AN ACELLULAR ASSAY TO ASSESS THE GENOTOXICITY OF COMPLEX MIXTURES OF ORGANIC POLLUTANTS BOUND ON SIZE SEGREGATED AEROSOL

Jan Topinka[1,2] , Jan Hovorka[2] , Alena Milcova[1] , Jana Schmuczerova[1] , Jiri Krouzek[2] , Radim J. Sram[1]

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Background

Much effort has been put into clarification of the adverse effects of environmental pollution on human health. Respirable ambient air particulate matter (PM) of an aerodynamic diameter <2.5_m (PM2.5) comprises a complex mixture consisting of a large number of chemicals, many of which are toxic and/or carcinogenic. 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. 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. 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. 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. We used an acellular test coupled with ^{32}P-postlabelling to compare DNA adduct forming activity of organic extracts (EOM) from size segregated aerosols (0.17–10μm).

Methods

Sampling

Coarse (1-10μm), fine (0.5-1μm) and condensation (0.17-0.5μm) aerosol fractions were collected on polyurethane foam (PUF) consecutively in four various localities by HiVol cascade impactors (BGI 900 samplers, U.S.A.) 24 hours daily during February – March of 2009. 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) 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 eight polycyclic aromatic hydrocarbons (PAHs) regarded as carcinogenic PAHs (c–PAHs) according to IARC, 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), dibenzo[a,h]anthracene (DB[ah]A), benzo[g,h,i]perylene (B[ghi]P), 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 C under aerobic conditions 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].

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BACKGROUND

Genotoxicity of the organic extracts (EOM) from the aerosols is connected with chemicals bound on the surface of the particles. One of the most important groups of toxic pollutants in the ambient air are carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) and their derivatives. The ability of c-PAHs to form after metabolic activation by cytochrome P450 enzymes DNA adducts was used in the acellular test based on the analysis of these adducts resulting from the covalent binding of some genotoxic EOM components with calf thymus DNA. The analysis was performed in the size segregated aerosols since the genotoxic potential may differ among various aerosol size fractions.

AIM

To determine
relative abundance
of various aerosol size fractions
and their abilities to bind c-PAHs

To compare
the genotoxicity
of the size segregated aerosols
collected in various localities
exhibiting different levels
of the air pollution

METHODS

Sampling

Coarse (1-10 μm), fine (0.5-1 μm) and condensation (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.) 24 hours daily during February – March of 2009. 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).

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RESULTS

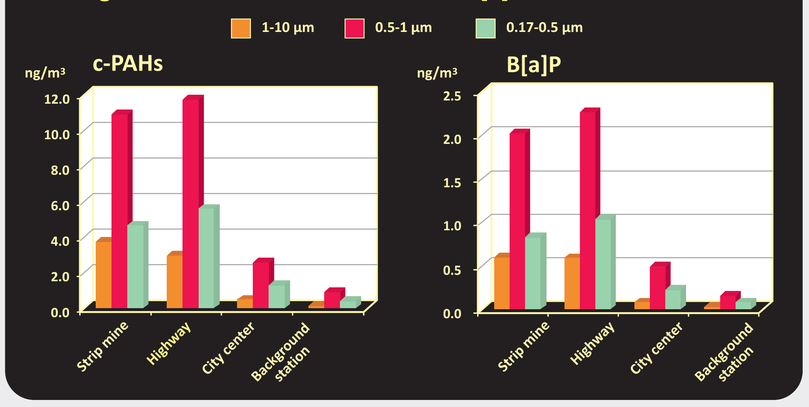
Basic aerosol sampling characteristics are shown in Table 1 demonstrating that 6,000 – 14,000 m^3 of the air was sampled in periods indicated. The relative mass distribution among size fractions differs substantially among various localities.

