Nanoparticles are not major carriers of carcinogenic PAHs in the size segregated aerosol

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Background

Some studies have suggested that genotoxic effects of the combustion related aerosol are induced by carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) and their derivatives forming organic fraction of the ambient air particulate matter. 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 is generally believed that ultrafine fraction of the ambient air PM is the most important carrier of c-PAHs.

Aims of the study

1. To determine relative abundance of various aerosol size fractions and their abilities to bind c-PAHs.
2. To compare the genotoxicity of the size segregated aerosols collected in various localities exhibiting different levels of the air pollution.
3. To evaluate the contribution of ultrafine particles (nanoparticles) to the total content of c-PAHs and their genotoxicity.

Methods

Sampling: Coarse (1-10 μm), fine (0.5-1 μm), condensation (0.17-0.5 μm), and ultrafine (<0.17 μm) aerosol fractions were collected on polyurethane foam (PUF) and teflon coated filters (for ultrafine fraction) consecutively in four various localities by HiVol cascade impactors (BGI 900 samplers, U.S.A.) 24 hours daily during January – March of 2010. Sampling sites include Brezno (industrial locality beside the strip mine), Dobre Stesti (beside the highway), city center of Prague and the background station Laz (located in a clean area south-west from the city of Pribram in forest).
EOM extraction and chemical analysis: Polyurethane foams (PUFs) and filters were extracted by dichlormethane. The chemical analysis of PAHs was performed in the laboratories of the certified company ALS Czech Republic s.r.o., Prague (EN ISO CSN IEC 17025). The concentrations of seven polycyclic aromatic hydrocarbons (PAHs) regarded as carcinogenic PAHs (c–PAHs) according to IARC, namely, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, and indeno[1,2,3-cd]pyrene (I[cd]P) were analyzed in each EOM sample.
In vitro acellular assay and DNA adduct analysis: Calf thymus DNA (1 mg/ml) was incubated with EOM samples (100 µg EOM/ml) for 24 hours at 37^{o} C under aerobic condititions with/without rat liver microsomal S9- fraction (Pardubice-Rybitví) [1]. DNA adduct levels were analyzed by ^{32}P-postlabeling with nuclease P1 method for adduct enrichment [2].

Results

Basic aerosol sampling characteristics are shown in **Table 1** demonstrating that 8,700 – 13,700 m^3 of the air was sampled in periods indicated. The relative mass distribution among size fractions differs substantially among various localities. Condensational fraction (0.5 - 1μm) is the major size fraction in all 4 localities (37 - 46%). Ultrafine fraction (< 0.17 μm) is relatively most abundant in background station (~27%) while in the proximity of strip mine is the lowest (~7%). The concentrations of c-PAHs and B[a]P in EOMs prepared from various size fractions indicate that fine fraction (0.5 – 1 μm) bounds the highest amount of these compounds in all localities, which might be particularly connected with a high mass proportion of this fraction in the total aerosols (**Table 1**). In contrast, ultrafine fraction bounds the lowest c-PAHs and B[a]P mass.

Table 1: Characteristics of HiVol samples of size segregated aerosols collected in various localities

Monitoring site [GPS coordinates]	Sampling period	Air volume [m^3]	Size fraction [μm]	PM [μg/m^3]	Mass fraction [%]
Strip mine (Brezno) [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, 13°18´9´´E]	29.1.-6.2. 2010	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 centre (Prague) [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, 13°53´45´´E]	20.2.-24.2. 2010	8,770	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

