly affects the skin, which is an organ in direct contact with the pollution. For example, toxic and cancerogenic dioxins readily penetrate the skin due to their lipophilic properties (4). On the other hand, pollutants reach the lungs by respiration, and their passage in the blood flow can affect other organs such as the skin (4). A link between pollution and chronic cutaneous inflammatory diseases such as atopic dermatitis (AD) or impaired wound healing has recently been reported (5). Similarly, it has been shown that long-term exposure to pollutants is associated with an enhanced incidence of lung as well as skin cancers (6). The aryl hydrocarbon receptor (AhR) transcription factor, which is expressed in all cutaneous cells, is central to all of these pathophysiological processes (7, 8). The AhR signaling pathway is induced by PM in particular, which have a high affinity for this receptor (7).

In light of the increasing concerns arising from pollution, there is ample merit in gaining a better understanding of the consequences of pollution at the level of the skin in response to

the AhR. We here document the effects and the interactions that occur in skin in response to pollution and that involve the AhR.

## **1. Biology of the AhR**

The skin is the largest organ of the human body. It protects the organism from exterior insults, and it plays a role as a sensor of the environment. This is why the cutaneous homeostasis and the integrity of the skin need to be tightly regulated.

The AhR is expressed in all of the organs that act as barriers, such as liver, lungs, intestine, and skin (7). The AhR is a helix-loop-helix domain transcription factor that responds to endogenous and exogenous signals, and it is, therefore, also able to modulate these effects. It is found in the cytoplasm of all cutaneous cells, both in the epidermis and the dermis, and it is essential for homeostasis of the skin (7).

The AhR is activated in the cytoplasm after binding of its ligand, which allows induction of the signal for its translocation to the nucleus. Thanks to chaperone proteins, the AhR is directed to the nuclear compartment where it heterodimerizes with the molecule ARNT (Aryl hydrocarbon Receptor Nuclear Translocator). This protein complex is able to activate the transcription of certain genes, by binding to their XRE (Xenobiotic Response Element) promotor regions (**Figure 1**). On the other hand, the AhR activates the expression of its own AhRR (Aryl hydrocarbon Receptor Repressor) repressor, thereby ensuring a negative feedback loop. AhRR binds to ARNT in the nucleus and prevents the binding and the action of the AhR/ARNT complex (7).

### **1.1. Nature of the AhR ligands**

Numerous ligands are capable of activating the AhR pathway. They are classified into three groups according to their nature.

### **1.1.1. Endogenous ligands**

UVB radiation is capable of generating dimers of tryptophan, called FICZ (6-formylindolo[3,2-b]carbazole), which can bind to the AhR (9). These are referred to as endogenous ligands. Activation of the AhR pathway by FICZ promotes pigmentation and hence improves protection of the DNA. Due to this, the AhR is sensitive to the environment and it acts as a sensor of UV radiation (7). When exposed to UVB radiation, mice lacking the AhR exhibit less cutaneous pigmentation than wild-type mice (10).

### **1.1.2. Natural exogenous ligands**

The AhR can bind with high affinity to ligands in food. Phytochemical antioxidants such as flavonoids, which are responsible for the color of fruits and plants, or glucosinolates that give rise to the bitterness of mustard and cauliflower, are exogenous natural ligands of the AhR. Their binding induces translocation of the nuclear factor Nrf2 to the nucleus, allowing expression of antioxidant enzymes such as Nqo1 (Quinone oxidoreductase) and oxygenase-1 (HO-1) (11-13) that protect cells from damage generated by ROS. A recent study has shown that these exogenous natural ligands exert a barrier function by regulating transepidermal water loss (14).

### **1.1.3. Synthetic exogenous ligands**

Lastly, particulates from pollution, such as PAHs or TCDD, are synthetic exogenous ligands with very high affinities. Their binding to the AhR promotes the generation of reactive oxygen species, which are known to cause DNA damage. Pollution gives rise to an increase in inflammation and it exacerbates cutaneous inflammatory diseases such as atopic dermatitis as well as psoriasis (15).

The nature of the ligand determines the AhR response. In humans, the specificity of the binding of the AhR depends on a valine residue situated at position 381. In case of

substitution by an alanine, the specificity increases, unlike substitution by an asparagine, which leads to abolition of the binding.

