Environment Protection Engineering

Vol. 35 2009 No. 1

KATARZYNA PIEKARSKA*

MUTAGENIC EFFECT OF MAIN GROUPS OF ORGANIC POLLUTANTS ADSORBED ON SUSPENDED PARTICULATE MATTER (PM10 AND PM 2.5) COLLECTED WITHIN WROCŁAW URBAN AREA

Mutagenic activity of pollutants adsorbed on suspended PM10 and PM2.5 particulates collected in springtime within the urban area of Wrocław (Poland) was identified by the *Salmonella* assay. Particulate matter was sampled on sintered-glass filters, using a high performance Staplex air aspirator. Pollutant extraction by dichloromethane was performed in a Soxhlet apparatus. The fractions obtained were separated by a column chromatography method. The assays were conducted in the presence of TA98 and TA100 strains as well as their derivative strains YG1041 and YG1042. It was found that the samples under examination contained pollutants that might affect directly or indirectly the genetic material. These are mutagens of the reading frame-shift type and the base-pair substitution type. In the assays, where PAH, nitro-PAH and dinitro-PAH fractions were introduced, the values of the mutagenicity ratio (MR) obtained were higher or lower compared to those obtained for the whole extracts. The mutagenic effect was not found only in the case of a dinitro-PAH fraction presence in the extract of PM2.5 particulates when the assay was conducted with metabolic activation.

1. INTRODUCTION

The problem of the effect of atmospheric pollution on human health and our environment has been existed for years. Atmospheric emissions, both from natural and anthropogenic sources, can spread over long distances and then permeate into water, soil and living organisms. Most of air pollutants are gases. Particulate pollutants constitute the second pollutant group and form a complex mixture of organic and inorganic substances. Dustiness of atmospheric air is one of the parameters describing air pollution. Most of genotoxic pollutants are adsorbed on suspended particles [1], [2]. Air is a carrier medium for xenobiotics that humans are exposed to through a respiratory tract. We breathe air unintentionally for 24 hours, taking up ca. 11 m^3 of air over

* Institute of Environmental Protection Engineering, Wrocław University of Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland. E-mail: katarzyna.piekarska@pwr.wroc.pl

that period [3]. The harmfulness of particulates to organisms depends on the particulate diameter. The finest particulate fraction, containing particles 2.5 μm in diameter and smaller, the so-called PM2.5 fraction, is most harmful to humans and animals. That fraction most deeply penetrates the respiratory tract and finally settles on the surface of alveoli. In this way, the pollutants enter the circulatory system [4].

It is very difficult to determine the type of substances that are responsible for the air mutagenicity because of a complex pollutant composition. A huge number of compounds representing different chemical classes can be found in polluted air, and those compounds can form very complex mixtures of unknown biological properties. As can be concluded based on literature analysis, mainly those more polar PAHs, i.e. the nitro-, amino- and oxy-PAH derivatives rather than the non-substituted PAHs, can be blamed for the mutagenic effect of the suspended particulates [5]. Because the identification of individual chemical compounds in such a mixture as the suspended particulates is quite hindered and often impossible, the separation of an extraction into fractions, each of them containing compounds of a particular class, as well as the assessment of mutagenicity of substances in the classes by bioindication assays become essential. Only the results obtained using biological methods can provide us with a reliable information about the effect of such substances on living organisms. Taking account of the fact that the compounds under discussion in many cases show a synergistic effect, this approach becomes especially important [6].

A short-term *Salmonella* assay (the Ames assay) has been found useful in the studies on the mutagenicity of environmental pollutants. The assay involves the determination of reverse mutations from histidine auxotrophy to prototrophy in specially designed mutants of *Salmonella typhimurium* LT2 strains [7]. The strains used in the assays show an increased sensitivity to the chemical compounds, the components of the atmospheric air. To those strains usually belong *Salmonella typhimurium* TA98, TA100, YG1041 and YG1042. S-9 fraction, consisting of microsomal enzymes isolated from rat liver induced by biphenyl mixture (Aroclor 1254), is used in the assay for metabolic activation of promutagens. The S-9 is used in order to obtain conditions similar to those in mammal cells [8]. The assay was applied many times to detect mutagenic effect of organic extracts of suspended particulates that had been collected in different towns around the world [9]. In Poland, genotoxicity of air pollutants was investigated especially in the Upper Silesia region and in the area of the cities of Warszawa and Wrocław [10].

The purpose of this paper was to compare the mutagenicity of main groups of organic pollutants adsorbed on suspended particulates of PM10 and PM2.5 fractions, collected in spring season over the Wrocław area (Poland).