As per the aerosol mass, B[a]P and c-PAHs were predominantly bound to the fine and condensational fractions to the same extent with exception of the higway, where B[a]P and c-PAHs concentrations were were equal or even higher in the ultrafine fractions**.**
In contrast to the total c-PAH concentrations, the ratios of individual c-PAHs did not vary significantly with exception of chrysene which is more abundant in course fraction in the background station. The most toxic c-PAH, B[a]P, formed 10-18% of total c-PAHs in all localities and PM size fractions.
The autoradiographs obtained by ^{32}P-postlabelling analysis of calf thymus DNA samples incubated for 24 hours with various EOMs from size segregated aerosols exhibit diagonal radioactive zones (DRZ), representing the total DNA adduct levels induced by the compex mixture. Since c-PAHs needs S9-mediated metabolic activation to exert their genotoxicity, we may estimate the substantial contribution of c-PAHs from the much higher intensities of DRZ for +S9-samples in comparison with –S9 samples.
The total DNA adduct levels induced by EOMs from size segregated aerosols and normalized per m^3 of sampled air suggest a crucial contribution of the fine aerosol fraction (0.5 – 1 μm) to the total PM genotoxicity despite the presence/absence of the metabolic activation (+S9/-S9). Correspondingly to the lowest c-PAHs content, ultrafine fraction represents the lowest genotoxicity.

To answer the question of which size fraction of the aerosol is the most efficient carrier of the genotoxic compounds bound on PM, it would be more appropriate to normalize

DNA adduct levels on the mass of corresponding aerosol fractions. Under such conditions our results indicate that condensational fraction (0.17 – 0.5 μm) is the most efficient to bind DNA adduct forming compounds in all sampling sites. Ultrafine fraction (< 0.17 μm) exhibited significant but lower genotoxicity.

Conclusions

1. When PM mass per m^3 of the air is taken into the consideration, fine particles (0.5 - 1 μm) are the most important carriers of c-PAHs and exhibits highest proportion of the genotoxicity of the total ambient air aerosol.

2. When PM mass itself is taken into the consideration, both fine (0.5 - 1 μm) and condensational fraction (0.17 – 0.5 μm) are the most efficient carriers of c-PAHs although condensational fraction induced the highest genotoxicity.

3. The results suggest that ultrafine fraction (< 0.17 μm) of various ambient air aerosols is neither most effective carrier of c-PAHs (with exception of sampling site in the proximity of highway) nor most effective inducer of their genotoxicity.

References

[1] Adams, S.P., Laws, G.M., Storer, R.D., DeLuca, J.G., Nichols, W.W., Mutat. Res. 368, 1996, 235-248.
[2] Reddy, M.V., Randerath, K., Carcinogenesis 7, 1986, 1543–1551.

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BACKGROUND

Some studies have suggested that genotoxic effects of the combustion related aerosol are induced by carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) and their derivatives forming organic fraction of the ambient air particulate matter. 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 is generally believed that ultrafine fraction of the ambient air PM is the most important carrier of c-PAHs.

AIMS

1. To determine relative abundance of various aerosol size fractions and their abilities to bind c-PAHs.
2. To compare the genotoxicity of the size segregated aerosols collected in various localities exhibiting different levels of the air pollution.
3. To evaluate the contribution of ultrafine particles (nanoparticles) to the total content of c-PAHs and their genotoxicity.

RESULTS

Table 1. Characteristics of HiVol samples of size segregated aerosols collected in various localities

Monitoring site fraction [GPS coordinates]	Sampling period	Air volume [m³]	Size fraction [µm]	PM [µg/m³]	Mass [%]
Strip mine (Brezno) [50°24´1´´N, 13°25´20´´E]	14.1.-24.1. 2010	13,670	1 - 10 0.5 - 1 0.17 - 0.50 <0.17	10.7 17.0 6.3 2.7	29.2 46.4 17.1 7.3
Highway (Dobre Stesti) [49°40´58´´N, 13°18´9´´E]	29.1. - 6.2. 2010	11,670	1 - 10 0.5 - 1 0.17 - 0.5 <0.17	6.8 9.1 3.0 1.5	33.5 44.4 14.9 7.2
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Background station (Laz) [49°39´35´´N, 13°53´45´´E]	20.2. - 24.2.2010	8,770	1 - 10 0.5 - 1 0.17 - 0.5 <0.17	1.3 2.4 1.0 1.7	20.3 37.5 15.6 26.6