## **2. Consequences of the activation of the AhR at the cutaneous level**

The epidermis is the most superficial layer of the skin. It is mainly composed of keratinocytes that become differentiated from the basal layer to the corneal layer. There are also melanocytes, which are responsible for pigmentation of the skin. The epidermis plays an essential role in the barrier function. It is sensitive and in direct contact with environmental particulates, particularly those found in pollution. The underlying dermis is also highly exposed. Park *et al.* exposed dermal fibroblasts to particulate pollution for 24 h, and they identified 1977 genes for which the expression was altered. We report here the consequences of this substantial transcriptional modification on certain cutaneous functionalities.

### **2.1. Loss of the barrier function of the skin**

It has been shown that reconstructed human epidermis exposed long-term to tobacco smoke undergoes histological changes. The spinous and granular layers were no longer visible, while the thickness of the corneal layer was increased (3). Another experiment performed on cultured keratinocytes reported an increase in the differentiation of the cells in response to TCDD (16). The development of the corneal layer at the expense of the granular and spinous layers can be explained by acceleration of the differentiation of the keratinocytes (3). By contrast, the ligand FICZ is capable of decreasing the thickness of the epidermis and of improving the transepidermal loss of water in mice with atopic dermatitis (17). On the other hand, mice that specifically lack the AhR in keratinocytes (K5AhR-KO) exhibited greater transepidermal water loss than the wild type mice (18). The AhR also targets the expression of filaggrin, *FLG*, which is essential for the barrier function of the skin by participation in the

formation of the corneal layer. Mice lacking the AhR (AhR KO) express lower levels of genes associated with the barrier function (14). The expression of filaggrin, occludin, and involucrin increase following mechanical stress in healthy mice, but this increased expression is not seen in mice lacking the AhR. The loss of AhR appears to result in less cutaneous resistance to mechanical stress. Moreover, the expression of genes coding for extracellular matrix anchoring proteins (collagens IV and VII) and the expression of genes coding for proteins involved in cell-cell junctions (corneodesmosin, desmocollin-1, and desmoglein-1) were found to be decreased in a reconstructed epidermis model after exposure to cigarette smoke (3). This led to a decrease in the efficacy of the epidermal barrier by the loss of cell cohesion. The AhR is, therefore, subject to very tight regulation at the level of the epidermis. Depending on the ligand and the context, the AhR can maintain or change the homeostasis of the epidermis.

## **2.2. Modulation of the proliferative and the migratory capacities of cutaneous cells**

The AhR also participates in the activation of growth factors such as EGF (epidermal growth factor) involved in cell differentiation or proliferation. AhR KO mice exhibit faster dorsal wound healing than normal mice. In this model, the re-epithelialization is faster due to faster keratinocyte migration. There are more fibroblasts, and the collagen level is higher at the level of the granulation tissue. Fibroblasts secrete high levels of active TGF- $\beta$  (transforming growth factor), which participates in enhancing wound healing (18). This study shows that decreasing in AhR activity could be a new potential way to treat chronic lesions. By contrast, activation of the AhR by PAHs results in a decrease in proliferation as well as entry into apoptosis of keratinocytes and fibroblasts, which could participate in impaired healing (19).



### 2.3. Modulation of MMPs

Matrix metalloproteinases (MMP) induce changes in the quantity and the structure of collagens. A model of reconstructed human skin, and *Hairless*  $-/-$  mice, exposed to tobacco smoke were shown to exhibit a significant increase in MMP-1 and MMP-3 (3). These proteins are responsible for the degradation of type I, II, and III collagen and type IV and V collagen, respectively. On the other hand, the expression of inhibitors of MMPs, TIMP-1 and TIMP-3, is not altered in cutaneous tissue. Tobacco smoke and pollutants alter the balance between the biosynthesis and the degradation of collagen. The balance tends toward degradation of collagen in favor of the induction of MMPs.

