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MUTAGENIC AND CYTOTOXIC PROPERTIES OF EXTRACTS OF SUSPENDED PARTICULATE MATTER COLLECTED IN WROCŁAW CITY AREA

A *Salmonella* assay in the presence of TA98 and YG1041 strains, and also when using human lung adenocarcinoma cells of the A549 line, has certified the mutagenic and cytotoxic properties of organic pollutants and fractions thereof adsorbed on suspended PM10 collected in winter and summer seasons in the Wrocław urban area. Particulates were sampled on sintered-glass filters using a high-performance Staplex air aspirator. Their extraction by dichloromethane was performed in a Soxhlet apparatus. Particulates were separated into three fractions: PAH, nitro-PAH and dinitro-PAH by a column chromatography method. The samples of particulates collected in winter season showed higher mutagenic and cytotoxic effects than the samples collected in summer time. Pollutants capable of direct and indirect affecting genetic material, classified as the mutagens of the reading frame-shift type and the base-pair substitution type, were found in the samples tested. Mutation ratio (MR) values obtained in the majority of experiments conducted in the presence of fractions of the pollutants tested were lower compared with the MR values obtained for the whole (unfractionated) extracts. No mutagenic effect was found in the case of fractions derived from the summer-collected sample of particulates, when the experiment was conducted with metabolic activation. The greatest amount of compounds responsible for a cytotoxic effect was found in the winter-collected nitro-PAH fraction, and also in the summer-collected nitro-PAH and dinitro-PAH fractions.

1. INTRODUCTION

There are enormous amounts of toxic, mutagenic and potentially carcinogenic pollutants in the environment nowadays. New, dangerous compounds appear throughout the environment as a result of human activities [1]. Air pollutants make a greatly diverse mixture of chemical compounds. Some of them occur in gaseous phase, some

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are adsorbed on various-size dust particles and can penetrate in this form into the respiratory system, then through alveoli to the circulatory system, to be finally diffused over entire body [2]. A large group among them, relatively well known, is constituted by polycyclic aromatic compounds (PAH), showing mutagenic and carcinogenic activity. Highly mutagenic are also nitro-, chloro- and oxy-PAH derivatives [3]. Their harmfulness depends on the diameter of particulate matter. Respirable particles of the diameter <10 μm remain suspended in the air for quite a bit of time, thus increasing the probability of their entering into the human respiratory system [4]. The risk of getting a pulmonary carcinoma, respiratory system diseases, arterial sclerosis, neurobehavioural changes as well as heart and cardiovascular system diseases grows due to a long-term exposure to air pollutants in high concentrations, whereas a short-term exposure can bring about intensification of respiratory system diseases, such as asthma, bronchitis and heart arrhythmia and can cause allergic reactions [5].

Detection and identification of pollutants based on chemical analyses is costly and requires using advanced analytical techniques. Therefore the mutagenicity and toxicity of mixtures, and not individual compounds contributing to the environment pollution, must be considered. Moreover, as identification of particular substances contained in such a mixture would be difficult or often impossible, separation of particulate matter extract into fractions containing compounds belonging to a particular category becomes essential. Reliable information about their impact on a living organism can be acquired only on the basis of the results obtained by biological methods, because many substances show synergistic or antagonistic effects [6].

A short-term bacterial test in *Salmonella* (the Ames assay) [7] is useful in the research focused on the assessment of air pollutants and their mutagenic properties. The assay is employed to determine the level of reverse mutations from histidine auxotrophy to prototrophy in the specially designed mutants of the *Salmonella typhimurium* LT2 strains [8]. Various cell lines, such as human lung carcinoma epithelioid cells (A549), are utilized for air pollution toxicity studies under in vitro conditions. The usefulness of cell lines for assessing cytotoxic properties of respirable particles has been proven in numerous studies [9].

The purpose of this paper was the assess the mutagenic and cytotoxic properties of organic pollutants adsorbed on airborne PM10 fraction from the samples collected in winter and summer seasons in the area of the city of Wrocław.

2. MATERIALS AND METHODS

Investigations were carried out based on airborne particulate matter samples collected using a high-performance PM-10 Staplex air aspirator. The air was aspired on sintered-glass TFAGF810 filters (20 cm × 25 cm), at the rate ranging from 71.7 m^3/h to 82.08 m^3/h. Filters in the aspirator were replaced every 24 hours. The sampling site

was situated in the Old Town (Stare Miasto) district, at Nowy Targ Square, which is a heavy traffic area. Samples were collected in summer (May through September) and in winter (November through March). Filters together with particulates from individual samplings were combined into one sample, cut into pieces and put into Soxhlet extractors. Then pollutants were extracted by dichloromethane in the darkness for 16 hours plus 15-minute reflux [10]. After that the extracts were thickened until dry in a vacuum evaporator, and finally blown through with nitrogen. The dry extracts obtained were analysed in order to determine the PAH, nitro-PAH and dinitro-PAH content therein and used as material in biological assays.