CONCLUSIONS

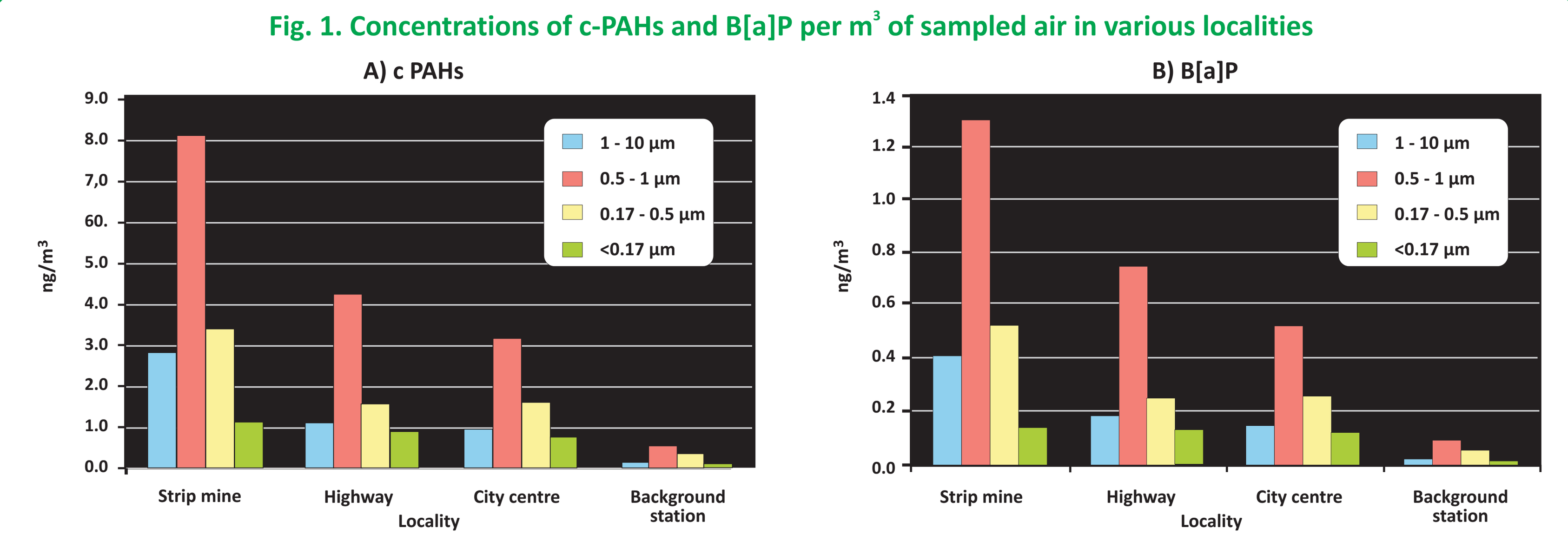
- When PM mass per m³ of the air is taken into the consideration, fine particles (0.5 - 1 µm) are the most important carriers of c-PAHs and exhibits highest proportion of the genotoxicity of the total ambient air aerosol.
- When PM mass itself is taken into the consideration, both fine (0.5 - 1 µm) and condensational fraction (0.17 - 0.5 µm) are the most efficient carriers of c-PAHs although condensational fraction induced the highest genotoxicity.
- The results suggest that ultrafine fraction (<0.17 µm) of various ambient air aerosols is neither most effective carrier of c-PAHs (with exception of highway) nor most effective inducer of their genotoxicity.

