Environment Protection Engineering

Vol. 37 2011 No. 2

KATARZYNA PIEKARSKA*, MARZENA ZACIERA**,
ANNA CZARNY***, EWA ZACZYŃSKA***

APPLICATION OF SHORT-TERM TESTS IN ASSESSMENT OF ATMOSPHERIC AIR POLLUTION

Results of analyses of airborne particulates collected in winter and summer seasons from two locations of Wrocław (city centre and the outskirts area) have been presented. Samples of particulate matter were collected on sintered-glass filters, using a high performance air aspirator Staplex-PM10. Extraction with dichloromethane was carried out in a Soxhlet apparatus. Mutagenicity of dichloromethane extracts was studied based on a conventional *Salmonella* assay. Two *Salmonella typhimurium* strains TA98 and YG1041 were employed in the assay. Genotoxicity of organic air pollutants was detected using a miniaturized SOS chromotest obtained from EBPI (Brampton, Ontario, Canada). Bacterial assays were carried out with and without metabolic activation by the S9 microsomal fraction. Cytotoxicity of the particulate extracts was studied by the method of their direct contact with one-layer culture of human lung carcinoma epithelial cells – A549.

1. INTRODUCTION

Atmospheric air is a carrier for xenobiotics, action of which human organism is exposed to through a respiratory tract [1]. Most of air pollutants are gaseous. Another group of pollutants constitutes of particular matter (PM), which is a complex mixture of organic and inorganic substances. Particulate matter can take the form from submicro particle aerosols to easy visible dust particles. Depending on size of particles, the following PM fractions can be distinguished: PM10 – with particles diameters below 10 μm, PM5 – with particle diameters below 5 μm, PM2.5 – with particle diameters below 2.5 μm, and PM1 – with particles smaller than 1 μm [2]. Pollutants present in air adsorb on airborne particulates of different sizes. The fine particle fraction, of grain

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

**Instiute of Occupational Medicine and Environmental Health, ul. Kościelna 13, 41-200 Sosnowiec, Poland.

***Institute of Immunology and Experimental Therapy, ul. Weigla 12, 53- 114 Wrocław, Poland.

size below 5 μm, shows the highest biological activity as it is able to penetrate to alveoli in the human and animal respiratory systems. Chemical compounds having effects on alveoli epithelial cells are gradually released from particulates retained in the alveoli. Then through the alveoli they get into the circulatory system and this way spread over the entire organism [3]. A major group, one relatively well recognized, includes polycyclic aromatic hydrocarbons (PAHs) – which are known of mutagenic and cancerogenic activity. It has been found that aromatic hydrocarbons are not the only cyclic compounds showing genotoxic effects and able to adsorb on dust particles. Also nitro-, chloro- and oxy- PAH derivatives as well as other yet unidentified chemical substances show significant mutagenic activity [3, 4].

Standard air tests do not cover determination of biological activity of pollutants present in the air. Assessment of air pollution level involves determination of concentrations for particulate matter and USEPA listed PAHs, followed by comparison of obtained values with those permitted by legal regulations. Such procedure provides knowledge only on current environment condition and does not give any indications as to the pollution effects on living organisms. Full chemical analysis of air pollution would be impracticable because of complexity of pollutant mixture composition and mutual interactions of pollutants in the mixture. Therefore a full chemical analysis cannot be taken as a basis for forecasting biological effects that might be produced by pollutants in humans and animals. Examination of atmospheric air particulate matter pollution using short-term tests may become an excellent tool in assessment of health hazard linked to the presence of chemical compounds adsorbed on airborne particulates [3, 4].

The purpose of this work was to determine genotoxicity and cytotoxicity of chemical pollutants adsorbed on particles from PM10 fraction collected in the Wrocław urban area. Wrocław is an urban-industrial agglomeration where air pollution comes from three sources: so called low-level emission, industrial nuisances and passenger car and goods traffic. The *Salmonella* plate test (Ames assay) of high forecasting power in relation to mutagenic and potentially cancerogenic agents was used in investigations and was considered the first short term test among the methods applied in genetic toxicology [5]. It is the most widely used bioassay in investigations on mutagenicity of atmospheric air particulates pollution [4]. Genotoxicity of organic air pollutants was detected by using the SOS chromotest. Few applications of the SOS chromotest have been reported in examination of particulate matter extracts and compared with respective results from the *Salmonella* assay [6, 7]. Cytotoxic effects of the particulate extracts in turn were investigated by the method of direct contact of the extracts with one layer culture of human lung carcinoma epithelial cells – A549. Lung epithelium cells are the first defense of the organism, ready to remove fine particles and bacteria from the respiratory system. Fitness of cell lines to evaluate cytotoxic properties of respirable particles was confirmed in numerous studies, and the recent investigations in Europe indicate that the smaller diameter of tested particles, the greater cytotoxic effect

is and thus their role in formation of DNA oxidative defects. The greatest cytotoxic effect can be observed in the case of particles from the PM0.2 fraction, and then the PM2.5–0.2 fraction; the least effect is produced by PM10–2.5 particles [8, 9].