It was recently shown that the active form of TGF- $\beta$  is an essential factor in homeostasis of the epidermis and growth of the dermis. Tobacco smoke, as well as PAHs, induce the non-functional form of TGF- $\beta$ , thereby leading to a reduction in the biosynthesis of extracellular matrix proteins (20). Similarly, tobacco smoke can result in oxidative stress by the induction of reactive oxygen species (ROS) in fibroblasts. ROS can then induce damage at the level of the DNA and stimulate the production of MMPs. The AhR is a transcription factor activated in response to pollutants, thereby giving rise to changes at the level of the skin. The activation of these transcription factors thereby leads to oxidative biological responses such as the formation of ROS (3) and it can also directly activate the production of MMP-1 and MMP-3, which are responsible for the degradation of collagen and elastin fibers (21).

The AhR pathway activated by the components of tobacco smoke and pollutants plays a central role in the process of extrinsic aging as a result of pronounced alterations at the level of the dermis.

## 2.4. Loss of the viscoelasticity

*In vivo* studies in mice and of cultured fibroblasts that have been exposed to tobacco smoke or to atmospheric pollutants have revealed changes in the biosynthesis of type III, IV, and VII collagens as well as elastin fibers (5). These changes translate into a significant decrease in the matrix components in the dermis and neosynthesis of abnormal elastin (22). Such dysmorphic elastic fibers can lead to elastosis or changes that lead to a loss of the viscoelasticity of the skin. Thus, these alterations appear to be responsible for the formation of wrinkles and hence early extrinsic aging.

On the other hand, the damaged skin of mice exposed to tobacco smoke exhibits a higher level of elastase than non-exposed mice. The elastic fibers are then degraded more, thereby perturbing the plasticity of the wound (23).

## 2.5. Hyperpigmentation

Recent studies have indicated that photoprotection by tanning can occur in the absence of UV radiation. Treatment of melanocytes with oligonucleotides such as the 7-mer pApGpTpApTpGpA is able to induce tyrosinase activity and stimulate the synthesis of melanin in the absence of UV light (24). Other studies have shown that ligands of the AhR, such as dioxin or PAHs, induce the proliferation of melanocytes (8). This mechanism can, therefore, provide scientific proof of the association between spots of pigmentation and exposure to FPs. Similarly, the exposure of primary melanocytes cultures to atmospheric particulates induces transcriptional expression of genes involved in the formation of skin spots (4). Lastly, activation of the AhR pathway in melanocytes in response to pollutants induces cell proliferation and high levels of melanin synthesis, leading to the appearance of pigment spots on the skin.

## 2.6. Inflammation

There AhR does not result in specific immune responses, due to the fact that the AhR is present in several types of immune cells and because the response differs depending on the bound ligand. The AhR can also activate the expression of pro-inflammatory or immunosuppressive cytokines such as interleukin (IL-) 1 $\beta$  (IL-1 $\beta$ ) or TGF- $\beta$  in keratinocytes. It has been shown that the AhR increases the expression of a pro-inflammatory cytokine, IL-8, in keratinocytes in a manner dependent on a response to oxidative stress induced by benzo[a]pyrene (BaP) in cigarette smoke (25). Exposure of a reconstructed epidermis to cigarette smoke induced a high degree of inflammation of the skin by the expression of IL-1 $\alpha$ , IL-8, and IL-18 (3). Keratinocytes treated with PAHs exhibited an increase in IL-1 $\alpha$  and IL-1 $\beta$ , which are interleukins that also perturb the dermis. Co-culture of these keratinocytes with fibroblasts revealed expression of MMP1 and COX-2 in dermal cells (26). In 2005, Masafumi Tauchi and their team generated transgenic mice that constitutively express the AhR in keratinocytes, under the control of the K promoter (27). The receptor is active in the complete absence of ligands, the aim, therefore, being to activate the transcription of target genes without a chemical entity (AhR ligands). These mice exhibited cutaneous inflammatory lesions and pronounced itching, and molecular analysis by microarrays revealed overexpression of pro-inflammatory genes. Non-immune cells are able to produce TNF alpha and IL-8 after activation of the Toll-like receptor 2 (TLR-2) (28). Hou *et al.* showed that TCDD increases the secretion of TNF alpha and IL-8 after induction of TLR-2. Suppression of the AhR by KO blocks the production of these pro-inflammatory cytokines (28).

The AhR hence plays a central role in the regulation of cutaneous inflammation, and its activation results in skin lesions.

