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Fine and ultrafine atmospheric particulate matter at a multi-influenced urban site: Physicochemical characterization, mutagenicity and cytotoxicity☆

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ABSTRACT

Particulate Matter (PM) air pollution is one of the major concerns for environment and health. Understanding the heterogeneity and complexity of fine and ultrafine PM is a fundamental issue notably for the assessment of PM toxicological effects. The aim of this study was to evaluate mutagenicity and cytotoxicity of a multi-influenced urban site PM, with or without the ultrafine fraction. For this purpose, $PM_{2.5\text{-}0.3}$ (PM with aerodynamic diameter ranging from 0.3 to 2.5 μm) and $PM_{2.5}$ were collected in Dunkerque, a French coastal industrial city and were extensively characterized for their physico-chemical properties, including inorganic and organic species. In order to identify the possible sources of atmospheric pollution, specific criteria like Carbon Preference Index (CPI) and PAH characteristic ratios were investigated. Mutagenicity assays using Ames test with TA98, TA102 and YG1041 *Salmonella* strains with or without S9 activation were performed on native PM sample and PM organic extracts and water-soluble fractions. BEAS-2B cell viability and cell proliferation were evaluated measuring lactate dehydrogenase release and mitochondrial dehydrogenase activity after exposure to PM and their extracts. Several contributing sources were identified in PM: soil resuspension, marine emissions including sea-salt or shipping, road traffic and industrial activities, mainly related to steelmaking or petro-chemistry. Mutagenicity of PM was evidenced, especially for $PM_{2.5}$, including ultrafine fraction, in relation to PAHs content and possibly nitro-aromatics compounds. PM induced cytotoxic effects at relatively high doses, while alteration of proliferation with low PM doses could be related to underlying mechanisms such as genotoxicity.

1. Introduction

Particulate Matter (PM) air pollution is now a well-recognized carcinogen for humans and is one of the most important environment and health concerns (Loomis et al., 2013). World Health Organization (WHO) estimates that exposure to PM may cause 3.7 million premature deaths worldwide (WHO, 2014). In the past decade, European and US authorities established new directives to reduce PM exposure limits (Krzyzanowski, 2008). Chronic and acute exposure to PM is related to lung cancer morbidity and mortality in industrialized countries (Carey et al., 2013; Katanoda et al., 2011; Li et al., 2015). However, both the underlying mechanisms of PM toxicity and the influence of PM composition and size distribution on such health effects still remain unclear.

Understanding the heterogeneity and complexity of fine and ultrafine PM is a fundamental issue for the assessment of PM toxicological effects. PM sampled at a multi-influenced site

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contains organic compounds, such as Polycyclic Aromatic Hydrocarbons (PAHs), furans and dioxins, inorganic components, such as ions and metals, black carbon, crustal and biogenic elements (Harrison and Yin, 2000). PM size and composition are linked to their origins, to the atmospheric photochemistry and meteorological conditions. Primary pollutants can indeed react together to form new xenobiotics, including compounds with low relative amount but suspected high toxic potential. These interactions also occur after inhalation. Additive, antagonist, and synergistic effects can modify PM toxicity (Claxton and Woodall, 2007; DeMarini, 2013). Furthermore, due to the diversity of emission sources and the chemical transformation of pollutants in the atmosphere, multiple pathways are often involved in the toxicity of PM and in the onset of adverse health effects (Ding et al., 2014; Longhin et al., 2016; Vaccari et al., 2015).

Lung carcinogenicity of PM is often related to their mutagenic and/or genotoxic properties (Sørensen et al., 2003). Several studies have demonstrated that $PM_{2.5}$ extractable organic matter could be mutagenic, using several *Salmonella typhimurium* strains in the Ames test (de Kok et al., 2006; Traversi et al., 2015). Short-term mutagenicity bioassays of particulate samples from different PM size fractions and/or different PM components (i.e. organic fraction, water-soluble fraction or total PM) may help understanding the contribution of different PM size fractions or chemicals on health (André et al., 2011; Skarek et al., 2007; Topinka et al., 2013).

In this context, the present work aimed to study the size-dependent toxicity of PM sampled at a multi-influenced site. PM was extensively characterized for physical and chemical properties, in order to identify the main contributing sources. Secondly, native $PM_{2.5\text{-}0.3}$ (PM with aerodynamic diameter ranging from 0.3 to 2.5 μm), as well as Organic Extract (OE) and Water-soluble Fraction (WF) of $PM_{2.5\text{-}0.3}$ and $PM_{2.5}$, were investigated for mutagenicity, cytotoxicity and cell proliferation. Mutagenicity was evaluated using the Ames test on three different *Salmonella typhimirium* strains able to evidence frameshift mutation, oxidative stress or PAHs effects. Cytotoxicity and effects on proliferation were measured on BEAS-2B bronchial epithelial cell line for which metabolic ability has been previously demonstrated (Uppstad et al., 2010).