Basic aerosol sampling characteristics are shown in Table 1 demonstrating that 8,700 – 13,700 m³ of the air was sampled in periods indicated. The relative mass distribution among size fractions differs substantially among various localities. Condensational fraction (0.5 - 1 µm) is major size fraction in all 4 localities (37 - 46%). Ultrafine fraction(< 0.17 µm) is relatively most abundant in background station (~27%) while in the proximity of strip mine is the lowest (~7%).

c-PAHs ANALYSIS

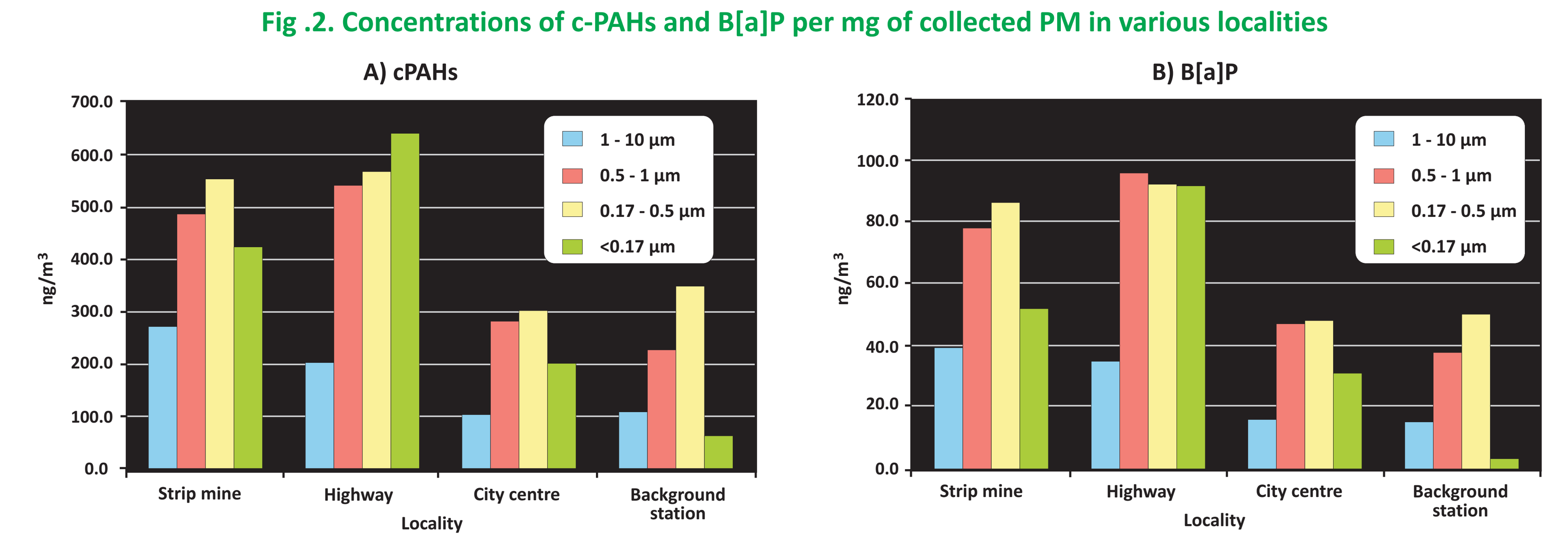
The concentrations of c-PAHs and B[a]P in EOMs prepared from various size fractions indicate that fine fraction (0.5 – 1µm) bounds the highest amount of these compounds in all localities (Fig. 1), which might be particularly connected with a high mass proportion of this fraction in the total aerosols (Table 1). In contrast, ultrafine fraction bounds the lowest c-PAHs and B[a]P mass.

Fig. 1. Concentrations of c-PAHs and B[a]P per m³ of sampled air in various localities



