MUTAGENICITY OF AIRBORNE PARTICULATE MATTER PM_{10}

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SUMMARY

Mutagenic activity of extractable organic matter (EOM), from airborne particles collected over winters in four towns of Czech Republic, was investigated by the means of Salmonella typhimurium indicator strains TA98 and YG1041 using the Ames plate incorporation assay. Mutagenicity of all tested samples showed significant dose-related increase in number of revertants per mg of EOM. The direct mutagenic potency detected with TA98 increased further in the presence of external metabolic activation. The mutagenic potency detected with YG1041 was about two orders of magnitude higher than that detected with TA98. The mutagenicity results correlated with the concentrations of the polycyclic aromatic hydrocarbons (PAHs) determined by GC/MS. Local differences in mutagenicity, expressed as numbers of revertants per m^3 of air, were observed with the highest values in Prague air samples. For routine ambient air mutagenicity monitoring, the use of YG1041 and the plate incorporation test are recommendable.

Key words: airborne particulate mutagenicity, Ames test, Salmonella/microsome

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INTRODUCTION

Atmospheric suspended particles are one of the major air pollutants. Many organic air pollutants adsorbed on their surface have mutagenic and carcinogenic potencies. In order to evaluate the potential health effects, short-term mutagenicity bioassays have been used, most widely the Ames Salmonella mutagenicity assay (1). Therefore, the mutagenicity monitoring of urban air particles has been included in the national-wide Environmental Health Monitoring System implemented in the Czech Republic since 1994 (2, 3). Our first published results from the period 1997–1998 indicated the presence of significant mutagenic potencies of EOM from the Czech urban air particles with distinctly increased values in winter months (4). In this study, we present data on the bacterial mutagenicity monitoring obtained in the winter periods 1999 to 2003.

MATERIALS AND METHODS

Sampling

Air particles PM_{10} were collected in four towns with different levels of industrial and commercial activities (Plzeň, Praha, Ústí n/L, Žďár n/S). Twenty-four hours samplings have been done during winter months (October – March) every eighteenth day using Hi-Vol air sampler Graseby-Anderson equipped with weighted Pallflex filter (20.3 x 25.3 cm, type T60A20). The mean flow rate of air was 1.13m^3/min, approx 1600 m^3 of air volume collected per one sample. In the same time intervals, air samples for the determination of PAHs were collected by a Hi-Vol air sampler Wedding and Assoc., USA, with a flow rate of 250 l/min on quartz filter and polyurethane foam cartridge.

Sample preparation

Extraction procedure was performed as described elsewhere (4, 5). Briefly, each filter was dried, cut into small pieces and extracted with 150 ml of dichloromethane in ultrasonic bath 3-times for 20 min each. The three extracts were combined, concentrated by a rotary vacuum evaporator and quantitatively transferred to 10 ml. Aliquots of 2 x 0.5 ml from this crude extract were used for gravimetric determination of extractable organic matter (EOM). Rests of extracts were redissolved in dimethyl sulfoxide (DMSO) and standard volume of 2 or 4 mg of EOM/ml DMSO was prepared for mutagenicity testing.

Mutagenicity assay

Mutagenicity was assayed by means of the Ames plate incorporation test in a modification of Maron and Ames (6) with indicator strains TA98 and its derivative YG1041 with elevated levels of both nitroreductase and O-acetyltransferase activities (7). The procedure was described in details previously (5). The genetic properties of the tester strains were checked according to recommended procedures. Following reference mutagens were used to check the sensitivities of the tester strains: 2-aminofluorene Sigma (5 μg/plate), 4-nitro-o-phenylendiamine Aldrich (10 μg/plate), 1-nitropyrene Sigma (0.5 μg/plate). All assays were done in duplicate plates; all positive and negative controls were tested in triplicate. Four doses ranging from 20 to 200 μg/plate for TA98 with and without S9mix, and from 4 to 20 μg/plate for YG1041 were used. After 70-hours incubation at 37°C, revertants (rev) were counted electronically with a Biotran II colony counter.

Determination of PAHs

Twelve PAHs - phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]-fluoranthene, benzo[a]pyrene, dibenz[a,h,]anthracene, benzo [g,h,i]perylene, indeno[c,d]pyrene were quantitatively analyzed by GC/MS Hewlett Packard according to the method TO-13A of the US EPA (8). TEQ values for carcinogenic PAHs were calculated using the TEFs recommended by the U.S.EPA (9).

Statistics

Maximum mutagenic potency (rev/μg EOM) of each sample was calculated as linear slope value using the Bernstein model of the GeneTox manager software (10,11). Mutagenic activity expressed as rev/m^3 was calculated using the following parameters: rev/μg EOM, total amount of EOM in sample and volume of sampled air.

