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Ultrafine particles are not major carriers of carcinogenic PAHs and their genotoxicity in size-segregated aerosols

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ABSTRACT

Some studies suggest that genotoxic effects of combustion-related aerosols are induced by carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) and their derivatives, which are part of the organic fraction of the particulate matter (PM) in ambient air. The proportion of the organic fraction in PM is known to vary with particle size. The ultrafine fraction is hypothesized to be the most important carrier of c-PAHs, since it possesses the highest specific surface area of PM. To test this hypothesis, the distribution of c-PAHs in organic extracts (EOMs) was compared for four size fractions of ambient-air aerosols: coarse ($1 < d_{ae} < 10\mu m$), upper ($0.5 < d_{ae} < 1\ \mu m$), and lower ($0.17 < d_{ae} < 0.5\ \mu m$) accumulation aerosol particles and ultrafine particles ($d_{ae} < 0.17$). High-volume aerosol samples were collected consecutively in four localities that differed in the level of environmental pollution. The genotoxicity of EOMs was measured by analysis of DNA adducts induced in an a cellular assay consisting of calf thymus DNA with/without rat liver microsomal S9 fraction coupled with ^{32}P-postlabelling. The upper accumulation fraction was the major size fraction in all four localities, forming 37–46% of the total PM mass. Per m^3 of sampled air, this fraction also bound the largest amount of c-PAHs. Correspondingly, the upper accumulation fraction induced the highest DNA-adduct levels. Per PM mass itself, the lower accumulation fraction is seen to be the most efficient in binding DNA-reactive organic compounds. Interestingly, the results suggest that the fraction of ultrafine particles of various ambient-air samples is neither a major carrier of c-PAHs, nor a major inducer of their genotoxicity, which is an important finding that is relevant to the toxicity and health effects of ultrafine particles, which are so extensively discussed these days.

1. Introduction

Recent scientific knowledge indicates gaps in the current policy framework, leaving health-relevant size fractions of particulate matter (PM) of ambient air pollution unregulated, thus jeopardizing public health through the continuing presence of particles with aerodynamic diameter (d_{ae}) of submicron ($d_{ae} < 1\ \mu m$) and ultrafine ($d_{ae} < 0.1\ \mu m$) dimensions. Although many studies indicate that the toxic effects of respirable aerosol particles are connected with inflammation and oxidative damage [1], other studies have suggested that particular genotoxic effects of combustion-related PM are mainly induced by carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) and their derivatives, which are part of the organic fraction of PM in ambient outdoor air [2–4]. The proportion of the organic fraction in PM mass is known to vary with particle size [5–7]. Therefore, concentrations of the c-PAHs and their toxic effects are also believed to vary with particle size. It is hypothesized that the ultrafine fraction of PM is the most important carrier of c-PAHs, since it dominates particle-number concentrations and possesses the highest specific surface area of the PM. The question arises whether or not the ultrafine particles, which are known to penetrate deeply into the lung [8], are the most significant (per m^3 of air) and most effective (per mg of PM mass) carriers of toxic organic compounds such as c-PAHs. Previous studies [9] summarizing data on the chemical characteristics of aerosols in Europe are mostly concentrated in the Mediterranean area and

Abbreviations: 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; 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; DMSO, dimethylsulfoxide; EOM, extractable organic matter; HPLC, high performance liquid chromatography; I[cd]P, indeno[cd]pyrene; PM, particulate matter; PM10, particulate matter of aerodynamic diameter <10 μm; WHO, World Health Organization.

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lack data from Eastern Europe, where specific sources of pollution may be expected, such as heavy industry based on old technology (ironworks, brown-coal mines, coke ovens), local heating systems combusting fossil fuels, and many old cars not equipped with up-to-date catalysts and particle filters.

The present study aimed to quantify the c-PAH content and the genotoxicity of the organic extracts (EOMs) in various PM fractions, including particles of $d_{ae} < 0.17$ μm, which comprise mainly the ultrafine fraction. We compared this fraction with three other PM size-fractions of d_{ae} between 0.17 μm and 10 μm in various sampling localities of the Czech Republic that differed in the extent and sources of air pollution.

2. Material and methods

2.1. Chemicals and biochemicals

Spleen phosphodiesterase was purchased from ICN Biomedicals, Inc.; micrococcal nuclease and 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 from 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 ($1 < d_{ae} < 10$ 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 ultrafine, were trapped on PTFE-coated Glass Micro-Fiber Absolute filters (Pallflex TX40). Aerosol was sampled by means of a HiVol cascade impactor (BGI 900, USA), which has the smallest attainable cut-off at 0.17 μm. Since particle-number concentration of such sizes are dominated by particles of $d_{ae} < 0.1$ μm, it is correct and appropriate for this study to term them ultrafine particles. The sampling was done consecutively in four localities for 24 h on daily basis in each locality, with interruptions needed for experimental set-up, during January–March 2010. Sampling localities included: Brezno (Chomutov district), 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 D5 highway; city center of Prague, where the device was in place on a roof-top station in a university botanic garden; and the background locality, Laz, which is located in a clean area south-west from the city of Pribram, in a temperate 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.