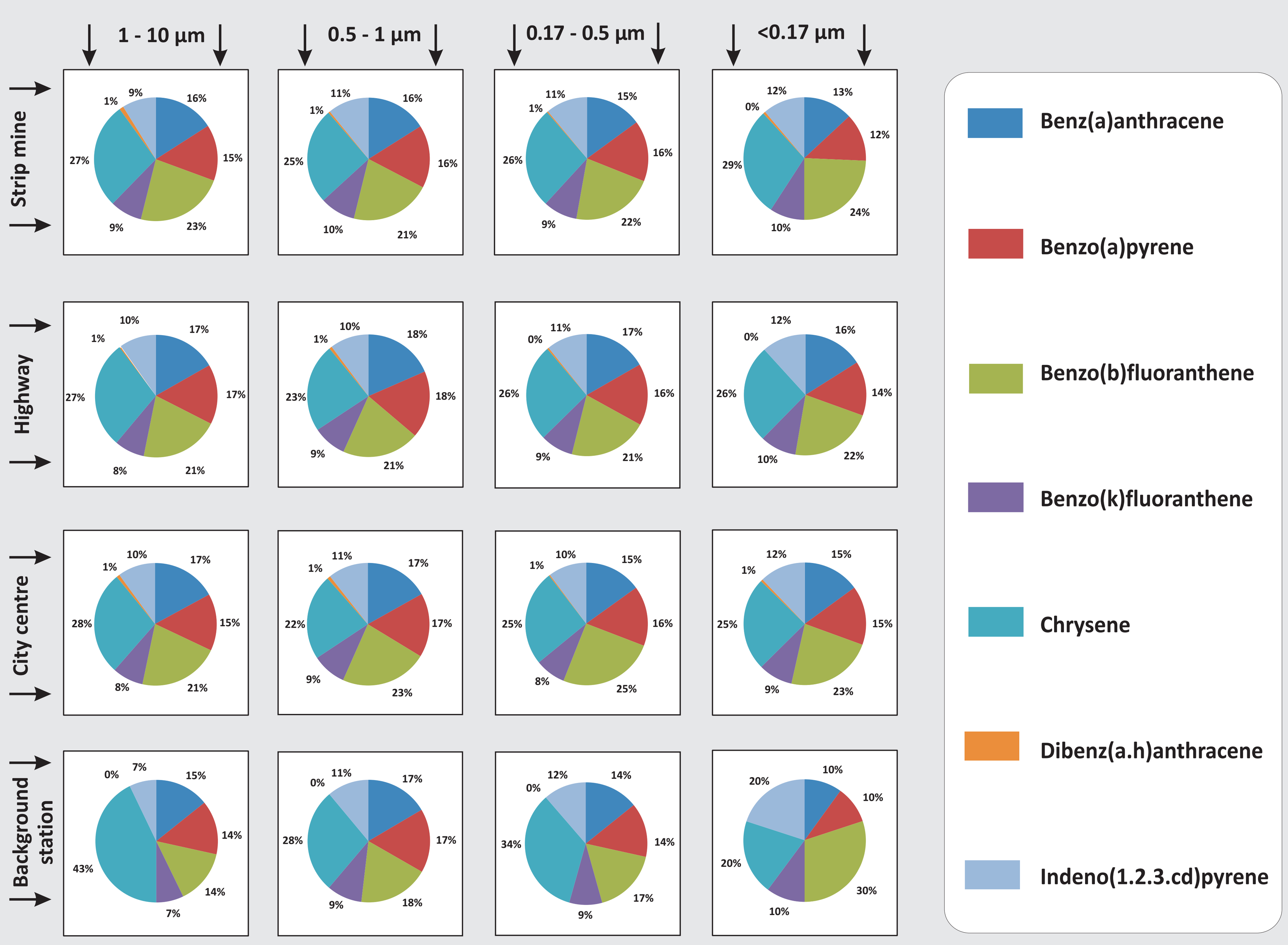
As per the aerosol mass, B[a]P and c-PAHs were predominantly bound to the fine and condensational fractions to the same extent with exception of the highway, where B[a]P and c-PAHs concentrations were were equal or even higher in the ultrafine fractions (Fig. 2).

Fig .2. Concentrations of c-PAHs and B[a]P per mg of collected PM in various localities



In contrast to the total c-PAH concentrations, the ratios of individual c-PAHs did not vary significantly with exception of chrysene which is more abundant in course fraction in the background station (Fig. 3). The most toxic c-PAH B[a]P 10 - 18% of total c-PAHs in all localities and PM size fractions.