### 240        **3. Link with cutaneous pathologies**

#### 241        **3.1. Atopic dermatitis**

242        Activation of the AhR by pollutants appears to be correlated with chronic inflammatory  
243        diseases such as atopic dermatitis (AD). AD is a skin disease that occurs mainly in childhood.  
244        It results in several clinical signs such as itching, erythema, and chronic xerosis. At the  
245        molecular level, the barrier function of the skin is altered due to, in certain cases, a loss of  
246        function of the gene coding for filaggrin. It was not until very recently that atmospheric  
247        particulates have been taken into account in the development and the exacerbation of the  
248        symptoms of AD. There is a correlation between the severity of AD in children and outdoor  
249        as well as indoor pollution such as particles present in paint (29). On the other hand, the  
250        expression of the AhR and ARNT are increased in the lesions of patients with AD relative to  
251        healthy skin (15).

252        A recent study used an AhR-CA mouse model that mimics AD. The keratinocytes of these  
253        mice constitutively express the AhR. This cytoplasmic receptor is, therefore, a key factor in  
254        AD (30).

255        In the presence of PAHs, AhR-CA mice overexpress the gene Artemin (*Artn*), which is a  
256        target of the AhR transcription factor, in the keratinocytes. The artemin protein (ARTN) is  
257        upregulated in the keratinocytes and is responsible for hyperinnervation and an increase in  
258        pruritis. The skin is, therefore, hypersensitive and the itchiness is exacerbated, in the same  
259        study, it was shown that the AhR is activated in DA lesions in humans (30). Moreover, it has  
260        been shown that *in vitro* exposure of human keratinocytes to pollutants leads to increased  
261        expression of ARTN (30). Hu *et al.* have shown that the level of AhR in the serum of AD  
262        patients is higher than that of healthy individuals. The expression of AhR mRNA is positively  
263        correlated with the severity of the disease as well as to the level of IL-6 present in the serum  
264        of AD patients (31).

### 3.2. Impaired wound healing

One of the main complications of diabetic patients is a lack of substantial wound healing, resulting in acute chronic lesions. It has been shown that smoking increases the risk of foot amputation in diabetic patients (32). Cigarette smoke is a direct activator of the AhR pathway as it contains more than 3800 components, including PAH (21). A number of clinical and experimental studies have shown the impact of cigarette smoke on chronic wound healing (33-35). Sorensen *et al.* made cutaneous excisions of smokers and non-smokers, and analysis of the lesions revealed perturbed inflammation and a decrease in the proliferation of fibroblasts that could participate in a delay of wound healing (35). Michaud *et al.* indicated that extracts of cigarette smoke inhibited vascular endothelial growth factor (VEGF), which perturbed the migration and the formation of capillaries formed by HUVECs (human umbilical vascular endothelial cells) (37). These studies suggest that for diabetic patients, evaluation of tobacco dependency and stopping smoking are key to improving chronic wound healing. Indeed, cessation of smoking of more than three weeks preoperatively reduced impaired wound healing after surgery (36).

The cutaneous vascular density and blood flow are decreased with aging or pathologies such as diabetes, or in the presence of chronic lesions, thereby increasing the expression of hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ) in tissues, which allows expression of angiogenic genes. Several studies have shown that the loss of HIF-1 $\alpha$  subunits in the endothelium leads to vascular anomalies. Under limiting oxygen concentrations, HIF-1 $\alpha$  is stabilized, which allows it to be translocated to the nucleus where it binds to ARNT, like AhR. The HIF-1 $\alpha$ :ARNT heterodimer binds to hypoxia response elements (HRE) in target genes inducible by hypoxia (37, 38). It has been shown that in the presence of pollutants such as PCB126, hypoxia significantly interferes with the functions of the AhR, which could negatively affect CYP1A1 mRNA expression, resulting in a lower protein level and hence less enzymatic

activity (39). This negative interaction between the AhR and hypoxia signaling pathways appears to depend at least in part on the availability of ARNT. Furthermore, it has been shown that ARNT KO mice exhibit vascular and angiogenic impairments that can contribute to impaired migration of immune cells during the first stages of wound healing (40). This negative interaction has been observed in liver (41, 42) and, unlike the study of Pollenz *et al.* on the induction of the AhR by TCDD, does not indicate competition for ARNT under hypoxic conditions (43).