Both PUFs and 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 use of HPLC with fluorescence detection according to the standard procedures set in ISO 11338-2. The concentrations of seven of the PAHs regarded by the IARC [10] as carcinogenic in experimental animals, 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), were analyzed in each EOM sample (among these, B[a]P is classified as carcinogenic to humans).

For the *in vitro* experiments, EOM samples were evaporated under a stream of nitrogen. 1,2-Propanediol (100 μl) was used as a keeper to avoid outflow of c-PAH in the course of the evaporation. The residue was re-dissolved in dimethyl sulfoxide (DMSO). The stock solution of each EOM sample contained 50 mg EOM/ml DMSO. Samples were kept in a freezer at −80 °C until analysis.

2.3. In vitro a cellular assay for DNA-adduct analysis

The assay was performed as previously described [11,3]. Briefly, calf-thymus DNA (1 mg/ml) was incubated with various EOM samples (100 μg EOM/ml) for 24 h at 37 °C with and without metabolic activation by use of an S9 fraction (1 mg protein/ml) from rat liver, which was purchased from Toxila (Pardubice, Czech Republic). Metabolic enzymes were induced in male Wistar rats (~250 g) by the mixture of polychlorinated biphenyls Delor 106 diluted in olive oil to the concentration of 200 mg/ml. Rats received a single i.p. injection of 0.25 ml/100 g bw. Animals were sacrified by cervical dislocation five days later under ether narcosis. B[a]P- and DMSO-treated calf-thymus DNA samples were used as positive and negative controls, respectively. DNA was isolated by phenol/chloroform/isoamylalcohol extraction and ethanol precipitation [12]; samples were kept at −80 °C until analysis.

[^{32}P]-postlabelling analysis was performed as previously described [13,14]. Briefly, DNA samples (6 μg) were digested with a mixture of micrococcal endonuclease and spleen phospho diesterase for 4 h at 37 °C. Nuclease P1 was used for adduct enrichment. The labeled DNA adducts were resolved by multidirectional thin layer chromatography on 10-cm × 10-cm PEI-cellulose plates. Solvent systems used for TLC were the following: D-1: 1 M sodium phosphate, pH 6.8; D-2: 3.8 M lithium formate, 8.5 M urea, pH 3.5; D-3: 0.8 M lithium chloride, 0.5 M Tris, 8.5 M urea, pH 8.0; D4 = D1. Autoradiography was carried out at −80 °C for 6–24 h. The radioactivity of distinct adduct spots and diagonal radioactive zones was measured by liquid scintillation counting. To determine the exact amount of DNA in each sample, aliquots of the enzymatic digest (1 μg of DNA hydrolysate) were analyzed for nucleotide content by reverse-phase HPLC with UV detection, which simultaneously allowed to control the purity of the DNA as well. DNA-adduct levels were expressed as number of adducts per 10^8 nucleotides. A BPDE-DNA adduct standard was run in triplicate in each postlabelling experiment in order to check for inter-assay variation.

3. Results and discussion

3.1. Distribution of mass and c-PAHs among PM size-fractions

In order to determine the relative abundance of various aerosol size-fractions and their ability to bind c-PAHs, we used a high-volume (HiVol) cascade impactor (BGI 900, USA) which aerodynamically classifies the particles in the ambient air into coarse ($1 < d_{ae} < 10$ μm), upper ($0.5 < d_{ae} < 1$ μm), and lower ($0.17 < d_{ae} < 0.5$ μm) accumulation, and ultrafine ($d_{ae} < 0.17$) size fractions. Detailed characteristics of the sampling and the PM samples, including the absolute and relative mass abundance of individual fractions of the aerosols collected, are summarized in Table 1. The mass distributions among the size fractions differ substantially among the various localities. Nevertheless, the upper accumulation fraction was the major size fraction in all four localities (37–46%). The ultrafine fraction was highly abundant at the

Table 1
Characteristics of samples of size-segregated aerosols collected in sampling localities.

Monitoring site [GPS coordinates]	Sampling period	Air volume [m^3]	Size fraction – d_{ae} [μm]	PM [μg/m^3]	Mass fraction [%]
Strip mine (Brezno) [50°24′1″ N°25′20″ E][50°24′1″ N, 13°25′20″ E]	14.1.–24.1.2010	13,670	1–10	10.7	29.2
			0.5–1	17.0	46.4
			0.17–0.5	6.3	17.1
			<0.17	2.7	7.3
Highway (Dobre Stesti) [49°40′58″ N°18′9″ E][49°40′58″ N, 13°18′9″ E]	29.1.–6.2.2010[a]	11,670	1–10	6.8	33.5
			0.5–1	9.1	44.4
			0.17–0.5	3.0	14.9
			≤0.17	1.5	7.2
City center (Prague) [50°4′19″ N°25′25″ E][50°4′19″ N, 14°25′25″ E]	4.3.–17.3.2010	11,610	1–10	8.9	31.2
			0.5–1	10.5	36.8
			0.17–0.5	5.2	18.2
			<0.17	3.9	13.8
Background station (Laz) [49°39′35″ N°53′455″ E][49°39′35″ N, 13°53′455″ E]	20.2.–26.2.2010	8770	1–10	1.3	20.3
			0.5–1	2.4	37.5
			0.17–0.5	1.0	15.6
			<0.17	1.7	26.6

[a] March 3–4 and 12–13 sample collection was interrupted for technical reasons.