2. MATERIALS AND METHODS

PM10 and PM2.5 samples were taken in late March and at the beginning of April 2007 on sintered-glass filters with a high-performance Staplex air aspirator. Filters in

the apparatus were replaced every 24 hours until all the material necessary for biological tests was collected. The sampling site was situated in the heavy traffic area of the campus of the Wrocław University of Technology (Norwida St./Wybrzeże Wyspiańskiego crossing) on a roof at the second floor level. Filters together with particulates from individual samplings were combined into one sample, cut into pieces and put into Soxhlet apparatuses. Then their extraction by dichloromethane was performed in the absence of light for 16 hours plus 15-minute reflux [11]. The extracts were thickened until dry in a vacuum evaporator, and finally blown through with nitrogen. The dry extracts obtained were analysed for PAH, nitro-PAH and dinitro-PAH content and also used as the material for the mutagenicity assays (*Salmonella*).

A crude extract was fractionated in glass columns filled with silica gel. First, a column was conditioned with cyclohexane. Then the thickened crude extract was placed at the head of the column and particular fractions were eluted following the procedure reported by ZACIERA [12]. Polycyclic aromatic hydrocarbons were determined by a high-performance liquid chromatography technique using fluorescence detection, whereas the nitro-PAH content – by the gas chromatography using mass detection. The dry residue of particulate extracts was intended for biological examinations, thus it was dissolved in sterile dimethyl sulfoxide (DMSO) in such a way that 1 cm^3 of stock solution contained pollutants from 1000 m^3 of atmospheric air. Four test strains: TA98, TA100, YG1041 and YG1042 [13] were used in *Salmonella* assays conducted according to the recommendations given by MARON and AMES [7] in two experiment versions: without their metabolic activation and with their metabolic activation by a microsomal fraction S9. The *Salmonella* strains tested were donated by Dr. T. Nohmi, the Division of Genetics and Mutagenesis, National Institute of Hygienic Sciences, Tokyo, Japan. A protein content in the microsomal fraction, determined by Lowry's method, was 64.44 mg/cm^3. In the experiments, the S9 content in the S9-mix was 4% (v/v). All organic pollutants present in the samples collected and the pollutants contained in three fractions (PAHs, PAH nitro derivatives and PAH dinitro derivatives) were tested and all the analyses were carried out in five replications. Cultures were incubated for 48 hours (TA98, TA100) or for 72 hours (YG1041, YG1042) at the temperature of 37 °C. After that time the number of (his^+)-revertant colonies growing on the Petri dishes were determined. Prior to starting the experiments, we checked each time the genetic markers of the strains and the degree of their spontaneous reversion (negative check) as well as the strain sensitivity to diagnostic mutagens (positive check – without S9: 0.2 μg of 2,4,7-trinitro-9-fluorenone per plate for the TA 98 strain and 50 μg of 2,6-dinitrotoluene per plate for the YG 1041 strain; with S9 addition: 5 μg of 2- aminofluorene per plate for both test strains).

Mutagenic effect of suspended particulates was expressed by a mutagenicity ratio (MR), which is the ratio of an average number of revertants grown in the presence of the sample tested and to an average number of spontaneous revertants. The samples were considered mutagenic if their $MR \geq 2$.

3. RESULTS AND DISCUSSION

In the spring 2007, the following amounts of particulates were collected from the sampling site in the Wrocław area: PM10 – 55.02 μg/m^3; PM2.5 – 36.74 μg/m^3. The volumes of air samples were 6739.24 m^3 (PM10) and 7642.08 m^3 (PM2.5), respectively. The concentration of the suspended particulates collected in springtime in the Wrocław area was close to that measured in the spring–summer seasons in different European towns. For instance, the PM10 concentration in Antwerp ranged from 31.4 to 41.6 μg/m^3 [14] and in Prague, Teplice and Prachatice (the Czech Republic) – between 21.2 and 57.1 μg/m^3 [15]. Earlier observations in Wrocław revealed the PM10 concentration oscillating between 19.9 μg/m^3 and 51.04 μg/m^3 [16]. Higher concentration of this fraction of particulates was measured in the Upper Silesia region [17], where in summertime its concentration ranged from 22.5 to 77.5 μg/m^3. In turn, the PM2.5 concentration was comparable with spring-related concentration observed in Italy. The PM2.5 concentration reported by CASSONI et al. [18] in six Italian towns ranged between 14.36 and 48.84 μg/m^3, whereas the respective concentrations in the Turin area, found by GILLI et al. [19], ranged from 20 μg/m^3 to 60 μg/m^3. They were lower than the permissible concentrations of particulate matter being collected over a 24-hour period, which according to the US EPA regulations [20] amount to 150 μg/m^3 (PM10) and 65 μg/m^3 (PM2.5).

The concentrations of particulate extracts were determined for twelve PAHs and eight PAH nitro derivatives, which are the most often occurring pollutants of the atmospheric air (table 1).