Thus, the presence of tissue hypoxia can significantly perturb the normal transcriptional response of the AhR after exposure to PCB 126, which could ultimately affect cell metabolism and contribute to the development of chronic lesions as a result of major cutaneous vascular defects. Moreover, cigarette smoke has adverse effects on vascular biology, inducing endothelial dysfunction and arterial stiffness (44). Cigarette smoke causes defective angiogenesis that can be linked to a lack of VEGF (45), a main pro-angiogenic factor for which the expression depends on HIF-1 $\alpha$  (46, 47). More extensive studies focused on skin and impaired wound healing in this toxic context of pollutants are necessary to improve the understanding of these multiple interactions.

### **3.3. Cancer**

Long-term exposure to indoor and outdoor air pollution is associated with an increased incidence of cancer, particularly lung cancer (6). PAHs exhibit cancerogenic properties by the formation of DNA adducts following the activation of cytochrome P450, which leads to the formation of diol-epoxide metabolites (48). Matsumoto and his team have shown that repeated topical application of PAHs on the back of AhR<sup>+/+</sup> mice led to the formation of epidermoid carcinomas after 29 weeks, unlike AhR<sup>-/-</sup> mice (49). The formation of cutaneous tumors in the AhR<sup>+/+</sup> mice was mediated by the expression of CYP1A1, induced by extracts of air pollution particles (49). The AhR plays a major role in the cutaneous carcinogenesis

induced by PAHs. The epithelial–mesenchymal transition (EMT) results in invasive cancers by the formation of metastasis, and it is known that Slug is a key transcription factor in the EMT. It inhibits the proteins required for cell-cell interaction, such as E-cadherins, and it allows cell migration. There are several XRE regions in the Slug gene promoter, to which the AhR can bind. Inhibition of the AhR leads to suppression of the expression of Slug (50). Prolonged exposure to different pollutants can lead to certain cancers becoming more aggressive due to an increased metastatic potential.

#### **4. Conclusion/perspectives**

The AhR is subjected to extremely tight regulation. Ligands of a synthetic exogenous nature, such as pollutants, can disrupt this balance. In a context of pollution, the AhR promotes the generation of ROS, which cause DNA mutations. Reactive oxygen species promote the secretion of pro-inflammatory cytokines and decrease the expression of filaggrin by the NFκB and AP1 pathways. The proliferation of melanocytes and melanogenesis are enhanced in the presence of pollutants. Lastly, at the level of the dermis, activation of the AhR by synthetic exogenous ligands generates ROS and the activity of metalloproteases is increased. Fibroblasts synthesize an abnormal form of elastin and an inactive form of TGF-β. Activation of the AhR by pollutants also leads to the formation of cutaneous tumors and it can promote the formation of metastasis.

The structure and the composition of the skin are modified in the presence of pollutants. The extracellular matrix is degraded, inflammation is increased, and pigmentation is stimulated. These changes are directly linked with early cutaneous aging, serious forms of AD, delayed wound healing, and the formation of cancer (**Figure 2**). Hidaka and their team have shown that the Artemin gene is a target of the AhR, inducing substantial pruritis in the presence of

340 PAH in mice. Inhibition of *Artemin* reduces the symptoms of AD (30). In humans, this gene  
341 is found in AD lesions, Strong activation of *Artemin* in response to pollutants is, therefore,  
342 possible. *Artemin* could also be a therapeutic target in AD. Interestingly, Van Den Bogaard *et*  
343 *al.* were the first to show a beneficial therapeutic effect of activation of the AhR on the  
344 barrier function of the skin by coal tar. The expression of filaggrin was restored in the  
345 keratinocytes of the reconstructed epidermis of AD patients with a heterozygous mutation  
346 (FLG<sup>-/+</sup>) (51). In 2020, Furue, assigned the beneficial effects of natural or medicinal ligands  
347 of the AhR to anti-oxidant properties via activation of NRF2 in AD patients (52). The  
348 AhR/NRF2 pathway is, therefore, a potential therapeutic target for AD patients.

