Environmental Pollution 230 (2017) 758–774

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Toxic organic substances and marker compounds in size-segregated urban particulate matter - Implications for involvement in the *in vitro* bioactivity of the extractable organic matter☆

Athanasios Besis [a], Alexandra Tsolakidou [a], Dimitra Balla [a], Constantini Samara [a, *], Dimitra Voutsa [a, **], Anastasia Pantazaki [b], Theodora Choli-Papadopoulou [b], Theodore S. Lialiaris [c]

[a] Environmental Pollution Control Laboratory, Department of Chemistry, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece
[b] Laboratory of Biochemistry, Department of Chemistry, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece
[c] Demokrition University of Thrace, Faculty of Medicine, Department of Genetics, Alexandroupolis 68100, Greece

ARTICLE INFO

Article history:
Received 12 January 2017
Received in revised form
28 May 2017
Accepted 28 June 2017
Available online 18 July 2017

Keywords:
Cytotoxicity
Genotoxicity
Nitro-PAHs
PAHs
PCBs
PBDEs

ABSTRACT

Toxic organic substances and polar organic marker compounds, *i.e.* polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs) and their nitro-derivatives (N-PAHs), as well as dicarboxylic acids (DCAs) and sugars/sugar anhydrites (S/SAs) were analyzed in size-segregated PM samples (<0.49, 0.49–0.97, 0.97–3 and >3 μm) collected at two urban sites (urban traffic and urban background) during the cold and the warm season. The potential associations between the organic PM determinants and the adverse cellular effects (*i.e.* cytotoxicity, genotoxicity, DNA damage, oxidative DNA adduct formation, and inflammatory response) induced by the extractable organic matter (EOM) of PM, previously measured in Velali et al. (2016b), were investigated by bivariate correlations and Principal Component Analysis (PCA). Partial Least Square regression analysis (PLS) was also employed in order to identify the chemical classes mainly involved in the EOM-induced toxicological endpoints in the various particle size fractions. Results indicated that particle size range <0.49 μm was the major carrier of PM mass and organic compounds at both sites. All toxic organic compounds exhibited higher concentrations at the urban traffic site, except PCBs and OCPs that did not exhibit intra-urban variations. Conversely, wintertime levels of levoglucosan were significantly higher at the urban background site as a result of residential biomass burning. The PLS regression analysis allowed quite good prediction of the EOM-induced cytotoxicity and genotoxicity based on the determined organic chemical classes, particularly for the finest size fraction of PM. Nevertheless, it is expected that other chemical constituents, not determined here, also contribute to the measured toxicological responses.

1. Introduction

Urban air particulate matter (PM) contains a broad range of organic substances with vastly different properties (oxidation state, volatility, hygroscopicity, polarity), and extremely diverse sources such as direct emissions from traffic, combustion activities, biogenic emissions, and secondary formation mechanisms in the atmosphere (Hallquist et al., 2009).

Toxic organic substances, such as combustion-derived polycyclic aromatic hydrocarbons (PAHs) and combustion by-products, organochlorine pesticides (OCPs), and banned or restricted industrial chemicals such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), are ubiquitous in urban air PM posing a hazard for human health (WHO, 2003). These substances are mainly accumulated in submicron particles, featuring a 50% or greater organic compound mass (Besis et al., 2015;

☆ This paper has been recommended for acceptance by Prof. von Hippel Frank A..

* Corresponding author.
** Corresponding author.
E-mail addresses: csamara@chem.auth.gr (C. Samara), dvoutsa@chem.auth.gr (D. Voutsa).

http://dx.doi.org/10.1016/j.envpol.2017.06.096
0269-7491/

Chrysikou and Samara, 2009; Degrendele et al., 2014; Lammel et al., 2010; Landlová et al., 2014). Particles in this size range are strongly related to a potential health risk since they penetrate into the deepest parts of respiratory tract, the alveoli.

Organic PM constituents play an important role in genotoxic mechanisms of PM such as adduct-forming potential and oxidative DNA damage (Karlsson et al., 2004; Healey et al., 2005; Hanzalova et al., 2010). Studies showed that the mutagenicity of airborne PM is mainly associated with moderately to highly polar classes of substances that tend to contain nitroaromatics, aromatic amines, and aromatic ketones produced in the atmosphere when organic compounds (even non-mutagenic) are exposed to NOx and sunlight (Valavanidis et al., 2008). Furthermore, several *in vitro* studies with fractionated crude organic complex mixtures clearly demonstrated that the highest mutagenic and DNA adduct-forming potential is associated with the neutral and slightly polar fractions, which contain most of the PAHs and nitro-PAHs derivatives (NPAHs) (Buschini et al., 2001; Topinka et al., 2000). PAHs have been classified as known, probable, or possible human carcinogens, and/or mutagens (IARC, 2012; US EPA, 2012). Moreover, NPAHs have even higher carcinogenic and mutagenic potential compared to parent compounds (10 times and 2×10^5 times, respectively, Durant et al., 1996) contributing significantly to the mutagenicity of atmospheric particles, particularly 1,8-dinitropyrene (Kawanaka et al., 2008). At present, only the class-1 carcinogen Benzo[a]pyrene (BaP) is regulated under the 4th Daughter European Directive 2004/107/EC.