2. MATERIALS AND METHODS

Air samples were collected using a high performance air aspirator Staplex PM-10, FC-2ETM model. The samples were taken during summer (samples I and II) and winter (samples III and IV) seasons from two city locations. The former sampling point was located in the Wrocław outskirts, at Strachocińska St. (samples I and III), in the low-rise residential housing area, close to quite a busy exit route from the town. The area has not been connected to a utility gas supply system, thus kitchen ranges are used in many houses all year round. The other sample collection point was located in the city centre, at Grunwaldzki Sq. which is cut along its long axis by the only transit route towards Warsaw. Heavy traffic is noted here 24 hours a day. Times of sample collection and sample volumes are presented in Table 1.

Table 1

List of data on collected PM10 samples

Type of sample	Collection time [h]	Air volume [m^3]	Mass of particulate matter [$\mu g/m^3$]	Mass of tar substances [$\mu g/m^3$]
I. Summer (Strachocińska St.)	67	4 542.6	57.17	19.90
II. Summer (Grunwaldzki Sq.)	96	6 474.9	38.33	26.68
III. Winter (Strachocińska St.)	119	8 035.0	138.93	145.15
IV. Winter (Grunwaldzki Sq.)	105	7 069.0	87.32	51.04

Antihygroscopic glass filters together with particulates from individual test cycles were combined into one sample, cut and placed in a Soxhlet apparatus. Then extraction was carried out using dichloromethane for 16 h plus 15 min reflux, without access of light. Extracts were thickened until dry in a vacuum evaporator and the dry residue was weighed in order to determine the amount of tar substances in samples. Obtained dry extracts were analysed to find PAH, nitro-PAH and dinitro-PAH content, they also were used in bioassays [4, 10, 11]. PAHs in the samples were determined by a high performance liquid chromatography technique using fluorescence detection, whereas the nitro-PAH content – by the gas chromatography using mass detection [12–14].

Mutagenicity of the particulate matter was evaluated based on a bacterial *Salmonella* test (Ames assay) [15]. It consists in checking whether the tested material causes reverse mutation (reversion) of special histidine dependent (his^-) strains of *Salmonella*

typhimurium LT2 bacteria. Two test strains were used in investigations: *Salmonella typhimurium* TA98 and YG1041*. The TA98 strain detects mutagens of the reading frame shift type. The YG1041 strain is a derivative of the TA98 strain and shows increased sensitivity to nitro-, amino- and hydroxylamino-PAH derivatives [16]. The assay was carried out in experiment versions without metabolic activation, and with the metabolic activation by a microsomal fraction S9 activated with Aroclor 1254, and derived from Wistar rat liver. The microsomal fraction S9 was used in assays to induce metabolic activation of promutagens. Protein content in the fraction, as determined by Lowry's method, was 64.44 mg/cm^3. S9-mix containing 4 vol. % of S9 was used in experiments. Each time before starting the experiments, genotypes of test strains were checked. Also the number of spontaneously induced revertants was determined (negative check) as was the number of revertants induced by the action of diagnostic mutagens (positive check). As diagnostic mutagens in experiments without metabolic activation by S9 fraction served: 2,4,7-trinitro-9-fluorenone (for TA 98 strain) and 2,6-dinitrotoluene (for YG 1041 strain), and in the case of experiments in the presence of the S9 fraction – 2-aminofluorene (TA98, YG1041).

Mutagenic effect of airborne particulates was presented in the form of the mutagenicity ratio (*MR*) calculated from the formula:

$$MR = \frac{n_i}{n_s},$$

where n_i denotes the number of induced revertants, and n_s – the number of spontaneous revertants. Samples were considered mutagenic if their mutagenicity ratios *MR* were equal or higher than 2.

Genotoxicity of organic air pollutants was detected using a miniaturized SOS Chromotest obtained from EBPI (Brampton, Ontario, Canada). The principle of the test is based on activation of DNA repair process – so called "SOS system", in cells of *Escherichia coli* K12 PQ37 bacteria. Activation of the process indicates that DNA has been damaged. In the test strains, the SOS system operator's gene had been fused with a structural gene of the β-galactozidaze enzyme. Expression of the SOS system genes is measured in the test under contact with the tested sample. Their action is measured by the β-galactozidaze activity [17]. Individual dilutions of organic extracts of air pollutants were dissolved in DMSO and placed in microwells on test plates. The test was performed following the instructions attached to the test set. Experiments were carried with and without metabolic activation with the microsomal fraction S9. As diagnostic mutagens were used 4-nitroquinoline-N-oxide and 2-aminoanthracene. Absorbance was measured at 615 nm (genotoxic activity) and 405 nm (bacteria survival) on

**Salmonella* test strains were obtained from Dr. T. Nohmi, Division of Genetics and Mutagenesis, National Institute of Hygienic Sciences, Tokyo, Japan.

a spectrophotometer Sunrise (Tecan, Austria). The genotoxic effect of airborne particulates was evaluated in the form of induction factor (*IF*):

$$IF = \frac{R(C)}{R(0)},$$

where: $R(C)$ – specific activity of β-galactozidaze at a given concentration c of the tested sample, $R(0)$ – specific activity of β-galactozidaze for the negative control. The tested sample was considered genotoxic if the *IF* value was higher than 1.5.