349 Food items can provide flavonoids that can compete with pollutants by exerting an anti-  
350 oxidative role (8). Haas and their team have shown that suppression of the AhR ligands in the  
351 food of mice decreased the barrier function similar to mice lacking the AhR. After being fed  
352 a diet enriched in I3C (indole-3-carbinol), these mice fully recovered their barrier function  
353 (14). Recently, studies have shown that a flavonoid, eupapholin, can attenuate the oxidative  
354 stress and inflammation induced in cultured keratinocytes by fine particulates (53).  
355 Eupapholin appears to reduce the generation of ROS and the activity of the MAP Kinase  
356 pathway, which is the basis for NFκB activation (54). Other than environmental or personal  
357 actions to reduce pollutants, one can imagine counteracting the effects of pollution by a  
358 specific diet that results in high levels of natural exogenous ligands of the AhR to limit the  
359 action of pollutants.



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## **Figure legends:**

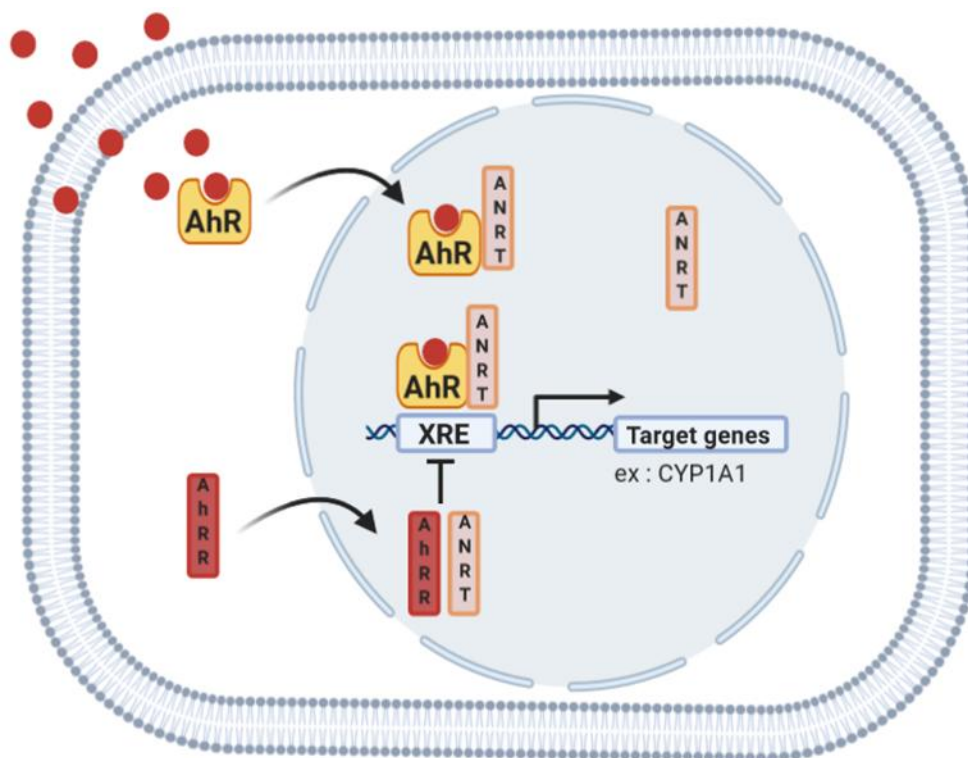
### **Figure 1. Signaling by the AhR in response to its ligand.**

AhR: Aryl hydrocarbon Receptor; ARNT: Aryl hydrocarbon Receptor Nuclear Translocator; XRE: Xenobiotic Response Element; AhRR: Aryl hydrocarbon Receptor Repressor. In the cytoplasm, the AhR binds its ligand and is then transported to the nucleus. In the nucleus, the AhR and its ligand heterodimerize with the ARNT to bind to the XRE in promoter regions of target genes such as, for example, cytochrome P450 1A1 (CYP1A1) (55).