In most *in vitro* studies, the toxic effects induced by the extractable organic matter (EOM) of PM have been investigated using cumulative size fractions, mostly $PM_{2.5}$ or PM_{10} (Chakra et al., 2007; Dergham et al., 2015; Gabelova et al., 2004; Oh et al., 2011; Perrone et al., 2010; Leung et al., 2014), whereas fewer studies have used size-segregated PM (Happo et al., 2013; Healey et al., 2005; Topinka et al., 2015).

Recently, the adverse cellular effects (cytotoxicity, genotoxicity, generic and oxidative DNA damage, and inflammatory mediator secretion), induced by size-segregated water-soluble and extractable organic PM fractions, were measured at an urban traffic and an urban background site of a large Greek city (Thessaloniki) using a variety of end-points (Velali et al., 2016a,b). The cytotoxicity induced by the extractable organic fractions appeared to be considerably lower (around half) than those induced by the water-soluble fraction, while both fractions induced equally significant DNA damage (Velali et al., 2016b).

The major objective of the present study was to investigate the potential associations between the effects induced by the organic PM fractions and the concentrations of low to highly polar classes of organic PM constituents. For this purpose, the size-segregated PM samples from the two urban sites were analyzed for a variety of toxic organic compounds, such as PAHs and NPAHs, PCBs, OCPs, and PBDEs. Dicarboxylic acids (DCAs) and sugars/sugar anhydrites (S/SAs) were also analyzed in the finest particle fraction since, although not toxic, these highly water-soluble compounds increase the solubility of toxic pollutants in aerosol particles, in addition, their increased polarity is thought to amplify the uptake and retention of fine particles within the respiratory system leading to undesirable effects (Perrone et al., 2013). Spatiotemporal variations and intracorrelations of target organic compounds were investigated. Moreover, their associations with the adverse cellular responses induced by the extractable organic PM were investigated in an attempt to better understand the biological mechanisms underlying the toxic effects.

2. Materials and methods

2.1. PM sampling

PM samples were acquired from two sites in the city of Thessaloniki, northern Greece (40°62′N, 22°95′E), representative of urban traffic (UT) and urban background (UB) air pollution. 48-h size-segregated PM sampling was achieved as previously described (Besis et al., 2015; Velali et al., 2016a,b) using two identical 5-stage high-volume cascade impactors (effective cut-off diameters at 7.2, 3.0, 1.5, 0.97 and 0.49 μm) and prebaked quartz filters. PM samples were collected during the cold and the warm months of 2013 (January and July at UT, March and May at UB). For the purposes of the study, the six particle fractions obtained from each sampling event were merged to four: the quasi-ultrafine mode <0.49 μm, the upper accumulation mode (0.49–0.97 μm), the upper fine mode (0.97–3 μm), and the coarse mode (>3.0 μm).

The meteorological conditions (T, RH and wind speed) during the sampling campaigns were as follows: 10 ± 1.8 °C, 64 ± 7.7%, 0.22 ± 0.05 m s^{-1} for UT-cold and 26 ± 2.5 °C, 51 ± 11%, 0.25 ± 0.10 m s^{-1} for UT-warm *vs.* 11 ± 2.8 °C, 66 ± 11%, 2.0 ± 1.0 for UB-cold and 22 ± 1.6 °C, 51 ± 3.6%, 2.4 ± 0.8 m s^{-1} for UB-warm (Samara et al., 2016).

2.2. Extraction and fractionation of organic PM components

Filter samples were extracted and fractionated according to previously developed and validated procedures (Chrysikou, 2009; Chrysikou and Samara, 2009). Briefly, filter segments (1/2 of slotted filters and 1/4 of backup filters) were extracted with DCM/n-hexane (3:2 v/v) in a microwave-assisted extraction unit (CEM MARSX, Model 907600) operated at 1600 Watt under the following conditions: temperature 110 °C, pressure 482.6 kPa, ramp time 20 min, hold time 10 min. After filtration to remove insoluble materials and concentration in a rotary evaporator, organic extracts were fractionated on a glass chromatography column (3 g silica + 2 g alumina + 0.5 g Na_2SO_4). Three organic fractions were successively eluted using n-hexane, DCM/n-hexane (3:2 v/v), and acetone/n-hexane (3:7 v/v) signifying the polar organic fraction (NPOF), the moderately polar organic fraction (MPOF) and the polar organic fraction (POF), respectively. Organic PM fractions were further concentrated by rotary evaporation and divided into two parts, for chemical analyses and *in vitro* measurements. NPOF, MPOF and POF fractions were analyzed for PCBs/OCPs, PAHs and NPAHs, respectively.

PBDEs were extracted separately according to a more intensive procedure described in Besis et al. (2015). Briefly, filter segments (1/2 of slotted filters and 1/4 of backup filters) were treated in a microwave extraction unit using DCM/n-hexane (1:1 v/v). After concentration, extracts were treated with concentrated H_2SO_4, cleaned up through a glass column packed with acidic and neutral silica gel, and fractionated through an activated silica column. This class of organic compounds was assumed to contribute to the bioactivity of the NPOF fraction.

Polar organic markers, including low molecular weight dicarboxylic acids (DCAs) and saccharides/anhydrosaccharides (S/SAs), were extracted by sonication of filter segments (1/4 of backup filters) in DCM/methanol (2:1 v/v). Due to the lack of adequate PM sample from slotted filters, polar organic markers were measured in the <0.49 μm particle size fraction only. This class of organic compounds was assumed to contribute to the bioactivity of the POF fraction.