Table 1

PAH and nitro-PAH concentrations in organic extract of suspended PM10 and PM2.5

PAHs	PM10	PM2.5	Nitro-PAH	PM10	PM2.5
	(ng/m^3)			(ng/m^3)	
Phenanthrene	1.001	0.107	1-nitronaphthalene	0.11	0.06
Anthracene	0.109	0.011	2-nitrofluorene	0.24	0.07
Fluoranthene	1.664	0.223	9-nitroanthracene	0.12	0.15
Pyrene	1.410	0.195	3-nitrofluoranthene	0.11	0.05
Benzo[a]anthracene	0.638	0.084	1-nitropyrene	0.05	0.03
Chrysene	0.432	0.111	1,3-dinitropyrene	n.d.	n.d.
Benzo[b]fluoranthene	0.820	0.383	1,6- dinitropyrene	n.d.	n.d.
Benzo[k]fluoranthene	0.300	0.110	1,8- dinitropyrene	n.d.	n.d.
Benzo[a]pyrene	1.870	0.316			
Dibenzo[a,h]anthracene	0.035	0.046			
Benzo[g,h,i]terylene	0.762	0.513			
Indeno[1,2,3-c,d]pyrene	0.733	0.222			
Total	9.778	2.322	Total	0.63	0.36

n.d. – not detected.

A total PAH amount detected in the PM10 sample was 9.778 ng/m^3, and in the PM2.5 sample – 2.322 ng/m^3. Especially polyaromatic hydrocarbons containing 3 to 6 condensed rings and adsorbed on particulates show carcinogenic activity [14]. Of the PAHs present in the extracts, three (benzo[a]anthracene, benzo[a]pyrene, dibenzo[a,h]anthracene) were classified by IARC into 2A group as probably carcinogenic to humans, and another three (benzo[b]fluoranthene, benzo[k]fluoranthene, indeno[1,2,3-c,d]pyrene) into 2B group, as possibly carcinogenic to humans [21]. The concentrations of individual PAHs were within the limits reported in the literature and very close to those detected in the urban regions of Belgium, Czech and Italy [14], [15], [18], [19], [22]. The proportion of benzo[a]pyrene in organic extracts was high, and its concentrations reached 1.870 ng/m^3 (in PM10 fraction) and 0.316 ng/m^3 (in PM2.5 fraction). Benzo[a]pyrene is an indicator compound used to assess the carcinogenic activity of other PAHs. A relative carcinogenicity index k assumed for that compound equals 1 [21]. The samples tested, contained also, at lower concentrations, dibenzo[a,h]anthracene – the compound of higher carcinogenic potential (k = 5), and benzo[g,h,i]perylene – the compound of lower carcinogenity (k = 0.01). The latter compound is regarded as the indicator of exposure to aromatic hydrocarbons emitted by diesel engines [9].

No dinitro-PAHs were found in the extracts within their determination limits. Instead, five mononitro-PAH derivatives were detected, among others 2-nitrofluorene and 1-nitropyrene, both classified into the 2B group [21]. 3-nitrofluoranthene was present in the extracts as well. 1-nitropyrene and 3-nitrofluoranthene are typical products of fuel combustion in diesel engines. Those compounds are not observed in any other reactions taking place in a gaseous phase [23]. A total amount of nitro-PAHs in the PM10 extract was 0.63 ng/m^3, and in the PM2.5 sample – 0.36 ng/m^3. The nitro-PAH concentrations in organic extracts of air pollutants are usually considerably lower as compared with the PAH content. However, such compounds are highly stable in a solid phase and some of them show higher mutagenicity (2×10^5 times) and carcinogenicity (10 times) compared to those of PAHs [24].

The *Salmonella* assay revealed the mutagenic properties of organic air pollutants adsorbed on suspended particles of the PM10 and PM2.5 fractions from the spring-collected samples in the Wrocław area (tables 2 and 3). High mutagenicity ratios (MR) obtained in the assays conducted for the integrated extracts, in all strains, with and without metabolic activation, proved that pollutants able to affect directly (direct mutagens) and indirectly (promutagens) the genetic material were present in the samples tested.

In the assays with TA98 strain, which is commonly used for testing the mutagenicity of air-polluting particulates and for detecting the mutagens of the reading frame-shift type [7], the highest MR values (MR = 9.47 at 50 m^3/plate) were obtained for the whole PM2.5 extract when the assays were conducted without metabolic activation (table 2). The smallest air volume indispensable for a mutagenic effect in the

Table 2

MR values for extracts of particulate pollutants, tested with TA98 and YG1041strains in presence of S9 (+F) and in absence of S9 (–F)