Cytotoxicity of the particulate extracts was studied by the method of their direct contact with one-layer culture of human lung carcinoma epithelial cells – A549 (American Type Culture Collection, Cell Culture Line – ATCC CCL 185). Cultures of A549 cells were grown in Dulbecco culturing medium with addition of 10% inactivated (30 min, 56 °C) veal serum and 100 u/cm^3 penicillin, 100 μg/cm^3 streptomycin and 2×10^{-3} M L-glutamine. One-layer culture of A549 cells, of the density 2×10^6/cm^3, was placed on plastic 96-well plates and incubated for 24 h at 37 °C, in the ambient atmosphere of 5% CO_2. After that time the fluid from above the cells was removed, and tested extracts in corresponding concentrations were placed over one-layer cultures of the A549 cells. Then the cultures were incubated for 24, 48 and 72 h at 37 °C, in the atmosphere of 5% CO_2. Cell cultures without addition of tested samples, and the cell cultures with addition of DMSO in the same amount as in tested samples served as reference. Quantitative and morphological changes that occurred as the effect of addition of tested extracts were estimated after 24, 48 and 72 h using an inverted microscope. Minimum concentration of tested samples, yet producing degeneration in 50% of cells, was considered a toxic dose (Tissue Culture Cytotoxic Dose – $TCCD_{50}$) [18]. Results of the cytotoxic tests were presented as volumes of tested air (in m^3) sufficient for obtaining the extracts that still would induce toxic effects.

3. RESULTS AND DISCUSSION

Concentrations of airborne particles in selected test points ranged from 38.33 μg/m^3 (II) to 138.93 μg/m^3 (III). Concentrations of tar substances ranged from 19.9 μg/m^3 (I) to 145.15 μg/m^3 (III). The highest amounts of particulates (138.93 μg/m^3) and tar substances (145.15 μg/m^3) were found in sample III (Table 1). The observed seasonal differences in those concentrations corresponded with literature reports [19, 20]. For the most part, the differences result from increased emission of particulates in urban agglomerations during winter seasons, due to combustion processes for heating purposes. Obtained PM10 amounts (38.33–57.17 μg/m^3 in summer and 87.32 –138.93 μg/m^3 in winter) were close to the levels measured in spring–summer and

autumn–winter seasons in various European cities (Czech Republic, Italy, Belgium) [10, 21–23].

Table 2

PAH concentration in PM10 organic extracts [ng/m^3]

Controlled WWA	Summer (I)	Summer (II)	Winter (III)	Winter (IV)
Fen	0.106	0.374	6.252	3.792
A	0.016	0.034	0.856	0.562
Flu	0.270	0.574	4.336	5.314
Pyr	0.150	0.360	11.244	4.58
B[a]A	0.088	0.174	4.24	1.32
Chr	0.120	0.220	3.1	2.28
B[b]F	0.518	0.654	2.8	0.372
B[k]F	0.164	0.222	1.844	0.85
B[a]P	0.514	0.438	7.788	2.65
D[a,h]A	0.242	0.194	0.364	0.224
B[g,h,i]P	0.154	0.366	0.984	2.19
I[1,2,3-c,d]P	n.d.	n.d.	0.384	1.69
Total	2.342	3.610	44.192	25.824

n.d. – not detected

Tested samples (Table 2) contained the same twelve PAHs as those in the list of regulated aromatic hydrocarbons. The highest PAH amount adsorbed on airborne particles was noted in winter season. Total PAH amount detected at that time ranged from 25.824 ng/m^3 to 44.192 ng/m^3, whereas in PM samples collected in summer – from 2.342 ng/m^3 to 3.61 ng/m^3. Three PAHs among those present in the samples, namely (benzo[a]anthracene, benzo[a]pyrene, dibenzo[a,h]anthracene) were classified by IARC in 2A group as probably carcinogenic to humans, and another three PAHs: (benzo[b]fluoranthene, benzo[k]fluoranthene, indeno[1,2,3-c,d]pyrene) were classified in 2B group, as possibly carcinogenic to humans [24]. All PM samples contained high percentage of benzo[a]pyrene, and its concentration ranged from 0.438 ng/m^3 (II) to 7.788 (III) ng/m^3. Benzo[a]pyrene is an indicator compound in relation to which compared is carcinogenic potential of other PAHs [24, 25]. A relative carcinogenicity index for that compound has been assumed 1. The only compound found to have higher carcinogenic potential is benzo[a]anthracene (k = 5), and its presence was also confirmed in the tested extracts. Benzo[a]pyrene content reached 1/5–1/10 of total content of all PAHs, i.e. from 10.3% (IV) to 21.9% (I) of total PAH concentration in tested extracts. Determined concentrations of individual PAHs were within the limits reported in the literature, and were very close to those detected in urban areas in Belgium and Prague [10, 22].

Also content of nitro-PAH derivatives was determined in tested PM samples (Table 3). Nitro-PAH derivatives because of their mutagenic activity are ranked among most hazardous chemicals for human health. The studies carried out so far indicate that the nitro-PAH concentration in the air is low, which brings about considerable analytic problems [12, 14]. Certain nitro-PAHs can be formed during combustion processes. They are present for instance in extracts of particles sampled from exhaust gas of diesel engines (e.g. nitrofluorene, nitroanthracene, nitrofluoranthene, nitrobenzo[a]pyrene). However, majority of them are produced in atmospheric air as a result of PAH reaction with nitrogen oxides in gaseous phase.