The crude extract was fractionated in glass columns filled with silica gel, following the procedure reported by ZACIERA [11]. 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 designed 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. All organic pollutants (C) that were present in the samples collected as well as the pollutants contained in three fractions: PAHs (II), PAH nitro derivatives (III) and PAH dinitro derivatives (IV) were introduced into the assays.

Two test strains: TA98 and YG1041 were used in *Salmonella* assays, conducted according to the recommendations given by MARON and AMES [8], in two experiment versions: without metabolic activation and with the metabolic activation by a microsomal fraction S9 that had been derived from Wistar rat's liver and induced with Aroclor 1254. The microsomal fraction S9 was used for assays to induce the metabolic activation of promutagens. Protein content in the microsomal fraction, as determined by Lowry's method, was 64.44 mg/cm^3. S9-mix containing 4% (v/v) of S9 was used in experiments. *Salmonella* test strains were donated by Dr. T. Nohmi, Division of Genetics and Mutagenesis, National Institute of Hygienic Sciences, Tokyo, Japan. All analyses were carried out in five replications. Samples were incubated for 48 hours (TA98) or for 72 hours (YG1041) at the temperature of 37 °C. After that time the number of (his^+) revertant colonies growing on Petri dishes were determined. Each time before starting the experiments, genetic markers of the test strains and the degree of their spontaneous reversion were checked (negative check), as well as the test strain sensitivity to diagnostic mutagens (positive check – without S9: 0.2 μg of 2,4,7-trinitro-9-fluorenone per plate in the case of 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 in the case of both test strains). The mutagenic effect of the extracts of airborne particulates was presented in the form of a mutation ratio (MR), which is the ratio of the mean number of revertants grown in the presence of the sample tested to the mean number of spontaneous revertants. Samples were considered mutagenic if their values $MR \geq 2$.

Cytotoxic effects of particulate matter extracts were tested, using the method of their direct contact with a one-layer culture of human lung carcinoma epithelioid cells – A549 (American Type Culture Collection, Cell Culture Line-ATCC CCL 185). A549 cell cultures were grown in Dulbecco's liquid medium supplemented with inactivated (30 min, 56 °C) calf serum (10%), penicillin (100 μg /cm^3), streptomycin (100 μg/cm^3), and 2mM of L-glutamine. A one-layer culture of A549 cells, of the density of $2\times10^6/cm^3$, was grown in plastic 96-well plates. The culture was incubated for 24 hours at the temperature of 37 °C, in an ambient atmosphere of 5% CO_2. Thereafter the liquid was removed from above the cells and replaced by the extracts tested at relevant concentrations, and then incubation followed for 24, 48 and 72 hours at 37 °C in an ambient atmosphere of 5% CO_2. Cell cultures without the addition of the samples tested and cell cultures with DMSO addition at the same amount as in the case of the samples tested served as control cultures. Quantitative and morphological changes caused by the extracts tested were estimated after 24, 48 and 72 hours, using an inverted microscope. Minimum concentration of the samples tested which caused degenerations in 50% of cells was considered as a toxic dose ($TCCD_{50}$, Tissue Culture Cytotoxic Dose) [12]. The result of the cytotoxic assay was presented as a volume of the air tested (in m^3) sufficient for obtaining the extract that still would induce toxic effects.

3. RESULTS AND DISCUSSION

PM10 concentration in the selected test point in Wrocław ranged from 43.74 μg/m^3 to 57.84 μg/m^3, while the concentration of organic compounds (tar substances) adsorbed on the airborne particulates varied between 7.56 μg/m^3 and 17.57 μg/m^3 (table 1). The concentrations of airborne particulates and organic pollutants adsorbed thereon were higher in winter than in summer. The seasonal differences in those concentrations corresponded with literature data [7]. For the most part, the differences result from an increased emission of airborne particulates in urban agglomerations during winter, due to combustion processes for heating purposes. The amount of PM10 was close to that measured in various European cities [13]–[17]. The permissible limits of the airborne particulate matter concentrations for the 24-hour period are as follows: in Poland, 125 μg/m^3; in EU, 50 μg/m^3; and according to the US EPA regulations, 150 μg/m^3 [18].

