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# Gene expression induction of volatile organic compound and/or polycyclic aromatic hydrocarbon-metabolizing enzymes in isolated human alveolar macrophages in response to airborne particulate matter ($PM_{2.5}$)

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**Abstract**

To contribute to improve the knowledge of the underlying mechanisms of action involved in air pollution particulate matter (PM)-induced cytotoxicity, we were interested in the metabolic activation of volatile organic compounds (VOC) and/or polycyclic aromatic hydrocarbons (PAH) coated onto Dunkerque City's $PM_{2.5}$ in human alveolar macrophages (AM) isolated from bronchoalveolar lavage fluid (BALF). This in vitro cell lung model is closer to the normal in vivo situation than other lung cell lines, notably in the characteristics that AM display in terms of gene expression of phase I and phase II-metabolizing enzymes. The bronchoscopic examinations and BAL procedures were carried out without any complications. After 24, 48 and 72 h of incubation, calculated lethal concentrations at 10% and 50% of collected airborne PM were 14.93 μg PM/mL and 74.63 μg PM/mL, respectively, and indicated the higher sensibility of such target lung cells. Moreover, VOC and/or PAH coated onto PM induced gene expression of *cytochrome P450* (*cyp*) *1a1*, *cyp2e1*, *nadph quinone oxydo-reductase-1*, *and glutathione S-transferase-pi 1 and mu 3*, versus controls, suggesting thereby the formation of biologically reactive metabolites. In addition, these results suggested the role of physical carrier of carbonaceous core of PM, which can, therefore, increase both the penetration and the retention of attached-VOC into the cells, thereby enabling them to exert a longer induction. Hence, we concluded that the metabolic activation of the very low doses of VOC and/or PAH coated onto Dunkerque City's $PM_{2.5}$ is one of the underlying mechanisms of action closely involved in its cytotoxicity in isolated human AM in culture.


*Keywords:* Healthy outpatients; Bronchoalveolar lavage; Alveolar macrophage; Particulate matter; Cytotoxicity; Metabolic activation; Volatile organic compounds; Polycyclic aromatic hydrocarbons

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## 1. Introduction

Ambient air pollution is an important environmental health risk factor for many different diseases. This is consistently indicated by numerous epidemiological studies on associations between air pollution particulate matter (PM) exposure and occurrence of acute respiratory infections, lung cancer and chronic respiratory and/or cardiovascular diseases (Brunekreef and Holgate, 2002; Dominici et al., 2007; Elliott et al., 2007; Hales and Howden-Chapman, 2007; Pope, 2004). Historically, health effect communities have faced formidable barriers to understanding the significance of ambient PM exposure, related to the highly complex nature of atmospheric aerosols and their anthropogenic sources and/or natural sources of origin (Alfaro-Moreno et al., 2002; Diociaiuti et al., 2001; Hetland et al., 2004; Liden et al., 2003; Nel et al., 2001, 2006). However, despite intensive investigation, the underlying mechanisms of PM-related adverse health effects are incompletely understood.

Hence, in order to contribute to a better knowledge of the underlying mechanisms of action involved in air pollution $PM_{2.5}$-induced lung cytotoxicity, we have undertaken an extensive investigation of the adverse health effects of $PM_{2.5}$, collected in Dunkerque, a French seaside city located on the southern coast of the North-Sea, and characterized by the proximity of important industrial activities and heavy motor vehicle traffic. We have also already shown that in vitro short-term exposure to Dunkerque City's $PM_{2.5}$ induced dose- and time-dependent oxidative damage, activation of nuclear factor-kappa B/inhibitory kappa B complex, inflammatory response, and apoptotic events in cells originated from the normal lung tissue of a human embryo in culture (L132 cell line) (Dagher et al., 2005, 2006, 2007; Garçon et al., 2006, 2007). Moreover, we have recently reported that the metabolic activation of the very low doses of volatile organic compounds (VOC) and/or polycyclic aromatic hydrocarbons (PAH) coated onto Dunkerque City's $PM_{2.5}$ constituted one of the underlying mechanisms of action closely involved in its cytotoxicity in A549 cell line. However, A549 cells derived from a type II-like human alveolar epithelial carcinoma, and several authors indicated that the profiles of phase I and phase II-metabolizing enzymes involved in the metabolic activation of organic chemicals will significantly vary depending on the considered cell types and/or tissue compartments of the lung (Hukkanen et al., 2000, 2002; Spivack et al., 2003; Thum et al., 2006).

Among the more than 40 different cell types, which are present in the respiratory tract in humans, one of the most susceptible lung cell types is alveolar macrophages (AM), which also plays a critical role in the lung defense against air pollution not only by clearing the airways of deposited PM but also by taking a great part in the detoxification of inhaled organic fraction through its metabolic activation by phase I and phase II enzymes (Castell et al., 2005; Dahl and Lewis, 1993; Hukkanen et al., 1997; Spivack et al., 2003; Willey et al., 1996). Hence, to improve the current knowledge about the role played by the organic chemical fraction coated onto PM in the lung cytotoxicity, in this work, we investigated the capacity of Dunkerque City's $PM_{2.5}$ to induce VOC and/or PAH-metabolizing enzymes in primary cultures of human AM isolated from bronchoalveolar lavage fluid (BALF), through the determination of the gene expressions of *cytochrome P450* (*cyp*) *1a1* (*cyp1a1*), *cyp2e1*, *and cyp2f1*, *microsomal epoxyde hydrolase* (*meh*), *nadph quinone oxydo-reductase-1* (*nqo1*), *and glutathione S-transferase-pi 1* (*gst-π1*) *and -mu 3* (*gst-μ3*). To better define the role of the organic fraction, titanium dioxide ($TiO_2$) and PM having undergone a thermal desorption (i.e. desorbed particulate matter; dPM) were also included in the experimental design of this work as negative controls.

## 2. Materials and methods

### 2.1. Chemicals

RPMI 1640 culture medium, Fetal Bovine Serum (FBS), penicillin/streptomycin solution, amphotericin B solution, and sterile phosphate-buffered solution (PBS) were from InVitrogen SARL (Cergy Pontoise, France). Titanium (IV) oxide powder (anatase; purity: 99%; primary particle size: 0.2 μm; surface not coated) was from Acros Organics (Noisy Le Grand, France). Benzene, toluene, and benzo(a)pyrene (B(a)P) were from Sigma–Aldrich (St.-Quentin Fallavier, France). Cytotoxicity Detection Kit (LDH) and Cell Proliferation Reagent (WST-1) were provided by Roche Diagnostics (Meylan, France). RNAqueous-4PCR kit, High Capacity cDNA Archive kit, Taqman fast universal PCR master Mix, No UNG, and TaqMan Gene Expression Assays were from Applera France SA (Courtaboeuf, France).

### 2.2. Methods

#### 2.2.1. PM sampling, physical and chemical characteristics, and outgassing

*PM sampling*: PM was collected in Dunkerque (51°04′N; 2°38′E; France), a French seaside city, using a high volume cascade impactor (Billet et al., 2007).

*Physical and chemical characteristics*: Scanning electronic microscopy coupled with energy dispersive X-ray analysis was used to assess PM composition and size distribution (Billet et al., 2007). Table 1 shows that the highest number of