Table 3

Table 3. Nitro-PAH concentration in PM10 dust extracts [ng/m^3]

Controlled nitro-PAH	Sample			
	Summer (I)	Summer (II)	Winter (III)	Winter (IV)
1-Nitronaphtalene	0.45	n.d.	0.83	0.45
2-Nitrofluorene	0.28	0.29	3.36	1.41
9-Nitroanthracene	n.d.	n.d.	1.84	0.74
3-Nitrofluoranthene	n.d.	n.d.	0.6	0.3
1-Nitropyrene	n.d.	n.d.	0.42	0.26
1,3-Dinitropyrene	n.d.	n.d.	n.d.	n.d.
1,6-Dinitropyrene	n.d.	n.d.	n.d.	n.d.
1,8-Dinitropyrene	n.d.	n.d.	n.d.	n.d.
Total	0.73	0.29	7.05	3.16

n.d. – not detected.

Nitro-PAHs occur in lower concentrations than non-substituted aromatic hydrocarbons, nevertheless they are very stable in solid phase, and as compared with PAHs – some of them have higher mutagenic (2×10^5 times) and cancerogenic (10 times) power [14]. Total content of those compounds in tested samples ranged from 0.29 ng/m^3 (II) to 7.05 ng/m^3 (III). The selected dinitro-PAHs were not found in extracts within their determination limits. Although observed were PAH mononitroderivatives, among others 2-nitrofluorene and 1-nitropyrene, both ranked in the 2B group. 2-Nitrofluorene was present in all tested samples, while 1-nitropyrene was found only in winter-collected samples. Large amounts of 2-nitrofluorene were detected in winter-collected samples. That compound comprised near half of the nitro-PAH content in those extracts. Its amount in the sample collected in summer (I) reached ca. 38%. Its largest amount was found in sample II (summer), even 100%, as it was the only mononitroderivative detected in that collection point. The largest number of compounds classified as PAH nitro derivatives was detected in winter-collected PM samples. Their highest concentration was found in sample III collected in winter at the Strachocińska St. The concentration reached 7.05 ng/m^3.

Table 4

MR ± S.D. for various concentrations of PM extracts from tested air samples, determined by *Salmonella* assay in TA 98 strain conducted in the presence and in the absence of S9 fraction[a]

Sample concentration [m^3/plate]	Sample							
	Summer (I)		Summer (II)		(Winter (III)		Winter (IV)	
	–S9	S9	–S9	S9	–S9	S9	–S9	S9
50	**3.49±0.2**	**2.62±0.2**	**0.43±0.2**	**0.57±0.1**	**9.29±0.3**	**17.7±0.4**	**31.73±0.6**	**18.28±0.4**
25	**2.2±0.2**	**2.32±0.2**	**1.78±0.3**	**0.85±0.2**	**12.49±0.5**	**13.14±0.2**	**28.52±0.5**	**15.98±0.5**
12.5	1.82±0.1	1.35±0.1	**2.05±0.1**	**11.04±0.2**	**13.63±0.4**	**11.52±0.2**	**20.68±0.7**	**13.41±0.3**
6.25	1.55±0.2	1.35±0.2	**2.67±0.2**	**17.9±0.3**	**9.19±0.3**	**10.29±0.3**	**10.31±0.4**	**10.9±0.4**
3.125	1.45±0.2	1.15±0.2	**15.5±0.3**	**16.34±0.4**	**5.91±0.3**	**4.85±0.4**	**10.15±0.4**	**6.95±0.2**
1.56	1.35±0.1	1.08±0.2	**8.41±0.2**	**9.64±0.2**	**4.27±0.2**	**3.85±0.2**	**5.93±0.2**	**6.8±0.1**
0.78	1.29±0.1	1.04±0.3	**2.94±0.4**	**8.5±0.2**	**3.7±0.3**	**2.87±0.2**	**5.1±0.2**	**6.34±0.3**
0.39	1.25±0.1	0.92±0.2	1.43±0.1	**5.12±0.2**	**2.76±0.2**	**2.77±0.2**	**5.09±0.3**	**4.73±0.4**
0.195	1.19±0.2	0.92±0.2	1.16±0.1	**3.13±0.1**	1.83±0.2	**2.23±0.1**	**4.6±0.1**	**2.94±0.2**
0.097	1.12±0.2	0.77±0.3	1.09±0.1	**2.27±0.1**	1.71±0.2	1.81±0.1	**4.04±0.2**	1.6±0.2
0.049	1.06±0.1	0.77±0.2	1.01±0.1	1.16±0.1	1.67±0.1	1.45±0.1	**2.41±0.2**	1.29±0.1
0.0245	1.06±0.3	0.73±0.2	0.93±0.1	0.67±0.1	1.07±0.1	1.25±0.1	1.42±0.1	1.08±0.1

[a]Negative check 22–36 revertants per plate. Positive check 3242–4552 revertants per plate (–S9), 1645–1886 revertants per plate (S9).