Sample concentration (m^3/plate)	Type of sample/ test strain															
	PM10								PM2.5							
	TA98															
	–F				+F				–F				+F			
	Whole	PAH	Nitro-PAH	Dinitro-PAH	Whole	PAH	Nitro-PAH	Dinitro-PAH	Whole	PAH	Nitro-PAH	Dinitro-PAH	Whole	PAH	Nitro-PAH	Dinitro-PAH
50	6.15	3.16	2.92	2.94	6.11	3.16	3.16	2.19	9.47	1.43	2.46	1.25	4.3	1.62	1.83	1.54
25	3.36	1.49	1.86	1.62	3.74	2.29	2.87	2.06	6.75	1.27	1.56	1.05	2.11	1.51	1.43	1.36
12.5	3.04	1.40	1.82	1.23	2.62	1.64	1.48	1.93	5.37	1.07	1.16	1.01	1.53	1.44	1.41	1.26
6.25	1.74	1.38	1.29	1.18	2.57	1.57	1.38	1.74	5.12	1.05	1.14	1.01	1.27	1.38	1.24	1.19
3.125	1.42	1.35	1.25	1.14	1.61	1.37	1.25	1.63	3.91	1.05	1.05	1.01	1.20	1.36	1.21	1.04
1.56	1.39	1.16	1.03	1.10	1.26	1.13	1.25	1.51	3.80	0.96	1.01	1.01	0.99	1.34	1.21	1.03
0.78	1.18	1.07	0.88	1.07	0.99	1.21	1.17	1.36	3.31	0.94	0.99	0.99	0.97	1.19	1.17	1.0
0.39	0.96	1.07	0.86	1.01	0.97	1.17	1.13	1.21	2.26	0.92	0.99	0.90	0.98	1.05	1.05	0.99
0.195	1.0	1.02	0.86	0.96	0.91	1.11	1.13	1.04	2.10	0.90	0.96	0.89	1.0	0.83	0.99	0.92
0.097	1.05	0.99	0.77	0.90	0.90	1.02	1.11	0.99	1.44	0.83	0.96	0.77	1.04	0.67	0.94	0.83
0.049	1.05				0.97				1.32				1.02			
	YG1041															
50	4.97	3.18	3.75	4.47	18.11	4.85	5.83	5.89	5.87	3.21	4.16	5.18	10.86	1.75	3.4	1.46
25	6.19	3.44	4.38	4.19	19.53	4.50	4.19	3.13	14.40	2.35	3.52	5.07	13.76	1.17	2.27	1.09
12.5	10.99	3.96	4.07	3.27	15.78	2.13	2.09	1.80	10.62	1.34	2.68	3.12	5.19	1.16	1.48	1.02
6.25	9.98	5.02	3.77	2.65	4.18	1.08	1.46	1.23	8.66	0.85	1.95	2.24	4.46	1.12	1.40	0.90
3.125	6.97	1.82	2.83	1.53	1.77	0.99	1.01	1.20	7.98	0.95	1.24	1.37	3.54	0.99	1.07	0.92
1.56	4.59	1.54	2.24	1.35	1.48	1.00	0.99	1.05	7.57	0.91	1.11	0.96	3.10	1.00	1.00	0.98
0.78	2.64	1.26	1.85	1.21	1.02				6.16				2.61			
0.39	1.80	1.13	1.24	1.13	0.82				5.00				2.29			
0.195	1.40	1.04	1.12	1.02	0.93				3.90				2.20			
0.097	1.02				1.01				2.72				2.06			
0.049	0.93				0.94				1.42				1.64			

Table 3

MR values for extracts of particulate pollutants, tested with TA100 and YG1042 strains in presence of S9 (+F) and in absence of S9 (–F)