Table 1

Data on collection of samples

Type of sample	Time of collection (h)	Capacity of air sample (m^3)	Mass of pollutants (μg/m^3)	Mass of tar substances (μg/m^3)
Winter (Stare Miasto)	960	122 326.0	57.8428	17.57
Summer (Stare Miasto)	1669	161 004.0	43.737	7.56

The highest amount of PAHs adsorbed on airborne particulates was recorded in wintertime (table 2). Total PAH amount detected in that period was 40.670 ng/m^3, whereas the respective amount in the sample collected in summer was 7.447 ng/m^3. Chemical compounds occurring most often as the constituents of air pollutants of adverse impact on human health [11] were selected for analysis. Three PAHs of those present in the extracts, namely benzo[a]anthracene, benzo[a]pyrene, dibenzo[a,h] anthracene, were classified by IARC as 2A group, probably carcinogenic to humans, and another three PAHs, i.e., benzo[b]fluoranthene, benzo[k]fluoranthene, indeno [1,2,3-c,d]pyrene, were classified as 2B group, possibly carcinogenic to humans [18]. The total PAH concentration as well as the PAH profile were consistent with data in the literature, relating to other European cities [13]–[17]. The total nitro-PAH concentration in turn (table 2) ranged from 1.6 ng/m^3 to 3.43 ng/m^3. The reachest profile of those compounds was obtained for the winter-collected sample. No dinitro-PAHs were found in the extracts within their determination limits. Instead, mononitro-PAHs representing the 2B group were detected, among others 2-nitrofluorene and 1-nitropyrene. 2-nitrofluorene was present in both samples tested, while 1-nitropyrene was found only in the winter-collected sample. 3-nitrofluoranthene was present in the winter extract 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 proceeding in a gaseous phase [11].

Table 2

PAH and nitro-PAH concentration in organic extract of air pollutants (ng/m^3)

PAH examined	Winter	Summer	Nitro-PAH examined	Winter	Summer
Phenanthrene	3.127	0.855	1-nitronaphthalene	0.58	0.47
Anthracene	0.360	0.044	2-nitrofluorene	1.88	0.31
Fluoranthene	7.488	1.069	9-nitroanthracene	0.87	0.52
Pyrene	8.340	0.988	3-nitrofluoranthene	0.50	n.d.
Benzo[a]anthracene	3.144	0.286	1-nitropyrene	0.48	n.d.
Chrysene	1.911	0.343	1,3-dinitropyrene	n.d.	n.d.
Benzo[b]fluoranthene	2.832	0.955	1,6- dinitropyrene	n.d.	n.d.
Benzo[k]fluoranthene	1.702	0.267	1,8- dinitropyrene	n.d.	n.d.
Benzo[a]pyrene	7.980	0.765			
Dibenzo[a,h]anthracene	0.284	0.112			
Benzo[g,h,i]terylene	1.776	1.233			
Indeno[1,2,3-c,d]pyrene	1.726	0.530			
Total	40.670	7.447	Total	3.43	1.6

n.d. – not detected.