Table 5

MR ± S.D. for various concentrations of PM extracts from tested air samples, determined by *Salmonella* assay in YG 1041 strain conducted in the presence and in the absence of S9 fraction[a]

Sample concentration [m^3/plate]	Sample							
	Summer (I)		Summer (II)		(Winter (III)		Winter (IV)	
	–S9	S9	–S9	S9	–S9	S9	–S9	S9
50	**2.96±0.4**	**2.63±0.4**	**2.85±0.2**	**2.34±0.1**	**3.69±0.3**	**1.16±0.1**	**9.54±0.4**	**10.45±0.3**
25	**7.29±0.2**	**4.56±0.2**	**4.84±0.3**	**2.86±0.3**	**6.06±0.2**	**5.06±0.2**	**15.81±0.5**	**11.9±0.4**
12.5	**4.75±0.3**	**2.52±0.3**	**6.4±0.4**	**3.66±0.2**	**8.57±0.4**	**7.33±0.4**	**16.48±0.4**	**12.52±0.3**
6.25	**2.34±0.2**	1.92±0.2	**3.21±0.1**	1.05±0.1	**5.96±0.3**	**6.61±0.3**	**13.71±0.3**	**15.0±0.2**
3.125	1.93±0.1	1.66±0.2	**2.42±0.1**	0.99±0.1	**5.39±0.2**	**4.25±0.3**	**10.64±0.2**	**14.49±0.3**
1.56	1.53±0.2	1.49±0.1	1.95±0.1	0.99±0.2	**4.35±0.2**	**3.58±0.2**	**10.36±0.2**	**12.43±0.2**
0.78	1.32±0.2	1.43±0.1	1.51±0.1	0.93±0.1	**3.92±0.2**	**3.41±0.2**	**10.33±0.2**	**11.92±0.3**
0.39	1.16±0.1	1.32±0.1	1.22±0.1	0.9±0.1	**2.91±0.2**	**2.39±0.2**	**9.23±0.3**	**6.3±0.2**
0.195	1.14±0.1	1.18±0.1	0.95±0.2	0.85±0.2	**2.58±0.1**	1.87±0.1	**7.75±0.4**	**5.2±0.2**
0.097	1.11±0.1	0.92±0.1	0.84±0.1	0.85±0.1	**2.41±0.1**	1.55±0.1	**4.89±0.2**	**4.3±0.2**
0.049	0.91±0.1	0.99±0.1	0.75±0.1	0.8±0.1	1.22±0.1	1.37±0.1	**3.87±0.2**	**3.64±0.2**
0.0245	0.83±0.1	1.00±0.1	0.74±0.2	0.86±0.1	1.10±0.1	1.01±0.1	**2.51±0.2**	**3.05±0.2**
0.0061	0.95±0.1	0.97±0.1	0.8±0.1	0.89±0.1	1.09±0.1	0.93±0.1	**2.11±0.1**	**2.32±0.1**
0.0015	0.97±0.1	1.00±0.1	0.9±0.1	0.91±0.1	1.11±0.1	0.94±0.1	1.23±0.1	1.36±0.1

[a]Negative check 128–179 revertants per plate. Positive check 1165–1396 revertants per plate (–S9), 1126–1216 revertants per plate (S9).

Tested extracts produced mutagenic effects in both used strains (Tables 4 and 5). The winter-collected PM samples showed higher mutagenic activity than those collected in summer, which is in line with results reported by other authors [4]. The tested samples contained pollutants able affect genetic material indirectly or directly as well. As the evidence for that can be considered high mutagenicity ratios (*MR*) obtained in experiments carried out in the presence of the microsomal fraction S9 and without it as well. For the YG1041 strain higher *MR* values were obtained from assays carried out without using the S9 fraction. Thus that strain was the most sensitive to direct mutagens present in the tested samples. Higher *MR* values were obtained for samples collected in the city centre than for samples from the outskirts of the town. Only from assays carried out for pollutants collected in summer and using the YG1041 strain, higher *MR* values were obtained for the sample collected on the outskirts of the town (I). The highest *MR* values were obtained for extracts of particulates collected in winter in the city centre, when the TA98 strain was employed in assays. The respective values were: 31.73 ± 0.6 in the case of assays carried out without the S9 fraction and 18.28 ± 0.4 in assays carried out in the presence of the S9 fraction. For that sample also the highest *MR* values were obtained from assays carried out using the YG1041 strain, respectively: 16.48 ± 0.4 (–S9) and 15.0 ± 0.2 (S9). Maximum *MR* values for individual tester strains were obtained at various concentrations of particulates introduced into assays which is evidence for their differentiated sensitivity to tested compounds. Within the tested range of air extract concentrations (50–0.0015 m^3/plate), a distinct dose–response curve was observed, describing fully the biological effects of pollutants present in extracts, depending on their concentration. Owing to a broad range of tested air sample concentrations in case of YG1041 strain (Table 6), it was possible to determine an inflexion point on the dose-response curve (toxic effect). In case of TA98 strain such effect was obtained with the sample II (summer – city centre) and the sample III (winter – town outskirts) from assays carried out in the absence of S9 fraction. In case of other extracts even at pollutant concentrations derived from 50 m^3 of air the threshold points of their toxic effects and thus the air volumes producing maximal mutagenic effect. The least volumes of polluted air, producing mutagenic effect in strains used in assays varied greatly. Detection threshold of the TA98 strain was in the order of 0.049–0.39 m^3 for winter conditions and 0.097–25 m^3 for summer conditions. And the respective detection thresholds of the YG1041 strain were 0.0061 –0.39 m^3 for winter conditions and 3.125–12.5 m^3 for summer conditions. There was no significant relation between the PM concentration and the polluted air volume that produced mutagenic effect. The highest particulate matter concentration, 138.93 μg/m^3, was obtained for the sample III (winter – town outskirts), whereas the highest concentration of adsorbed mutagenic compounds was found for the sample IV (winter – city centre). The particulates concentration for that sample was 1.5 times lower, i.e. 87.32 μg/m^3. Similar situation was observed for pollutants collected in summer. The highest particulate matter concentration was obtained for the sample I (summer – town out-