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]	5. 2. - 9. 2. 2009	6.127	1-10 0.5-1 0.17-0.5	10.9 6.9 1.9	55.3 35.1 9.6
Highway (Dobre Stesti) [49°40´58´´N, 13°18´9´´E]	13. 2. - 19. 2. 2009	10.121	1-10 0.5-1 0.17-0.5	3.5 6.4 3.4	26.6 48.0 25.4
City center (Prague) [50°4´19´´N, 14°25´25´´E]	4. 3. - 14. 3. 2009	13.830	1-10 0.5-1 0.17-0.5	6.4 5.5 2.6	44.4 37.9 17.7
Background station (Laz) [49°39´35´´N, 13°53´45´´E]	18. 3. - 26. 3. 2009	12.542	1-10 0.5-1 0.17-0.5	2.8 3.4 1.7	35.6 42.9 21.5

The concentrations of B[a]P and c-PAHs in EOMs prepared from various size fractions indicate that fine fraction 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)

Fig.1: Concentrations of c-PAHs and B[a]P in various localities



Relative distribution of individual c-PAHs did not vary significantly with size or sampling locality

Fig.2: Relative distribution of c-PAHs in fine fraction (0.5-1 μm) for individual localities

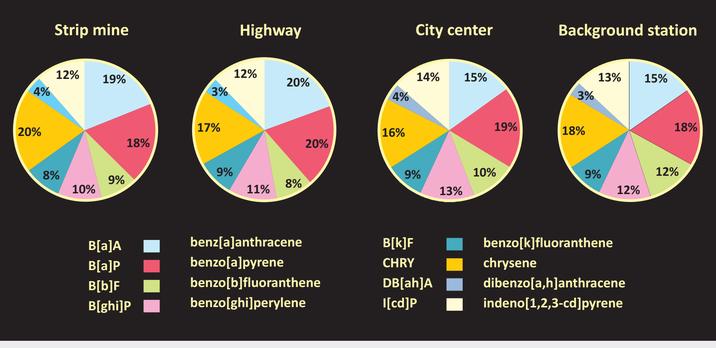
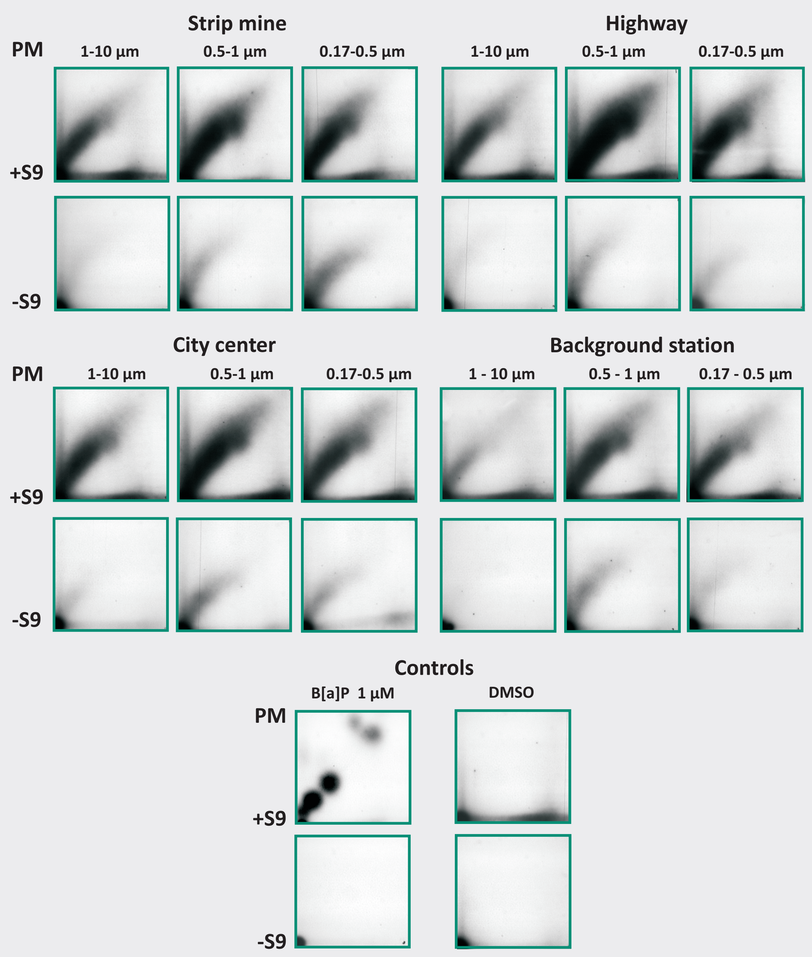
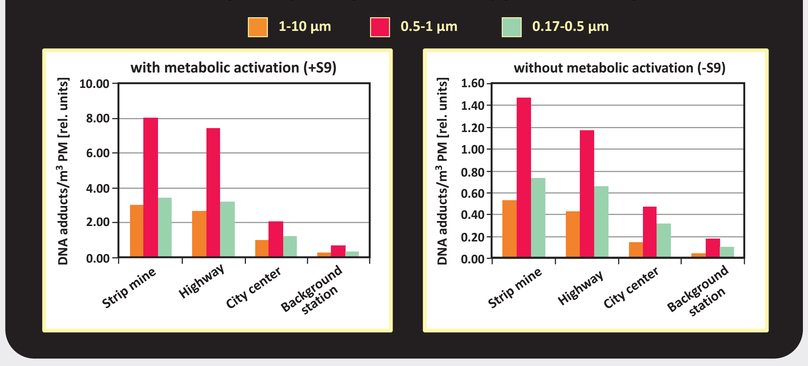