Fig. 3: Relative distribution of c-PAHs in EOMs derived from PM of various aerodynamic diameter and collected in various localities



GENOTOXICITY - DNA ADDUCTS

The total DNA adduct levels induced by EOMs from size segregated aerosols and normalized per m³ of sampled air (Fig. 4) suggest a crucial contribution of the fine aerosol fraction (0.5 – 1 µm) to the total PM genotoxicity despite the presence/absence of the metabolic activation (+S9/-S9). Correspondingly to the lowest c-PAHs content, ultrafine fraction represents the lowest genotoxicity.

Fig. 4. Total DNA adduct levels induced by EOMs extracted from size segregated aerosols in a cell-free system (calf thymus DNA±S9) per m³ of sampled air.
The values represent the mean from two replicates varying by < ±10%.

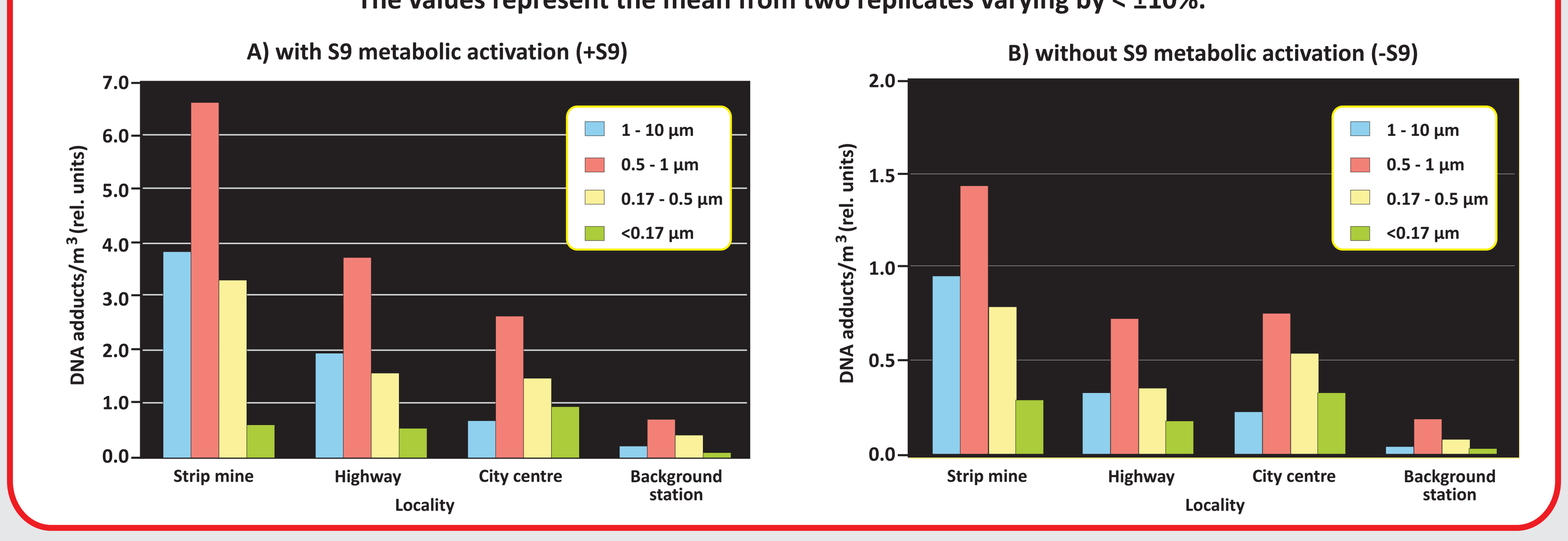