skirts): 57.17 μg/m^3, while the least volume of polluted air, producing mutagenic effect was found for the sample II (summer – city centre). The concentration of particulates for that sample was also about 1.5 times lower, which was 38.33 μg/m^3. Particulate matter collected in the city centre area (samples II and IV) contained higher total PAH amount, although percentages of individual PAHs in the samples varied considerably.

Table 6

IF values for particulate pollution extracts determined with SOS Chromotest[a]

Sample concentration [m^3/well]	Sample							
	Summer (I)		Summer (II)		Winter (III)		Winter (IV)	
	–S9	S9	–S9	S9	–S9	S9	–S9	S9
50	**1.89**	1.32	**4.12**	**3.80**	TOX	TOX	TOX	TOX
25	**1.56**	1.24	**3.90**	**2.50**	TOX	**3.78**	TOX	**5.27**
12.5	1.40	1.14	**3.20**	**1.95**	**5.12**	**3.46**	TOX	**5.01**
6.25	1.32	1.09	**2.45**	**1.54**	**4.97**	**2.57**	**9.34**	**4.56**
3.125	1.27	1.12	**1.65**	1.40	**3.20**	**1.80**	**7.17**	**3.12**
1.56	1.20	1.08	1.30	1.38	**2.69**	**1.65**	**5.61**	**2.01**
0.78	1.17	1.00	1.21	1.25	**1.68**	1.45	**3.98**	**1.76**
0.39	1.12	0.98	1.15	1.20	**1.54**	1.21	**2.19**	1.40
0.195	1.10	1.05	1.05	1.15	1.40	1.19	**1.63**	1.32
0.097	1.00	0.95	0.95	1.05	1.34	1.13	1.42	1.30
0.049	1.05	0.91	1.10	0.95	1.25	1.17	1.41	1.23
0.0245	1.10	1.03	1.00	1.00	1.19	1.08	1.32	1.10
0.0061	0.97	1.11	0.89	1.00	1.05	1.00	1.11	1.07
0.0015	1.00	1.00	0.95	1.05	0.97	1.05	1.02	1.01

[a]TOX – toxic sample.

Results of investigations on genotoxic activity of PM extracts, carried out using a miniaturized bacterial screening test called the SOS Chromotest are presented in Table 6. Tested extracts revealed genotoxic properties in the *Escherichia coli* K12 PQ37 strain used in the test. The *IF* values obtained from all conducted tests for samples collected in summer and in winter alike exceeded 1.5 and showed a linear dose-response relationship. For pollutants present in tested extracts, higher *IF* values were obtained from tests carried out without metabolic activation, and furthermore, the obtained *IF* values were higher for samples collected in winter as compared to the *IF* values for summer-collected samples. Thus the results indicate that chemical compounds effecting directly on genetic material prevail in tested extracts. The direct genotoxicity of organic air pollution is related to the presence of such compounds as nitro-, hydroxy- and oxy- PAH derivatives in the tested extracts [4, 26, 27]. Moreover, higher *IF* values were obtained for extracts of particulate matter collected in the city

centre area (II, IV – Grunwaldzki Sq.) as compared with the *IF* values obtained for samples collected on outskirts of the town (I, III – Strachocińska St. Volumes of polluted air that induced the mutagenic effect were larger than those obtained from the *Salmonella* assay; they oscillated around 3.125–25 m^3 in summer and 0.195–1.56 m^3 in winter.

Table 7

Effects of particulate pollution extracts on A549 line human lung cells, obtained from *in vitro* tests[a]

Contact duration	Type of sample	Toxic effect [m^3]							
		50	25	12.5	6.25	3.13	1.56	0.78	0.39
24	Summer (I)	t	t	n	n	n	n	n	n
	Summer (II)	t	t	t	n	n	n	n	n
	Winter (III)	t	t	t	t	t	n	n	n
	Winter (IV)	t	t	t	t	t	t	n	n
	Control sample A549	n	n	n	n	n	n	n	n
48	Summer (I)	t	t	n	n	n	n	n	n
	Summer (II)	t	t	t	n	n	n	n	n
	Winter (III)	t	t	t	t	t	n	n	n
	Winter (IV)	t	t	t	t	t	t	n	n
	Control sample A549	n	n	n	n	n	n	n	n
72	Summer (I)	t	t	n	n	n	n	n	n
	Summer (II)	t	t	t	t	n	n	n	n
	Winter (III)	t	t	t	t	t	t	t	n
	Winter (IV)	t	t	t	t	t	t	t	n
	Control sample A549	n	n	n	n	n	n	n	n

[a]n – non-toxic sample, t – toxic sample.

