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An acellular assay to assess the genotoxicity of complex mixtures of organic pollutants bound on size segregated aerosol. Part I: DNA adducts

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ABSTRACT

An acellular assay consisting of calf thymus DNA with/without rat liver microsomal S9 fraction was used to study the genotoxicity of complex mixtures of organic air pollutants bound to size segregated aerosols by means of DNA adduct analysis. We compared the genotoxicity of the organic extracts (EOMs) from three size fractions of aerosol ranging from 0.17 μm to 10 μm that were collected by high volume cascade impactors in four localities of the Czech Republic differing in the extent of the environmental pollution: (1) small village in proximity of a strip mine, (2) highway, (3) city center of Prague and (4) background station. The total DNA adduct levels induced by 100 μg/ml of EOMs were analyzed by ^{32}P-postlabelling analysis with a nuclease P1 method for adduct enrichment. The main finding of the study was most of the observed genotoxicity was connected with a fine particulate matter fraction (<1 μm). The concentrations of carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) in EOMs indicate that fine fractions (0.5–1 μm) bound the highest amount of c-PAHs in all aerosol sampling sites, which might be related to the higher specific surface of this fraction as compared with a course fraction (1–10 μm) and higher mass as compared with a condensational fraction (0.17–0.5 μm). As for aerosol mass, both fine and condensational fractions are effective carriers of c-PAHs. Similarly, the DNA adduct levels per m^3 of air were highest for the fine fraction, while the condensational fraction (strip mine site and city center) revealed the highest DNA adduct levels in cases where aerosol mass is taken into consideration. A strong correlation was found between the c-PAHs and DNA adduct levels induced by EOMs in all the localities and for various size fractions ($R^2 = 0.98$, $p < 0.001$). It may be concluded that the analysis of total DNA adducts induced in an acellular assay with/without metabolic activation represents a relatively simple method to assess the genotoxic potential of various complex mixtures.

1. Introduction

Much effort has been put into clarification of the adverse effects of environmental pollution on human health (Lewtas, 2007). Respirable ambient air particulate matter (PM) of an aerodynamic diameter <2.5 μm (PM2.5) comprises a complex mixture consisting of a large number of chemicals, many of which are toxic and/or carcinogenic (Harrison et al., 2004). Investigation of the biological effects of ambient air PM has involved a number of different approaches, including the study of particle induced genotoxicity. The genotoxic and mutagenic effects of the ambient air PM is most frequently connected with chemicals bound on the surface of the PM and/or with the particles themselves (Binkova et al., 2003; Karlsson et al., 2004; Claxton and Woodall, 2007). Some studies have suggested that genotoxic effects of the PM are induced by carcinogenic polycyclic hydrocarbons (c-PAHs) and their derivatives forming organic fractions of the PM (Topinka et al., 2000; Binkova and Sram, 2004; Lewtas, 2007). Other studies indicated that some metals and/or organic compounds forming PM may catalyze the oxidative damage of DNA (Ghio et al., 1999; Prahalad et al., 2001; Knaapen et al., 2002; Hanzalova et al., 2010). Complex mixtures of organic compounds, which the general population is exposed to via air pollution, are not completely characterized

Abbreviations: B[a]P, benzo[a]pyrene; B[b]F, benzo[b]fluoranthene; B[k]F, benzo[k]fluoranthene; B[a]A, benz[a]anthracene; B[ghi]P, benzo[ghi]perylene; BPDE, benzo[a]pyrene-r-7,t-8-dihydrodiol-t-9,10-epoxide[±]; c-PAHs, carcinogenic polycyclic aromatic hydrocarbons; CHRY, chrysene; DRZ, diagonal radioactive zone; DB[al]P, dibenzo[al]pyrene; DB[ah]A, dibenz[ah]anthracene; DCM, dichlormethane; 7,12-DMBA, 7,12-dimethylbenz[a]anthracene; EOM, extractable organic matter; HPLC, high performance liquid chromatography; I[cd]P, indeno[cd]pyrene; PM2.5, particulate matter < 2.5 μm; RAL, relative adduct labelling; SDS, sodium dodecyl sulfate; TLC, thin layer chromatography.

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since complex chemical analysis is a very difficult task. Therefore, bioassay-directed investigations of mutagenicity and/or direct or indirect reactivity of DNA with complex mixture components might represent a suitable alternative (Marvin and Hewitt, 2007; Binkova et al., 2007).

As mentioned above, one of the most important components of the complex mixtures of the air pollutants are c-PAHs. These compounds are formed during various combustion processes and might be metabolized to corresponding diol-epoxides exhibiting mutagenic and carcinogenic effects (IARC, 1983; Mersch-Sundermann et al., 1992; White, 2002). It has been repeatedly demonstrated that c-PAHs, formed after metabolic activation by cytochrome P450 enzymes, produce stable bulky DNA adducts that are detectable by ^{32}P-postlabelling (Hemminki et al., 1994; Dipple, 1995; Khalili et al., 2000). This activation system was also used in an acellular assay of genotoxicity based on the analysis of DNA adducts in calf thymus DNA in the presence/absence of the rat microsomal S9 fraction containing a mixture of PAH-metabolizing enzymes (Adams et al., 1996; Reddy et al., 1997; Pohjola et al., 2003; Binkova et al., 2007; Sevastyanova et al., 2008).