Sample concentration (m^3/plate)	Type of sample/ test strain															
	PM10								PM2.5							
	TA100															
	–F				+F				–F				+F			
	Whole	PAH	Nitro-PAH	Dinitro-PAH	Whole	PAH	Nitro-PAH	Dinitro-PAH	Whole	PAH	Nitro-PAH	Dinitro-PAH	Whole	PAH	Nitro-PAH	Dinitro-PAH
50	2.85	1.71	1.36	1.33	4.44	2.22	1.67	1.63	3.96	1.34	1.26	1.17	3.07	1.41	1.35	1.34
25	2.62	1.48	1.23	1.20	3.30	2.05	1.35	1.63	2.74	1.26	1.21	1.20	2.5	1.4	1.27	1.08
12.5	2.50	1.47	1.23	1.18	2.80	1.66	1.32	1.61	2.14	1.12	1.14	1.15	1.46	1.23	1.16	1.00
6.25	2.41	1.26	1.21	1.06	2.38	1.24	1.22	1.38	1.93	1.12	1.14	1.12	1.27	1.11	1.15	0.85
3.125	2.14	1.18	1.18	1.00	1.48	1.02	1.11	1.15	1.16	0.88	1.13	1.00	1.06	1.10	1.04	0.75
1.56	2.01	1.02	1.05	0.99	1.41	1.00	1.06	1.02	1.03	0.95	1.06	0.99	1.04	1.03	1.0	0.84
0.78	1.96				1.25				1.01				1.04			
0.39	1.86				1.21				1.00				0.94			
0.195	1.74				1.16				0.99				1.01			
0.097	1.53				1.11				0.89				1.00			
0.049	1.02				1.00				1.01				1.00			
	YG1042															
50	1.67	1.74	1.66	3.82	2.69	2.75	3.00	4.88	2.70	4.83	3.76	3.31	2.47	4.91	2.53	1.87
25	3.80	6.35	6.14	3.94	4.70	7.50	4.12	3.23	2.97	3.68	5.15	1.88	9.72	3.63	2.4	1.61
12.5	4.41	5.26	6.07	3.87	5.24	2.57	2.33	2.25	5.36	2.28	3.3	1.32	4.84	3.12	1.49	1.31
6.25	4.59	3.56	4.35	2.31	8.08	1.86	1.08	1.78	4.79	1.36	2.03	0.99	2.35	1.84	0.97	1.29
3.125	3.38	2.17	2.65	1.63	2.28	1.52	0.82	1.35	4.01	1.13	1.32	0.82	1.87	1.12	0.90	1.29
1.56	2.39	1.82	2.15	1.24	1.68	1.32	0.97	1.12	2.49	1.08	1.13	0.94	1.72	1.03	0.99	1.03
0.78	1.32	1.34	1.75	1.17	1.59	1.21			2.05				1.45			
0.39	1.22	1.17	1.35	1.08	1.57	1.13			2.03				1.25			
0.195	1.06	1.04	1.12	1.01	1.17	1.04			1.59				1.23			
0.097	0.95				0.87				1.32				1.04			
0.049	1.06				0.97				1.19				0.84			

TA98 strain, equal to 0.195 m^3, was also obtained in the same experiments. Thus, direct mutagens prevailed in the PM2.5 fraction of the particulate sample. In the case of assays conducted with individual component fractions of the PM2.5, a positive result was obtained only for the PAH nitro derivatives in the assay carried out without metabolic activation at the highest concentration of the air pollutants. In the case of the integrated PM10 extract, the assays conducted in the presence of the S9 fraction and without metabolic activation gave quite close MR values. 12.5 m^3 of air contained the lowest concentration of that extract necessary for producing a mutagenic effect in the assay conducted without the S9 fraction, while in the case of the assay with the metabolic activation, 6.25 m^3 of air contained the lowest extract concentration. The mutagenic effect was also observed for the PM10 particulate sample in the case of all the component fractions tested, both with and without metabolic activation. The mutagenicity ratios were however lower than those obtained for the unfractionated extract.

The highest MR values were obtained in the assays with the *Salmonella tyhimurium* YG1041 strain (table 1). As a TA98 derivative, this strain shows an increased sensitivity to nitro-, amino- and hydroxylamino PAH derivatives, because in its cells plasmids are found which are connected with the overproduction of nitroreductase and *O*-acetyltransferase [13]. Using this strain, a large number of revertants were obtained in the assays on whole extracts, both in the presence of the S9 fraction and without it. Within the tested range of air pollutant concentrations (from 0.049 m^3 to 50 m^3) a clear dose–response relationship was observed for this strain, which fully explains the relationship between biological effect of pollutants in the samples and their concentration. It was observed that the smallest air volume produced the mutagenic effect in YG1041 strain in the assays on PM2.5. In the experiments conducted with and without metabolic activation alike, that volume was 0.098 m^3. In the case, where the fractionated PM2.5 extract was used in the assays without S9, a mutagenic effect of PAH fraction and both fractions containing PAH nitro derivatives was observed. In the assays with metabolic activation, such an effect was obtained only for the nitro-PAH fraction. However, the MR values were lower compared with those obtained for the integrated extract. On the other hand, in the case of the integrated PM10 extract, we observed the mutagenic effect of the air pollutants derived from 0.78 m^3 air volume in the experiments without the S9 fraction, or the mutagenic effect of the air pollutants derived from 6.25 m^3 air volume – in the experiments with metabolic activation. All the fractions of the PM10 extract also demonstrated the mutagenic activity. And in this case, the MR values obtained were also lower compared with those measured in an unfractionated extract.

When the air particulate pollutants were examined in the presence of TA100 strain (table 3), known for undergoing a reversion, mainly when affected by mutagens of the base-pair substitution type [7], the MR values obtained were the lowest compared with those obtained for other strains. The highest MR (MR = 4.44) was obtained in

the assay with metabolic activation and the pollutants from the integrated PM10 extract contained in 50 m^3 of air. The lowest air volume, i.e. 1.56 m^3, still produced the mutagenic effect in TA100 strain in the assays without metabolic activation conducted on the integrated PM10 extract. The mutagenic effect caused by the integrated PM2.5 extract was less noticeable and visible in the assay without metabolic activation when the pollutants were contained in 12.5 m^3 of air and in 25 m^3 of air in the assay with the S9 fraction. In the experiments with fractionated extracts, the mutagenic effect of PM10 was obtained only for the PAH-containing fraction, in the assay conducted with metabolic activation.