Fig. 3: Autoradiographs of TLC maps of ^{32}P-labelled DNA digest after incubation of calf thymus DNA (±S9 fraction) with 100 μg/ml extractable organic matter (EOM) from size segregated aerosols



The autoradiographs obtained by ^{32}P-postlabelling analysis of calf thymus DNA samples incubated for 24 hours with various EOMs from size segregated aerosols (Fig. 3) exhibit diagonal radioactive zones (DRZ), representing the total DNA adduct levels induced by the compex mixture. We found the substantial contribution of c-PAHs from the much higher intensities of DRZ for +S9-samples in comparison with –S9 samples.

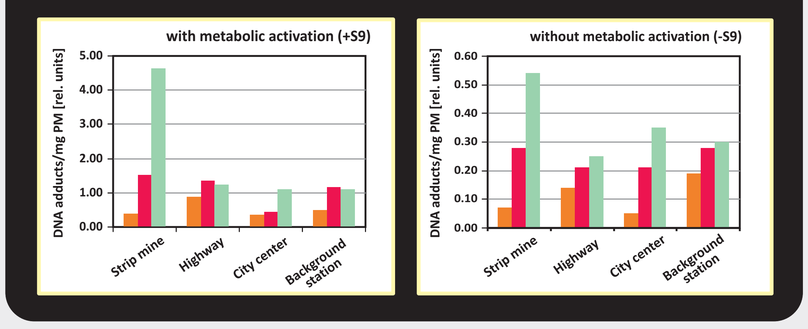
Fig. 4: Total DNA adduct levels induced by EOMs extracted from size segregated aerosols in acellular system (calf thymus DNA ± S9) per m^3 of sampled air



The total DNA adduct levels induced by EOMs from size segregated aerosols and normalized per m^3 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).

To answer the question 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 fraction (Fig. 5). Under such conditions our results indicate that condensational fraction (0.17 – 0.5 μm) is the most efficient to bound DNA adduct forming compounds in almost all sampling sites.

Fig. 5: Total DNA adduct levels induced by EOMs extracted from particles of various diameter in acellular system (calf thymus DNA ± S9) per mg of collected PM.

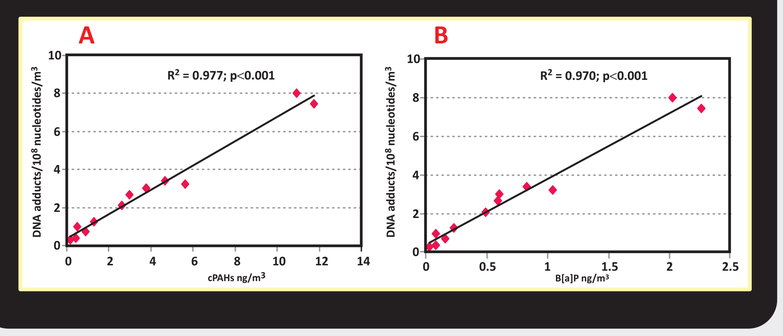


Highly significant correlation was found between B[a]P and c-PAHs bound on aerosols and total DNA adduct levels (Fig. 6).

CONCLUSIONS

1. The genotoxic potential of the ambient air aerosols depends on the size of particles.
2. The genotoxicity is substantially higher for a fine fraction of the aerosols.
3. Highly significant correlation was found between B[a]P and c-PAHs bound on aerosols and total DNA adduct levels.
4. A significant induction of DNA adducts in all EOM samples even without enzymatic metabolic activation by S9 fraction suggests some contribution of directly acting genotoxic compounds.
5. A model system based on the analysis of total DNA adducts induced in acellular assay with/without metabolic activation by microsomal cytochrome P450 enzymes represents a simple method of choice to assess and compare genotoxic potentials of various complex mixtures containing directly and indirectly acting genotoxic compounds.