For statistical analysis, nonparametric Kruskal–Wallis one-way ANOVA was chosen using the software Unistat 5.1. Correlations were performed by the Spearman rank correlation test.

RESULTS AND DISCUSSION

All individual samples showed significant dose-response effects in mutagenic potency of EOM ($p<0.01$). Table 1 presents the results observed at the monitored sites in median values and CI of mutagenicity (rev/m^3) and of concentrations of benzo[a]pyrene and TEQ values (ng/m^3) representing carcinogenic PAHs. The mutagenicity results showed weak-to-moderate significant correlations with concentration levels of individual PAHs and TEQ values (Table 2). Generally, the highest values for YG1041-S9 mutagenicity were obtained in Prague samples with significant difference ($p<0.001$) from the results obtained in other monitored localities. These results are in agreement with the data obtained in other study on genotoxicity of urban air pollutants in the Czech Republic (12) and pointed out that the capital of the Czech Republic, Prague, appears today to be one of the most polluted residential areas in the country. The increase in indirect-acting mutagenicity detected with TA98 is usually attributed to carcinogenic PAHs and may relate to the local heating facilities during the winter months. However, the only local significant difference ($p<0.007$) in this parameter as well as in the levels of carcinogenic PAHs was observed between Prague and Žďár n/S. About two orders of magnitude higher mutagenicity detected with the YG1041 strain as compared to TA98 results led to the suggestion that nitroarenes are also an important source of genotoxic contamination in the urban air (13) in the Czech Republic and that traffic may be a major emission source.

Table 3 summarizes the data for individual years of monitoring. Increasing time-trends in the TA98±S9 mutagenicity ($p<0.001$) as well as in the concentrations of carcinogenic PAHs ($p<0.03$) were obtained. Our data support the relation between the mutagenicity and potential adverse health effects of PAHs and the necessity of preventive measures in this field.

In conclusion, our results indicate that the use of YG1041 is to be recommended for the mutagenicity monitoring of ambient

Table 1. *Urban dust in winter months 1999–2003**

N	Praha 45	Plzeň 40	Ústí n/L 39	Žďár n/S 41
YG1041-S9 (rev/m^3)	1252 (941–1563)	716 (525–907)	812 (556–1068)	653 (492–815)
TA98- S9 (rev/m^3)	16.4 (10-22.9)	12.2 (5.4-19.0)	14.7 (9.6-19.8)	11.9 (6.8-17.0)
TA98+ S9 (rev/m^3)	28.6 (16.6 - 40.7)	20.9 (10.2 - 31.5)	21.9 (13.3 - 30.5)	17.1 (9.2 - 25.0)
B(a)P (ng/m^3)	2.6 (1.5 - 3.6)	1.3 (0.3 - 2.3)	1.6 (0.8 - 2.4)	1.0 (0.5 - 1.4)
TEQ	4.0 (2.6 - 5.3)	2.0 (0.5 - 3.6)	2.5 (1.3 - 3.6)	2.0 (1.2 - 2.7)
Σ PAHs (ng/m^3)	76.5 (58.5-94.4)	53.7 (26.3-81.0)	75.3 (60.3-90.2)	45.9 (32.2-59.6)

* only the first part of the year 2003
Medians and confidence intervals in parentheses

Table 2. *Urban dust in winter months 1999–2003*

ng/m^3 / rev/m^3	B[a]P	TEQ	Σ PAHs	B[a]A
TA98-S9	0.495	0.523	0.500	0.544
TA98+S9	0.479	0.487	0.469	0.540
YG1041-S9	0.602	0.594	0.549	0.616

Spearman correlation coefficients

Table 3. *Urban dust in winter months*

	1999/2000	2000/2001	2001/2002	2002/2003
TA98-S9 (rev/m^3)	4.02 (1.0-7.1)	16.73 (12.9-20.5)	16.77 (9.9-23.7)	18.89 (14.9-22.9)
TA98+S9 (rev/m^3)	5.10 (1.5-8.7)	29.35 (20.8-37.9)	29.53 (20.6-38.4)	29.27 (24.2-34.4)
YG1041-S9 (rev/m^3)	653 (378-929)	877 (591-1163)	866 (562-1171)	828 (674-982)
BaP (ng/m^3)	1.27 (0.9-1.7)	2.00 (0.9-3.1)	1.20 (0.2-2.2)	1.45 (0.008-2.9)
TEQ	2.04 (1.2-2.9)	3.37 (1.4-5.3)	2.00 (0.6-3.4)	2.85 (0.7-5.0)
Σ PAHs (ng/m^3)	50.6 (36.8-64.4)	81.6 (58.5-104.7)	59.8 (37.9-81.8)	71.1 (42.6-99.6)

Medians and confidence intervals in parentheses

air particles and that the plate incorporation test seems to be convenient for monitoring purposes.

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