The relative proportion of the organic fraction of PM mass is known to vary with particle size. Therefore, concentrations of the c-PAHs and appropriate genotoxic effects are also supposed to vary with particle size. It has been repeatedly demonstrated that lung deposition of the ambient air aerosols and the PAHs bound on them depends on the aerodynamic diameter (Venkataraman and Raymond, 1998; Kawanaka et al., 2009). Therefore, it might be of great interest to quantify the effect of particle size on the c-PAH quantities bound on aerosols and the genotoxicity of organic extracts from PM samples segregated in three size fractions. For this purpose, aerosols were collected using high volume cascade impactors at four localities of the Czech Republic differing by their extent of the air pollution. Sampling was carried out during the winter period when high air pollution levels were expected. Extracted organic matter (EOM) of the aerosol samples were analyzed for c-PAHs and were used in genotoxic tests. In this part of the study (Part I) we used an acellular test coupled with ^{32}P-postlabelling to compare DNA adduct forming activity of organic extracts (EOM) from size segregated aerosols (0.17–10 μm). To further analyze the possible contribution of some EOM components and their metabolites to oxidative DNA damage, we simultaneously analyzed 8-oxodG levels in the same model system (Part II, see tandem paper in this issue).

2. Materials and methods

2.1. Chemicals and biochemicals

Spleen phosphodiesterase was purchased from ICN Biomedicals, Inc.; micrococcal nuclease, nuclease P1 from Sigma (Deisenhofen, Germany); polyethylene-imine cellulose TLC plates (0.1 mm) from Macherey–Nagel (Düren, Germany); c-PAHs (99% pure) from Supelco, Inc.; T4 polynucleotide kinase (USB); and γ-^{32}P-ATP (3000 Ci/mmol, 10 μCi/μl) from Perkin Elmer. All other chemicals and solvents were of HPLC or analytical grade.

2.2. Air sampling, EOM extraction and chemical analysis

Coarse (1–10 μm), fine (0.5–1 μm) and condensational (0.17–0.5 μm) aerosol fractions were collected on polyurethane foam (PUF) consecutively in four various localities by HiVol cascade impactors (BGI 900 samplers, U.S.A.) for 24 h daily during February 2009 to March 2009. Sampling sites included: Brezno (Chomutov district), which is a village in a highly industrialized region of northern Bohemia in proximity to an open cast lignite mine and coal power station; Dobre Stesti (Pilsen district), which is in proximity to the D-5 highway; city center of Prague, where the device was in place on a roof-top station in a university botanic garden; and the background station Laz, which is located in a clean area south-west from the city of Pribram in a forest. The impactor was positioned on the roof of a mobile station at a height of 4 m. In Prague, the roof-top station was at a height of 25 m above street level.

Polyurethane foams (PUFs) were extracted by dichlormethane. 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). The concentrations of

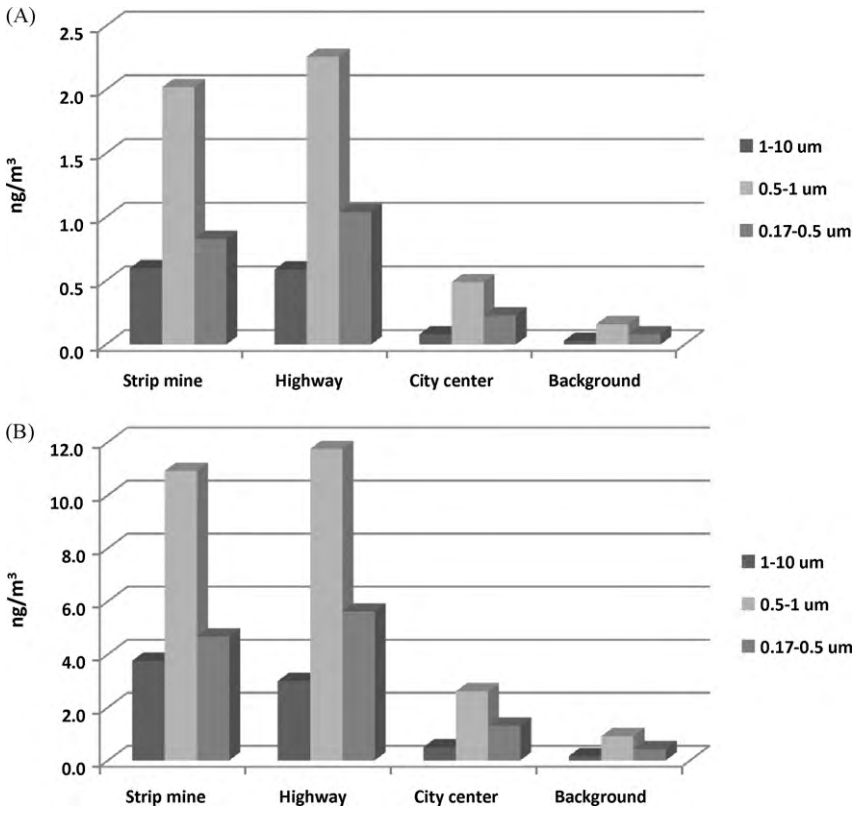

Fig. 1. B[a]P (A) and the sum of carcinogenic PAHs (B) in the air. Carcinogenic PAHs bound on the aerosols collected on polyurethane foams (PUFs) were extracted by dichlormethane and analyzed as described in Section 2. The values are normalized per m^3 of the air.