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Day-to-day variability of toxic events induced by organic compounds bound to size segregated atmospheric aerosol

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ABSTRACT

This study quantified the temporal variability of concentration of carcinogenic polycyclic aromatic hydrocarbons (c-PAHs), genotoxicity, oxidative DNA damage and dioxin-like activity of the extractable organic matter (EOM) of atmospheric aerosol particles of aerodynamic diameter (d_{ae}, μm) coarse ($1 < d_{ae} < 10$), upper- ($0.5 < d_{ae} < 1$) and lower-accumulation ($0.17 < d_{ae} < 0.5$) and ultrafine (<0.17) fractions. The upper accumulation fraction formed most of the aerosol mass for 22 of the 26 study days and contained ~44% of total c-PAHs, while the ultrafine fraction contained only ~11%. DNA adduct levels suggested a crucial contribution of c-PAHs bound to the upper accumulation fraction. The dioxin-like activity was also driven primarily by c-PAH concentrations. In contrast, oxidative DNA damage was not related to c-PAHs, as a negative correlation with c-PAHs was observed. These results suggest that genotoxicity and dioxin-like activity are the major toxic effects of organic compounds bound to size segregated aerosol, while oxidative DNA damage is not induced by EOM.

1. Introduction

Complex mixtures of organic compounds, including polycyclic aromatic hydrocarbons (PAHs), are present in various size fractions in air particulate matter (PM) and have been associated with many adverse health effects in humans, such as respiratory diseases and cancer. These conclusions from experimental and epidemiological findings were the major reasons why the International Agency for Research on Cancer (IARC) in 2013 classified outdoor air pollution as carcinogenic to humans (Group 1) (IARC, 2013).

Organic aromatic compounds bound to atmospheric PM may exert their toxicity by various mechanisms including genotoxicity (formation of DNA adducts, DNA strand breaks) caused mainly by c-PAH metabolism leading to DNA-reactive metabolites such as PAH-diolepoxides (Melendez-Colon et al., 2000), oxidative DNA damage, proteins and lipids induced mostly by alternative PAH-metabolism leading to the formation of quinones (Park et al., 2006; Hanzalová et al., 2010). Moreover, non-genotoxic mechanisms of toxicity of airborne complex mixtures associated with aryl hydrocarbon receptor (AhR)-mediated gene expression ("dioxin-like activity") were observed (Andrysík et al., 2011; Líbalová et al., 2012).

Most previous studies on the toxic effects of ambient air pollution used either cumulative PM size fraction (mostly $PM_{2.5}$ or PM_{10}) (Perrone et al., 2010; Leung et al., 2014), although the deposition of PM in human lung strongly depends on PM particle size (Heyder et al., 1986). Some studies used pooled PM samples collected during long-term sampling periods (Topinka et al., 2010, 2011), which may have been a limitation particularly for short-

Abbreviations: 8-oxodG, 8-oxodeoxyguanosine; d_{ae}, aerodynamic diameter; B[a]P, benzo[a]pyrene; B[b]F, benzo[b]fluoranthene; B[k]F, benzo[k]fluoranthene; B[a]A, benz[a]anthracene; BPDE, benzo[a]pyrene-r-7,t-8-dihydrodiol-t-9,10-epoxide [±]; c-PAHs, carcinogenic polycyclic aromatic hydrocarbons; CHRY, chrysene; CT DNA, calf-thymus DNA; DRZ, diagonal radioactive zone; DB[al]P, dibenzo[a,l]pyrene; DB[ah]A, dibenz[a,h]anthracene; DCM, dichlormethane; 7,12-DMBA, 7,12-dimethylbenz[a]anthracene; DMSO, dimethylsulfoxide; EOM, extractable organic matter; HPLC, high performance liquid chromatography; I[cd]P, indeno[1,2,3-c,d]pyrene; PAH, polycyclic aromatic hydrocarbons; PM, particulate matter; PM_{10}, particulate matter of aerodynamic diameter <10 μm; TEQ, toxic equivalency factor; WHO, World Health Organization.

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term effects. Other studies tried to use chemical characterisation of PM, mostly at the PAH-level, and then calculated total relative toxic equivalency factors (TEF) for mixtures based on the relative toxic potencies of the mixture's components relative to a standard compound, i.e. benzo[a]pyrene (B[a]P) (Halek et al., 2008; Fisher et al., 2011). However, this approach did not take into account the interactions of components leading to synergistic and antagonistic effects of PM components.

In contrast to previous studies, we used a 4-stage cascade impactor to simultaneously sample ambient air particles of different aerodynamic diameter at a sampling site representing one of the highest air pollution levels in Europe, the district of Ostrava-Radvanice and Bartovice (Czech Republic). Coal combustion, iron producing technologies, and traffic were sources of atmospheric aerosols in the district during the winter of 2012 (Pokorná et al., 2015). The c-PAHs bound to PM, namely benz[a]anthracene (B[a]A), chrysene (CHRY), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo[a]pyrene (B[a]P), dibenz[a,h]anthracene DB[ah]A, and indeno[1,2,3-cd]pyrene (I[cd]P), genotoxic potential, oxidative DNA damage and dioxin activity of organic extracts from PM (EOM) were determined on a daily basis for 26 consecutive days. To our knowledge, this is the first time that c-PAHs together with the toxicity markers listed above were determined in size fractionated PM on a daily basis to assess size and time variability of all toxicity parameters. We aimed to use relatively simple methods covering major toxic events to relate PM mass in 4 size fractions and c-PAH bound to PM with 3 major toxic endpoints determined in vitro (genotoxic potential, oxidative DNA damage and dioxin-like activity). Moreover, these relationships were assessed in 26 consecutive daily PM samples, i.e. in a relatively dense time period.