In the assays with integrated extracts conducted on the YG1042 strain (table 3), a derivative of the TA100 strain, being as sensitive to nitroaromatic compounds as YG1041 [13], toxic effects appeared, proving that biological effect of pollutants depends on their concentration. The highest MR values were obtained in the assay with metabolic activation, for the integrated PM10 extract (MR = 8.08 at 6.25 m^3/plate), and in the assay with the S9 fraction, for the integrated PM2.5 extract (MR = 9.72 at 25 m^3/plate). In the assay without metabolic activation, conducted on the integrated PM2.5 extract, the smallest air volume, i.e 0.39 m^3/plate, still produced a mutagenic effect. In the case of assays for main groups of pollutants contained in the extracts of particulates tested, the mutagenic effect was not observed only for the PM2.5 dinitro-PAH fraction in the assay with metabolic activation. MR values obtained in the case of this strain for certain fractions tested were higher than those obtained for the integrated extracts at the same concentrations of pollutants introduced on a plate.

Although both samples tested differed in total content and percentage of individual PAHs and nitro-PAHs, their extracts were mutagenic for all of the strains tested. The strains tested responded more strongly to the mutagens contained in the PM2.5 sample. In the case of this sample, the air volume able to produce mutagenic effects was also larger. As has been mentioned above, biological effects, including the mutagenic effect of suspended particles, depend greatly on their size. Particulates enter the organism mainly through the respiratory tract. As has been reported in many studies, mutagenicity increases with a decrease in the suspended particle aerodynamic diameter [25], which means that the PM2.5 fraction contains greater amount of adsorbed organic compounds than the PM10 fraction. This can be explained as follows: finer particles of larger surface can adsorb more pollutants in terms of the mass of particulates [9]. It is impossible to determine a full spectrum of mutagenic pollutants present in atmospheric air. The particulate extracts tested comprise promutagen-like compounds and direct mutagens. An indirect impact of pollutant on genetic material is linked with the presence of non-substituted PAHs, whereas a direct impact – with the presence of nitro-, amino- and oxy-PAH derivatives [26]. The highest mutagenicity ratios were induced by PM2.5 extracts in the assays conducted without metabolic activation (the prevalence of direct-acting mutagens) and by PM10 extracts in the assays with the S9 fraction (the prevalence of indirect-acting mutagens). The studies confirmed a high sensitivity of YG series strains

in detecting mutagenic compounds in the atmospheric air [17]. From the assays with the above mentioned strains, both the highest mutagenicity ratios and the smallest air volumes still producing mutagenic effects were obtained, thus providing the evidence of the presence of nitroaromatic compounds in the extracts tested. Most articles dealing with this subject claim that both moderately and highly polar compounds are largely responsible for the mutagenic effect of suspended particles. In more polar fractions, the PAH nitro derivatives are usually present at higher concentrations than non-substituted PAHs [7]. Therefore, by testing the YG1041 and YG1042 strains, it is possible to detect nitro-PAH derivatives in the extracts tested. The usefulness of the YG1041 strain in air pollution monitoring was proved in the studies conducted in North Bohemia [15], Prague [27], Upper Silesia [17] and Wrocław [16]. There are many different pollutants adsorbed on suspended particles. They can act synergistically or their cumulative effect can be smaller than each effect separately. The response obtained from an assay is the resultant of such interactions [28]. When using TA98, TA100 and YG1041 strains in experiments, the MR values obtained were lower in the assays with pollutant fractions present in the samples (PAHs, nitro-PAHs and dinitro-PAHs) compared with the assays with integrated extracts. In the assays with the YG1042 strain, in the case of certain fractions, the MR values turned out to be higher than those obtained for integrated extracts. No mutagenic effect was observed only for the dinitro-PAH fraction derived from the PM2.5 extract. Mutagenic activity was also revealed by PAH fractions and fractions of polar compounds separated from particulates collected by other authors [29].

4. CONCLUSIONS

1. The extracts of particulate air pollutants differed in a total content and in the percentage of individual compounds, depending on the aerodynamic diameter of their particles.

2. The extracts of PM10 and PM2.5 sampled in spring led to mutations in the *Salmonella typhimurium* strains TA98, TA100, YG1041 and YG1042 in the assays conducted either with metabolic activation, or without it. Thus, the mutagens of the reading frame-shift type and the base-pair substitution type were present in the samples tested, both as promutagens and direct mutagens.