2. Materials and methods

2.1. PM sampling

PM were sampled from March to July 2011 in the city center of Dunkerque, northern France (latitude: 51 °2′10″N; longitude: 2 °22′46″E), a coastal, industrial and urban site with 200,000 inhabitants (Kfoury et al., 2016). A high volume (68 m^3/h) five-stages plus back up cascade impactor (model 235 TFIA-2, Staplex®, USA) was used to collect $PM_{2.5\text{-}0.3}$, as described in Cazier et al. (2016). Briefly, a quartz fiber filter (TFAQS810, Staplex®, USA) was used as impaction substrate on the first stage while the following stages were kept nude to collect particles in their native form. During the whole sampling campaign, two impaction systems were run in parallel to maximize the mass of $PM_{2.5\text{-}0.3}$ available to perform physicochemical characterization and toxicological studies. Particles were continuously collected for seven days; impaction plates were then removed and placed in a laminar flow clean bench for two days to allow complete drying. Finally, particles impacted on stage 2–5, were brushed from the plates and pooled together. All particle batches were gathered in Teflon-PFA vessels and carefully homogenized using two Teflon coated magnetic balls and a magnetic stirrer for 2 h. The resulting sample was finally stored at −20 °C until use.

$PM_{2.5}$ were collected in parallel using the high volume (30 m^3/h) aerosol sampler DHA-80 (DHA-80, DIGITEL®, Switzerland) equipped with the $PM_{2.5}$ inlet (DPM2,5/30/00, DIGITEL®, Switzerland). The sampler was loaded with quartz fiber filters (QM-A, Whatman®, GE Healthcare Life Sciences, United Kingdom), pre-heated to 450 °C for 8 h before sampling in order to reduce blank carbon values. After sampling, filters were kept in a horizontal laminar flow clean bench for 24 h before storage at −20 °C until further handling.

2.2. Characterization of PM

Both $PM_{2.5\text{-}0.3}$ and $PM_{2.5}$ samples were extensively characterized as published elsewhere (Billet et al., 2007; Cazier et al., 2016; Ledoux et al., 2006). $PM_{2.5\text{-}0.3}$ size distribution, morphology and single particle analysis were performed using the scanning electron microscopy (438 VP; LEO Electron Microscopy Ltd, UK) coupled with energy dispersive X-ray analysis (IXRF, Oxford Instruments, UK) (SEM-EDX). Metals and ionic species were quantified in PM and WF by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES, iCAP 6000 Series, Thermo Scientific, UK), ICP-Mass Spectrometry (ICP-MS, Varian® 820-MS, Varian, USA) and Ion Chromatography (IC, Dionex® DX 100, Thermo Scientific, UK), respectively. Polycyclic aromatic hydrocarbons (PAHs) and linear alkanes were quantified after PM Soxhlet extraction using dichloromethane by gas chromatography-mass spectrometry (GC-MS, model 1200 TQ, Varian, USA). The carbon content was measured using the CHNS/O analyzer (FLASH, 2000; Fisher Scientific®, UK).

2.3. Sample preparation for toxicological studies

To better elucidate the specific role of the organic and/or water soluble component of PM, two fractions were considered. $PM_{2.5\text{-}0.3}$ and $PM_{2.5}$ organic extracts (OEs) were obtained by Soxhlet extraction using dichloromethane at 40 °C for 16 h. Dichloromethane was then evaporated under nitrogen flow and the organic extract reconstituted in dimethyl sulfoxide (DMSO). Water-soluble fractions (WFs) were obtained by lixiviation of $PM_{2.5\text{-}0.3}$ and portions of $PM_{2.5}$ filters in ultra-pure water, followed by 30 min of sonication.

Mutagenicity tests were performed on OEs and WFs of $PM_{2.5\text{-}0.3}$ and $PM_{2.5}$. Native $PM_{2.5\text{-}0.3}$ particulate samples were also tested. In this latter case, $PM_{2.5\text{-}0.3}$ was suspended in DMSO (final concentration <0.5%) and saline buffer, then irradiated using UV lights for 2 h, in order to reduce contamination by the biological fraction.

For the cytotoxicity and proliferation tests, OE and WF were directly mixed with the cell culture medium. Total $PM_{2.5\text{-}0.3}$ was suspended in cell culture medium and dispersed by sonication (5 min) just prior to the exposure. In experiments using OE, the final DMSO concentration in the culture medium does not exceed 0.1%.

2.4. Mutagenicity

Mutagenicity was evaluated using the Ames test with 3 *Salmonella typhimurium* tester strains (TA98, YG1041 and TA102), with (+S9mix) or without (−S9mix) addition of Aroclor-induced S9, using the pre-incubation method detailed in André et al. (2011). For each sample, triplicate measurements were performed at three concentrations: (i) 5, 20 and 50 μg/plate for $PM_{2.5\text{-}0.3}$; (ii) 5, 20 and 50 μg PM-equivalent/plate for $PM_{2.5\text{-}0.3}$ and $PM_{2.5}$ OEs; (iii) 4.1, 8.2, and 16.3 μg PM-equivalent/plate for $PM_{2.5\text{-}0.3}$ WF; (iv) 1.5, 2.9, and 5.8 μg PM-equivalent/plate for $PM_{2.5}$ WF. Positive controls were 2-nitrofluorene (−S9mix) and 2-aminofluorene (+S9mix) for TA98 and YG1041 and *tert*-butyl hydroperoxide (−S9mix) and 2-aminoanthracene (+S9mix) for TA102. Negative controls were