2. Materials and methods

2.1. Chemicals and biochemicals

Spleen phosphodiesterase was purchased from ICN Biomedicals, Inc.; micrococcal nuclease, and calf thymus DNA (CT DNA) were from Sigma (Deisenhofen, Germany); nuclease P1 was from Wako Chemicals, Japan; polyethylene-imine cellulose TLC plates (0.1 mm) were from Macherey–Nagel (Düren, Germany); c-PAHs (99% pure) were from Supelco, Inc.; T4 polynucleotide kinase (USB); and γ-^{32}P-ATP (3000 Ci/mmol, 10 μCi/μl) were from Perkin Elmer. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) was purchased from Cambridge Isotope Laboratories (Andover, MA); All other chemicals and solvents were of HPLC or analytical grade.

2.2. PM sampling

Prior to air sampling, the PUF substrates were cleaned in accordance with the BGI manual (BGI 900 High Volume Cascade Impactor, Guidance Manual, 2008). The PUF's cleaning consisted of consecutive leaching, hourly for each solvent, in ultrapure water, hexane, methanol, and dichlormethane in an ultrasonic bath. After the sonication, the substrates were dried in a clean air hood for 48 h. For weighing, the substrates and the ultra-filters were equilibrated for 24 h in a desiccator (~50% humidity, saturated $Mg(NO_3)_2$*$6H_2O$, T = 21 °C) and weighed at air-conditioned weighing room, wrapped in aluminum foil, placed in double sealed plastic bags with a zipper and stored in a sealed container in a laboratory. Immediately after the air sampling, the exposed substrates were weighed under the same conditions and stored at −20 °C till the chemical analysis. Two sets of field blanks were prepared and were manipulated as real samples except air sampling. Both aerosol mass and chemical species concentrations were blank corrected; nevertheless blank values were always well below 1% of concentrations of all the parameters determined. Coarse ($1 < d_{ae} < 10$ μm), upper accumulation ($0.5 < d_{ae} < 1$ μm), lower accumulation ($0.17 < d_{ae} < 0.5$ μm) aerosol particles were collected on polyurethane foam (PUF) and the smallest aerosol particles of $d_{ae} < 0.17$ μm, in this study termed quazi-ultrafine particles (qUF), were trapped on PTFE-coated Glass Micro-Fiber Absolute filters (Pallflex 70 TX40). The aerosol was sampled at a flow rate of 900 l/min by means of a HiVol cascade impactor (BGI 900, USA), which has the smallest attainable cut-off at 0.17 μm. Aerosol samples were consecutively collected with an integrating time of 23 h from January 26th to February 21st 2012 in a residential district of Ostrava-Radvanice and Bartovice (49°48′40.4″N, 18°20′15.8″E). The impactor was positioned on the roof of a mobile station at a height of 4 m. The PUF's substrates trap in some extent also gaseous PAH, i.e. exhibit positive sampling artifact. To evaluate this artifact, an additional PUF substrate layer was placed after the ultra-filters to trap exclusively gaseous PAH. Then, the after-filter PUF substrate was analyzed for 13 PAH including c-PAH. The positive sampling artifact for specific PAH varied with an ambient air temperature and diameter of aerosol particles and due to complexity will be subject of separate report. Nevertheless, sampling artifact was found negligible for PAH of the vapor pressure below 10^{-4} Pa, which applies for all the c-PAH evaluated in this paper.

2.3. PUF and filter extraction and PAH analysis

Both the PUFs and the absolute filters were extracted by dichloromethane. The chemical analysis of PAHs was performed in the laboratories of a certified company, ALS Czech Republic s.r.o., Prague (EN ISO CSN IEC 17025), by HPLC with fluorescence detection according to the standard procedures set in ISO 11338-2. The recovery of PAH extraction is controlled by laboratory control spikes. It is permanently stable (~80%). The PAH concentrations were blank corrected. The concentrations of seven of the PAHs regarded by the IARC as carcinogenic, namely B[a]A, CHRY, B[b]F, B[k]F, B[a]P, DB[ah]A, and I[cd]P, were analyzed in each EOM sample (of these, B[a]P is classified as carcinogenic to humans). For the in vitro experiments, EOM samples were evaporated under a stream of nitrogen. The residue was redissolved in dimethyl sulfoxide (DMSO). Samples were kept in a freezer at −80 °C until analysis.

2.4. Incubation of EOMs with CT DNA (in vitro acellular assay)

The assay was performed as previously described (Binkova et al., 2007). Rat liver S9 fraction was purchased from Toxila (Pardubice, Czech Republic). B[a]P (1 μM) and DMSO treated CT DNA samples were used as positive and negative controls, respectively.

2.5. DNA adduct analysis

Calf thymus DNA (CT DNA) was isolated by phenol/chloroform/isoamylalcohol extraction and ethanol precipitation (Gupta, 1985). ^{32}P-postlabeling analysis of DNA adducts was performed as described previously (Phillips and Castegnaro, 1999; Reddy and Randerath, 1986). A BPDE-DNA adduct standard was run in triplicate in each postlabeling experiment in order to check for inter-assay variation.

2.6. Oxidative DNA damage

After incubation with/without the S9 fraction described in 2.4., DNA was purified as reported by Rossner (Rossner et al., 2009) omitting the RNAse and proteinase K incubation steps. Levels of 8-oxodeoxyguanosine (8-oxodG) in DNA were analyzed using the