3. The studies have confirmed a high suitability of YG series strains for detecting mutagenic activity of nitro derivatives of aromatic compounds. These strains allowed positive results to be obtained in the tested groups of pollutants in some assays, in spite of negative results obtained in the presence of the basic strains TA98 and TA100.

4. A real health hazard posed by mutagens and carcinogens adsorbed on suspended particulates can be shown only by biological examinations, as they take into account the resultant effect of the pollutants on living organisms. In the assays conducted in the presence of TA98, TA100 and YG1041 strains, the mutagenic effect of the whole extracts of suspended particulates was greater than that of the main groups

of pollutants occurring in the samples tested. On the contrary, in the majority of the assays conducted with the YG1042 strain, the mutagenic effect of individual fractions turned out to be greater than that of the whole extracts.

5. The indicators of atmospheric air pollution used in a standard monitoring (determination of suspended particulate concentration and the concentration of EPA-listed PAHs) give only approximate information on the health hazards. The monitoring of atmospheric air pollution should be supplemented with the examination of mutagenic activity of organic pollutants by the *Salmonella* assay.

ACKNOWLEDGEMENT

The project was carried out within the No. 305 096 31/3476 grant awarded by the Polish Ministry of Science and Higher Education.

REFERENCES

[1] BRUNEKREEF B., HOLGATE S.T., *Air pollution and health*, Lancet, 2002, 360, 1233–1242.
[2] FEILBERG A., NIELSEN T., BINDERUP M.L., SKOV H., POULSEN M.W.B., *Observations of the effect of atmospheric processes on the genotoxic potency of airborne particulte matter*, Atmospheric Environment, 2002, 36, 4617–4625.
[3] SEŃCZUK W. et al., *Toksykologia*, Wydawnictwo Lekarskie PZWL, Warszawa, 1994.
[4] De KOK T.M.C.M., DRIECE H.A.L., HOGERVORST J.G.F., BRIEDE J.J., *Toxicological assessment of ambient and traffic-related particulate matter: A review of recent studies*, Mutation Researches, 2006, 613, 103–122.
[5] COHEN A.J., *Outdoor air pollution and lung cancer*, Environmental Health Perspectives, 2000, 108 (4), 743–750.
[6] CLAXTON L.D., WOODALL Jr G.M., *A review of the mutagenicity and rodent carcinogenicity of ambient air*, Mutation Research, 2007, 636, 36–94.
[7] MARON D.M., AMES B.N., *Revised methods for the Salmonella mutagenicity test*, Mutation Research, 1983, 113, 173–215.
[8] MORTELMANS K., ZEIGER E., *The Ames Salmonella/microsome mutagenicity test*, Mutation Research, 2000, 455, 29–60.
[9] CLAXTON L.D., MATTHEWS P.P., WARREN S.H., *The genotoxicity of ambient outdoor air, a review: Salmonella mutagenicity*, Mutation Research, 2004, 567, 347–399.
[10] PIEKARSKA K., KARPIŃSKA-SMULIKOWSKA J., *Effect of microsomal fraction induction on the detectability of mutagenic air pollutants by means of the Ames bacterial mutagenicity test*, Environment Protection Engineering, 2006, 32 (4), 15–23.
[11] LENICEK J., SEKYRA M., BADNARKOVA K., BENES I., SIPEK F., *Fractionation and chemical anaslysis of urban air particulate extracts*, Intern. J. Environ. Anal. Chem., 2000, 77(4), 269–288.
[12] ZACIERA M., *Metoda oznaczania nitrowych pochodnych WWA w powietrzu*, [in:] *Ochrona powietrza w teorii i praktyce*, J. Konieczyński (ed.), Instytut Podstaw Inżynierii Środowiska Polskiej Akademii Nauk, Zabrze, 2006.
[13] WATANABE M., ISHIDATE M. Jr, NOHMI T., *Sensitive method for the detection of mutagenic nitroarenes and aromatic amines: new derivatives of Salmonella typhimurium tester strains possessing elevated O- acetyltransferase levels*, Mutation Researches, 1990, 234, 337–348.