Investigations on organic air pollution by application of the SOS chromotest have been reported in the literature by far less in comparison with those based on the *Salmonella* assay. However all such reports emphasize great usefulness of this test in monitoring of air pollution. Research conducted in Czech Republic and in Bosnia and Herzegovina by Škarek et al. [6, 7] has shown considerable variation of results obtained in the test. In Czech Republic, particulates were sampled in July 2002 in heavily urbanized regions (2 locations) and poorly urbanized regions (2 locations). Positive results of the tests were obtained only in two urban locations, from tests carried out without metabolic activation. Although in tests carried out in May 2004 in a poorly industrialized Sarajevo and heavily industrialized Tuzla – all tests yielded positive results, irrespectively of whether the S9 fraction was present or not. However, similar as in the research presented here, greater genotoxic power was observed for samples collected in heavily industrialized area and tested without metabolic activation.

Results of investigation regarding the effect of tested PM extracts in the lung carcinoma cells of the A549 line have been given in Table 7. According to many researchers, in the assessment of harmful effects of chemical compounds on human organism, results of mutagenicity and genotoxicity studies should be considered in correlation with corresponding results from toxicity studies. When testing substances that penetrate the organism through a respiratory tract, cell lines derived from pulmonary alveoli epithelial cells as well as the lines derived from macrophages are used. Epithelial cells of the respiratory system and macrophages of the pulmonary alveoli, through the process of respirable particle phagocytosis, constitute the organism defense against penetrating pollutants. Cytotoxicity and mutagenicity studies under *in vitro* conditions have proved that the human A549 cell line produced from neoplastic cells of pulmonary carcinoma will be useful in examination of properties of respirable particles [9, 11, 28]. The essence of mutation consists in irreversible damage in genetic material of a cell, which leads to changes in its functioning, which in turn results in its insusceptibility to mechanisms that regulate the rate of cell division, growth and differentiation. If the modified cells that build the human body are not eliminated in the early phase by the immune system, they will begin to proliferate, which may lead to a neoplasm formation [12].

The research covered toxic effects of organic pollutants adsorbed on particular matter collected in winter and in summer alike on cells of the A549 line under *in vitro* conditions. Air concentrations that caused toxic effects varied, depending on sampling season and contact time of the tested pollutants with the cell line. Stronger toxic effect was noted in the case of PM extracts collected in winter as compared with those summer-collected. Similar seasonal variability of the cytotoxic effect was observed in other cities [9, 11, 28]. After 24, 48, as well as after 72 h in observation, the lethal effect in 50% of tested cells was caused by smaller doses of winter-sampled air versus the air doses collected in summer. In the case of extracts of airborne particulates sampled in winter the air volumes were: 1.56 m^3 (IV) and 3.13 m^3 (III) after 24 and 48 h of contact, and 0.78 m^3 for both samples after 72 h of contact. In the case of samples collected in summer, the toxic effect in A549 cells was observed after 24 and 48 h of exposure to pollutants derived from 12.5 m^3 of air collected at Grunwaldzki Sq. location (II) and from 25 m^3 of air collected at Strachocińska St. (I). The strongest toxic effect induced by the summer-collected samples was observed for sample II after 72 h in exposure. The air dose inducing such effect after that time of exposure was 6.25 m^3. Whereas the dose of PM pollutants collected at Strachocińska St. and inducing the toxic effect was 25 m^3, which is the same as after 24 and 48 h exposure.

4. SUMMARY

The investigations have confirmed high sensitivity of employed *Salmonella typhimurium* TA98 and YG1041 tester strains as well as the *Escherichia coli* K12 PQ37 to

organic pollutants adsorbed on airborne particulates of the PM10 fraction, which provided evidence for occurrence of PAHs and their nitro-, amino- and hydroxylamino-derivatives in atmospheric air. *MR* and *IF* values obtained in both bacterial tests indicated that chemical compounds of both promutagen and direct mutagen nature were present in tested samples, and moreover, similar *MR* values were obtained in the *Salmonella* assay from experiments carried out with metabolic activation by the microsomal fraction S9 as well as in the absence of the S9 fraction. By including the YG series strains in the assays, it was possible to confirm that moderately and highly polar classes of compounds are responsible for the mutagenic effect of air pollutants, and besides that, to detect the presence of mutagens in very small volumes of sampled air, of the range of 0.0061–0.049 m^3. Considering the results obtained from the SOS Chromotest, higher genotoxicity was found in tests carried out without metabolic activation than in the presence of the microsomal S9 fraction. Among chemical compounds capable of producing such effects are nitro- and amino PAH derivatives, polar aromatic compounds, heterocyclic compounds and phenols.

It was also confirmed in the presented studies that organic pollutants adsorbed on airborne particulate matter and sampled in winter and in summer alike show toxic effects in A549 line cells under *in vitro* conditions. After 24, 48, as well as after 72 h in observation, the lethal effect in 50% of tested cells was caused by smaller doses of air when the air samples were collected in winter than in the case of air samples collected in summer.

The only way to provide integrated and quick estimation of human exposure to chemicals adsorbed on airborne particulate matter is implementation of biological monitoring. Because of complexity of the carcinogenesis, it is necessary to adopt suitable organisms, cells and short-term test systems. Use of human cell cultures in research, along with bacterial assays, will provide opportunity to get a correct response of mammal cells to organic compounds that are pollutants of the atmospheric air.