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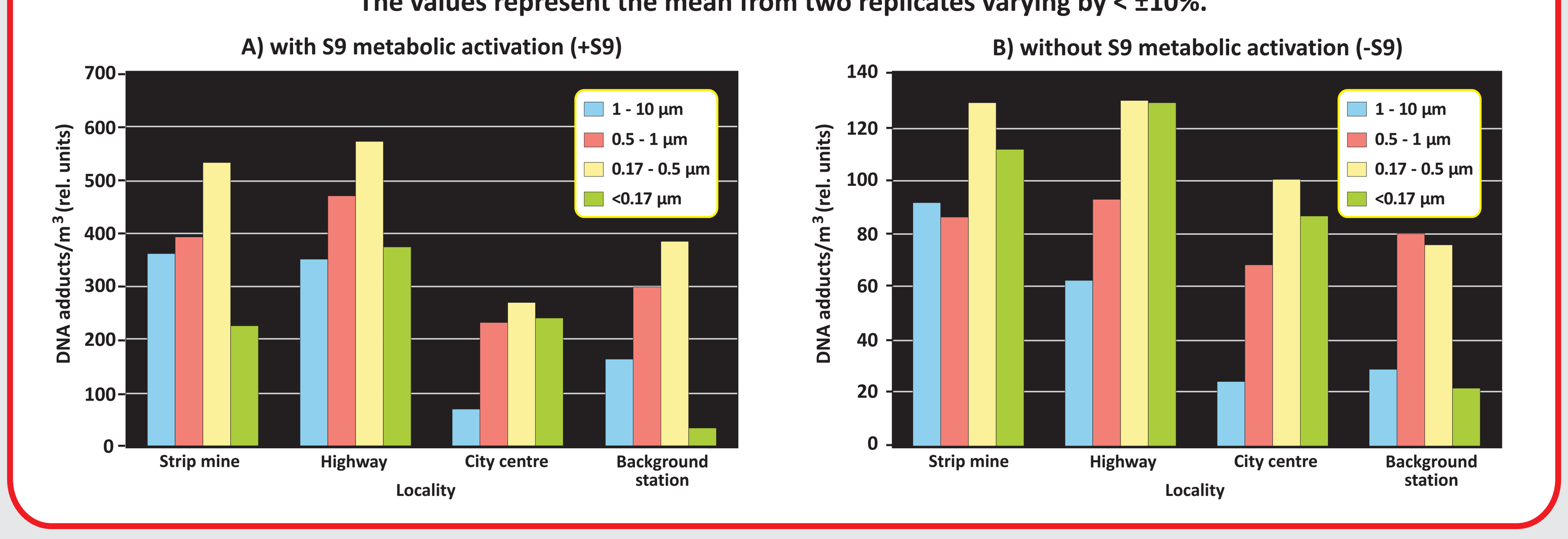
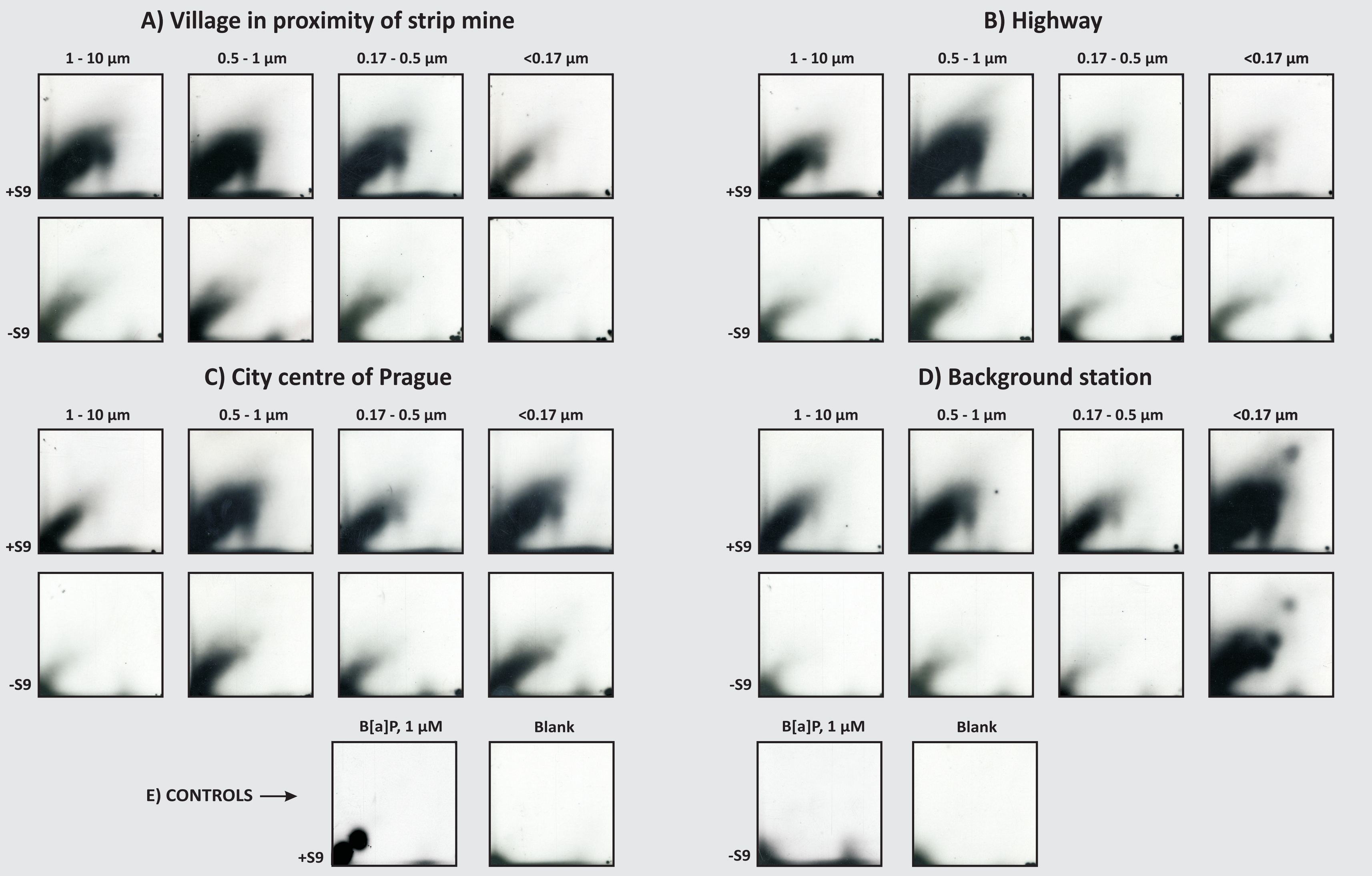
Fig. 5. Total DNA adduct levels induced by EOMs extracted from size segregated aerosols in a cell-free system (calf thymus DNA±S9) per mg of collected PM.
The values represent the mean from two replicates varying by < ±10%.



The autoradiographs obtained by ³²P-postlabelling analysis of calf thymus DNA samples incubated for 24 hours with various EOMs from size segregated aerosols (Fig. 4) exhibit diagonal radioactive zones (DRZ), representing the total DNA adduct levels induced by the compex mixture. Since c-PAHs needs S9-mediated metabolic activation to exert their genotoxicity, we may estimate the substantial contribution of c-PAHs from the much higher intensities of DRZ for + S9-samples in comparison with –S9 samples.

Fig. 6. Autoradiographs of TLC maps of ³²P-labelled DNA digest after incubation of calf thymus DNA (±S9 fraction) with 100 µg/ml extractable organic matter (EOM) from size segregated aerosols



METHODS

Sampling

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EOM extraction and chemical analysis

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In vitro acellular assay and DNA adduct analysis

Calf thymus DNA (1 mg/ml) was incubated with EOM samples (100 µg EOM/ml) for 24 hours at 37° C under aerobic conditition with/without rat liver microsomal S9- fraction (Pardubice-Rybitví) [1]. DNA adduct levels were analyzed by ³²P-postlabeling with nuclease P1 method for adduct enrichment [2].

References
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