[14] Du FOUR V.A., Van LAREBEKE N., JANSSEN C.R., *Genotoxic and mutagenic activity of environmental air samples in Flanders, Belgium*, Mutation Researches, 2004, 558, 155–167.
[15] ČERNA M., POCHAMANOVA D., PASTORKOVĂ A. BENEŠ I., LENIČEK J., TOPINKA J., BINKOVĂ B., *Genotoxicity of urban air pollutants in Czech Republic*. Part I. *Bacterial mutagenic potencies of organic compounds adsorbed on PM 10 particulates*, Mutation Researches, 2000, 469, 71–82.
[16] PIEKARSKA K., KARPIŃSKA-SMULIKOWSKA J., *Mutagenic activity of environmental air samples from the area of Wrocław*, Polish Journal of Environmental Studies, 2007, 16 (5), 757–764.
[17] MIELŻYŃSKA D., SIWIŃSKA E., KAPKA L., *Efekt mutagenny pyłów zawieszonych w powietrzu jako wskaźnik jakości powietrza*, Instytut Medycyny Pracy i Zdrowia Środowiskowego, Sosnowiec, 2002.
[18] CASSONI F., BOCCHI C., MARTINO A., PINTO G., FONTANA F., BUSCHINI A., *The Salmonella mutagenicity of urban airborne particulate matter (PM 2.5) from eight sites of the Emilia-Romagna regional monitoring network (Italy)*, Sci Total Environ., 2004, 324, 79–90.
[19] GILLI G., PIGNATA C., SCHILIRO T., BONO R., LA ROSA A., TRAVERSI D., *The mutagenic hazards of environmental PM 2.5 in Turin*, Environ. Res., 2007, 103 (2), 168–175.
[20] Research Note No 94-22 – *Mutagenic activity of air pollutants*, California Environmental Protection Agency, 1994.
[21] NISBET I.C.T., LAGOY P.K., *Toxic (TETs) for polycyclic aromatic hydrocarbons (PAHs)*, Reg. Toxicol. Pharmacol., 1992, 16, 290–300.
[22] BRITS E., SCHOETERS G., VERSCHAEVE L., *Genotoxicity of PM 10 and extracted organic collected in an industrial, urban and rural area in Flanders, Belgium*, Environ. Res., 2004, 96, 109–118.
[23] PITTS Jr. J.N., VAN CAUWENBERGHE K.A., GROSJEAN D. SCHMID J.P., FITZ D.R., BELSER JR. W.L., KNUDSON G.B., HYNDS P.M., *Atmospheric reactions of polycyclic aromatic hydrocarbons: facile formation of mutagenic nitro derivates*, Science, 1978, 202, 515–519.
[24] MÖLLER L., *In vivo metabolism and genotoxic effects of nitrated polycyclic aromatic hydrocarbons*, Environ. Health Perspect., 1994, 102 (4), 139–146.
[25] BUSCHINI A., CASSONI F., ANCESCHI E., PASINI L., POLI P., ROSSI C., *Urban airborne particulate: genotoxicity evaluation of different size fractions by mutagenesis tests on microorganisms and comet assay*, Chemosphere, 2001, 44, 1723–1736.
[26] SIENRA M. del R., *Oxygenated polycyclic aromatic hydrocarbons in urban air particulate matter*, Atmospheric Environment, 2006, 40, 2374–2384.
[27] BINKOVÁ B., ČERNA B., PASTORKOVÁ A., JELÌNEK R., BENEŠ I., NOVÁK J., ŠRÁM R.J., *Biological activities of organic compounds adsorbed onto ambient air particles: comparison between the cities of Teplice and Prague during the summer–winter seasons 2000–2001*, Mutation Research, 2003, 525, 43–59.
[28] KÜNZLI N., KAISER R., MEDINA S., STUDNICKA M., CHANEL O., FILLIGER P., HERRY M., HORAK Jr. F., PUYBONNIEUX-TEXIER V., QUÉNEL P., SCHNEIDER J., SEETHALER R., VERGNAUD J.C., SOMMER H., *Public-health impact of outdoor and traffic-related air pollution: a European assessment*, Lancet, 2000, 356, 795–801.
[29] DUCAATTI A., VARGAS V.M.F., *Mutagenic activity of airborne particulate matter as an indicative measure of atmospheric pollution*, Mutation Research, 2003, 540, 67–77.

EFEKT MUTAGENNY GŁÓWNYCH GRUP ZANIECZYSZCZEŃ ORGANICZNYCH ZAADSORBOWANYCH NA CZĄSTKACH PYŁU ZAWIESZONEGO PM10 I PM 2,5 POBRANEGO NA TERENIE WROCŁAWIA

Korzystając z testu *Salmonella*, stwierdzono mutagenne działanie zanieczyszczeń zaadsorbowanych na pyle zawieszonym PM10 i PM2,5 pobranym wiosną na terenie Wrocławia. Testy prowadzono w obec-

ności szczepu TA98 oraz jego pochodnej YG1041. W badanych próbkach stwierdzono zanieczyszczenia o charakterze mutagenów typu zmiany fazy odczytu, które mogą oddziaływać bezpośrednio i pośrednio na materiał genetyczny. Uzyskano niższe wartości współczynnika mutagenności (MR) w testach, do których wprowadzano frakcje WWA, nitro-WWA i dinitro-WWA, w porównaniu z wartościami MR otrzymanymi dla całkowitych ekstraktów. Jedynie frakcje WWA i dinitro-WWA obecne w ekstrakcie pyłów PM2.5 nie miały efektu mutagennego.