Fig. 6: Relation of c-PAHs (A) and B[a]P (B) content in EOMs from various localities and size fractions to DNA adduct levels induced by EOMs in calf thymus DNA + S9 fraction



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[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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Results and Discussion

The major finding of this study is that the genotoxic potential of the ambient air aerosols depends on the size of particles. Genotoxicity is substantially higher for a fine fraction of aerosols (0.5–1μm). This finding corresponds to the highest c-PAH content in the fine fraction and might also be related to the higher specific surface of the fine aerosol fraction in comparison with a course fraction and thus higher capacity to adsorb, on the surface, genotoxic compounds such as c-PAHs. Condensational fractions of the aerosol (0.17–0.5μm) are also very efficient carriers of c-PAHs, but its mass in total PM is relatively low compared to the fine fraction. Thus, the contribution of the condensational fraction to the total DNA adduct levels per m3 of the air is proportionally lower. However, the DNA adduct levels normalized per mass of the aerosol in individual fractions (Fig.1) indicate that condensational fractions contribute to the total genotoxicity more than corresponds to their mass.

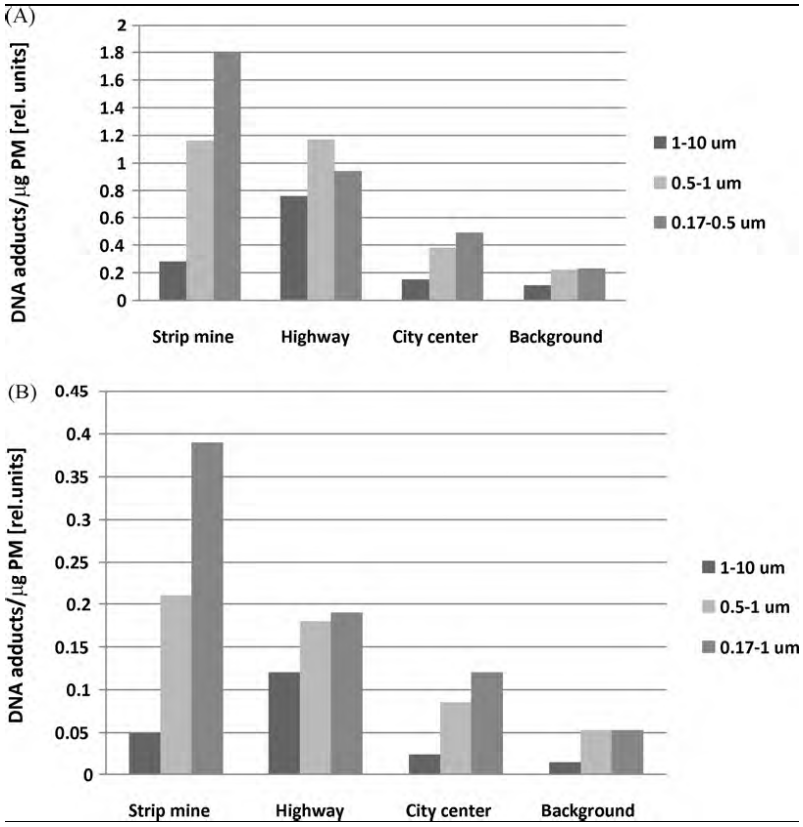

Fig. 1. Total DNA adduct levels induced by EOMs extracted from particles of various diameters in an acellular system (calf thymus DNA±S9) per μg of collected PM. DNA adducts were analyzed with metabolic activation by microsomal S9 fraction (A) and without the metabolic activation (B). The values represent the mean from two replicates varying by <±10%.

It may be concluded that the model system, based on the analysis of totalDNAadducts induced in the acellular assay with/without metabolic activation by microsomal cytochrome P450 enzymes used in this study, represents a relatively simple method of choice to assess and compare genotoxic potentials of various complex mixtures containing directly and indirectly acting genotoxic compounds.

References

[1] Adams, S.P., Laws, G.M., Storer, R.D., DeLuca, J.G., Nichols, W.W., Mutat. Res. 368, 1996, 235-248.
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