ACKNOWLEDGEMENTS

The project was carried out within the grant No. N N523 612939 (2010–2013) awarded by the Polish Ministry of Science and Higher Education.

REFERENCES

[1] BRUNEKREEF B., HOLGATE S.T., Lancet, 2002, 360, 1233.
[2] DE KOK T.M.C.M., DRIECE H.A.L., HOGERVORST J.G.F., BRIEDE J.J., Mutat. Res.-Rev. Mut. Res., 2006, 613 (2–3), 103.
[3] COHEN A.J., Environ. Health Persp., 2000, 108 (4), 743.
[4] CLAXTON L.D., MATTHEWS P.P., WARREN S.H., Mutat. Res., 2004, 567, 347.
[5] MORTELMANS K., ZEIGER E., Mutat. Res., 2000, 455, 29.

[6] ŠKAREK M., JANOŠEK J., ČUPR P., KOHOUTEK J., NOVOTNĂ-RYCHETSKĂ A., HOLOUBEK I., Environ. Int., 2007, 33, 859.
[7] ŠKAREK M., ČUPR P., BARTOŠ J., KOHOUTEK J., KLÁNOVÁ J., HOLOUBEK I., Sci. Total Environ., 2007, 384, 182.
[8] RISOM L., MØLLER P., LOFT S., Mutat. Res., 2005, 592, 119.
[9] GÁBELOVÁ A., VALOVICOVÁ Z., BACOVÁ G., LÁBAJ J., BINKOVÁ B., TOPINKA J., SEVASTYANOVA O., ŠRÁM R J., KALINA I., HABALOVÁ V., POPOV T.A., PANEV T., FARMER P.B., Mutat. Res., 2007, 620, 103.
[10] BINKOVÁ B., ČERNA B., PASTORKOVÁ A., JELÌNEK R., BENEŠ I., NOVÁK J., ŠRÁM R.J., Mutat. Res., 2003, 525, 43.
[11] PIEKARSKA K., ZACIERA M., CZARNY A., ZACZYŃSKA E., Environ. Prot. Eng., 2009, 35 (1), 37.
[12] ZACIERA M., *Nitro-PAH determination in air*, [In:] *Protection of Air in Theory and the Practice*, J. Konieczyński (Ed.), Institute of Environmental Engineering of the Polish Academy of Sciences, Zabrze, Poland, 2006, p. 212, (in Polish).
[13] PRYČEK J., CIGANEK M., ŠIMEK Z., J. Chromatogr., 2004, 1030, 103.
[14] BAMFORD H.A., BEZABEH D.Z., SCHANTZ M.M., WISE S.A., BAKER J.E., Chemosphere, 2003, 50, 575.
[15] MARON D.M., AMES B.N., Mutat. Res., 1983, 113, 173.
[16] HAGIWARA Y., WATANABE M., ODA Y., SOFUNI T., NOHMI T., Mutat. Res., 1993, 291, 171.
[17] QUILLARDET P., HOFNUNG M., Mutat. Res., 1985, 147, 65.
[18] MENDYKA B., RADEK P., WARGACKA A., CZARNY A., ZACZYŃSKA E., PAWLIK M., Med. Środ., 2005, 8 (2), 139.
[19] CREBBELLI R., FUSELLI S., BALDASSARRI L.T., ZIEMACKI G., CARERE A., BANIGNI R., Int. J. Environ. Health Res., 1995, 5, 19.
[20] WOROBIEC A., ZWOŹDZIAK A., SÓWKA I., ZWOŹDZIAK J., STEFANIAK E. A., BUCZYŃSKA A.,. KRATA A., VAN MEEL K., VAN GRIEKEN R., GÓRKA M., JĘDRYSEK M.-O., Environ. Prot. Eng., 2008, 34 (4), 81.
[21] GILLI G., TRAVERSI D., ROVERE R., PIGNATA C., SCHILIRO T., Environ. Res., 2007, 103 (1), 1.
[22] DU FOUR V.A., JANSSEN C.R., BRITS E., LAREBEKE N.V., Mutat. Res.-Genet. Toxicol. Environ. Mutag., 2005, 588 (2), 106.
[23] DU FOUR V.A., VAN LAREBEKE N., JANSSEN C.R., Mutat. Res., 2004, 558, 155.
[24] NISBET I.C.T., LAGGY P.K., Reg. Toxicol. Pharmacol., 1992, 16, 290.
[25] ATKINSON R., AREY J., Environ. Health Perspect., 1994, 102, 117.
[26] ČERNA M., POCHAMANOVA D., PASTORKOVĂ BENEŠ I., LENIČEK J., TOPINKA J., BINKOVĂ B., Mutat. Res., 2000, 469, 71.
[27] BRITS E., SCHOETERS G., VERSCHAEVE L., Environ. Res., 2004, 96, 109.
[28] GÁBELOVÁ A., VALOVICOVÁ Z., HORVÁTHOVÁ E., SLAMEŇOVÁ D., BINKOVA B., ŠRÁM R.J., Farmer P.B., Mutat. Res., 2004, 563, 49.