### **Figure 2. Impact of pollution on the cutaneous functionality involving the AhR.**

Pollution and cigarette smoke are composed of numerous ligands capable of activating the AhR. Oxidative stress induced by pollution perturbs the overall balance of the skin. This results in inflammation of the epidermis by the secretion of pro-inflammatory cytokines (1), greater trans-epidermal water loss (2) due to the loss of intercellular junctions (3), and destabilization of the dermal-epidermal junction (DEJ) (4). The keratinocytes of the basal layer lose their capacity for proliferation and their differentiation is increased, thereby enhancing the supra-basal layers (5). The proliferation and the migration of fibroblasts are altered (6), which can lead to impaired wound healing. Within the derma, the extracellular matrix is degraded (7) by the presence of metalloproteases (MMPs) induced by the inflamed keratinocytes in particular. However, endogenous or exogenous natural entities are capable of counteracting the adverse effects of pollution (8).

**Figures :**



**Figure 1. Signaling by the AhR in response to its ligand.**

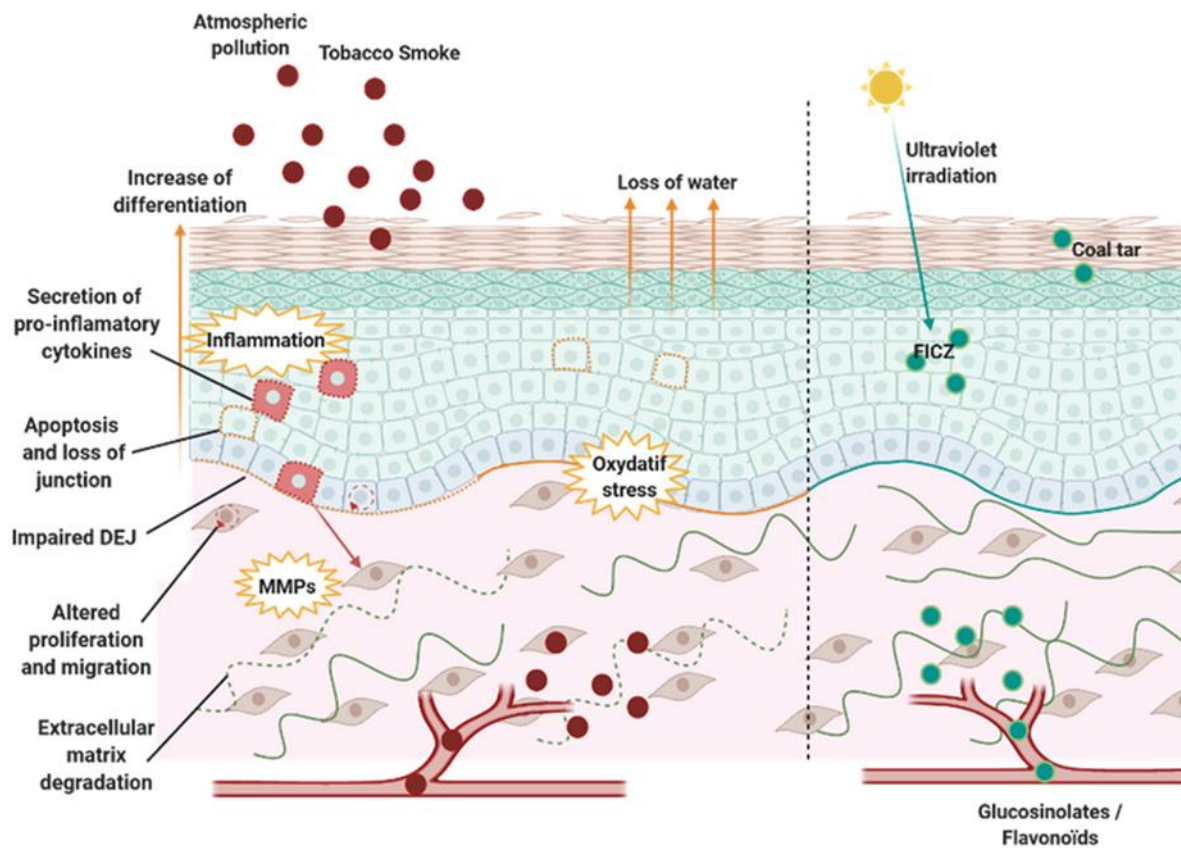


Figure 2. Impact of pollution on the cutaneous functionality involving the AhR.