Table 3

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

(m^3/ plate)	Type of sample/test strain															
	Winter								Summer							
	TA 98															
	–F				+F				–F				+F			
	C	II	III	IV	C	II	III	IV	C	II	III	IV	C	II	III	IV
50	**19.68**	**3.71**	**5.46**	**3.90**	**12.76**	**2.77**	**3.38**	**2.71**	**4.90**	**2.22**	**2.91**	1.72	**3.29**	1.40	1.46	1.89
25	**15.64**	**2.38**	**4.86**	**3.25**	**8.85**	1.71	**2.39**	**2.15**	**2.58**	1.89	**2.44**	1.56	**2.68**	1.27	1.37	1.45
12.5	**11.05**	1.23	**3.67**	**2.03**	**6.63**	1.69	1.95	1.88	**2.24**	1.72	**2.34**	1.29	**2.27**	1.17	1.35	1.38
6.25	**7.25**	1.07	**2.21**	1.22	**4.93**	1.41	1.88	1.55	1.67	1.58	**2.18**	1.19	1.58	1.14	1.33	1.36
3.125	**3.35**	1.01	1.51	1.07	**3.59**	1.41	1.57	1.25	1.43	1.47	1.78	1.05	1.40	1.02	1.29	1.18
1.56	**2.51**	0.94	1.09		**2.34**	1.23	1.28	1.11	1.37	1.11	1.14	0.95	1.25	1.00	1.11	0.99
0.78	1.76	0.86	0.99		1.66	1.16	1.26		1.06				1.29			
0.39	1.55	0.83	0.96		1.39	1.15	1.18		0.97				1.01			
0.195	0.98	0.79	0.94		1.05	1.13	1.17		0.97				1.01			
0.097		0.72	0.86			0.93	1.09									
	YG1041															
50	**4.39**	**3.30**	**4.12**	**2.7**	**5.91**	**2.90**	**3.40**	**2.80**	**3.49**	**7.84**	**9.93**	**2.77**	**3.23**	1.86	1.83	1.91
25	**8.30**	**3.36**	**5.16**	**4.8**	**12.83**	**3.43**	**5.65**	**4.64**	**3.93**	**5.08**	**5.70**	**2.18**	**4.95**	1.56	1.59	1.74
12.5	**29.24**	**4.78**	**7.80**	**5.67**	**46.21**	**7.82**	**9.81**	**2.65**	**4.14**	**3.39**	**3.88**	1.76	**2.09**	1.32	1.42	1.50
6.25	**31.50**	**5.08**	**10.3**	**4.66**	**36.77**	**5.10**	**7.54**	1.24	**5.93**	1.94	**2.74**	1.62	1.70	1.24	1.28	1.23
3.125	**35.50**	**3.71**	**4.78**	**3.35**	**32.32**	**4.76**	**5.82**	1.15	**3.62**	1.08	**2.05**	1.47	1.29	1.05	0.98	1.13
1.56	**30.88**	**2.20**	**4.09**	**2.8**	**20.46**	**3.77**	**3.61**	0.98	**2.09**		1.87	1.22	1.18			
0.78	**26.31**	1.67	**3.35**	1.67	**11.51**	**2.10**	**2.12**	1.02	1.76		1.54	1.08	1.00			
0.39	**20.96**	1.17	**2.12**	1.10	**5.22**	1.29	1.36		1.24		1.24					
0.195	**12.93**	1.02	1.67	0.98	**4.03**	1.06	1.17		1.03		1.09					
0.097	**5.52**		1.23		**2.80**	0.92			1.09							
0.049	1.77		1.09		0.91				0.93							

C – whole sample; II – PAH fraction; III – nitro-PAH fraction; IV – dinitro-PAH fraction.

In all the *Salmonella* assays, the highest values of the mutation ratio (MR) were measured in the winter-collected sample (table 3). Similar seasonal variability can be observed in other countries. Mutagenicity of air-polluting particulates increases in the heating season, and drops in summer [7]. The response of the YG1041 strain to mutagens present in the extracts of particulate matter was stronger compared with that of the TA98 strain, which means that those mutagens were, for the most part, nitroaromatic compounds, as the YG1041 strain is sensitive to them [20].

In the assays using TA98 strain (table 3), the highest MR values (e.g. MR = 19.68 at 50 m^3/plate) were obtained for the whole (unfractionated) extract of the sample collected in winter season, when the assays were conducted without metabolic activation (–F). So direct mutagens of the reading frame-shift type prevailed over indirect mutagens in that extract. The lowest concentration of the extract prepared from the winter-collected sample, at which the mutagenic effect (MR $\geq$ 2) was still observed in all experiments (–F, +F), was 1.56 m^3/plate. When considering the MR values obtained in experiments wherein separate fractions of particulate matter (II, III, IV) were introduced, one can see that the MR values obtained turned out to be significantly lower (e.g., MR = 3.71; MR = 5.46; MR = 3.9 at 50 m^3/plate), and the highest mutation ratios were obtained for the nitro-PAH fraction. As regards the whole extract prepared from the sample collected in summer, the MR values appeared to be lower than those obtained in the case of the winter-collected sample. The fact is easy to explain, as the particulate concentration in the sample collected in summer is lower, the amounts of the PAHs tested and their nitro-derivatives in such a sample are also smaller. The lowest concentration of the extract prepared from airborne particulates sampled in summer, at which the mutagenic effect was still observed in the experiments carried out with and without metabolic activation, was derived from 12.5 m^3 of air. The tests on separate fractions of the summer-collected particulate matter did not give evidence for the presence of mutagenic compounds in the experiments carried out with metabolic activation. In the experiments without metabolic activation, the mutagenic effect was obtained only in the case of fractions containing PAHs and nitro-PAHs.

Extremely high MR values were obtained from the assays with YG1041 strain (table 3), especially for the winter-collected sample. The highest MR values (MR_{-F} = 35.50 at 3.125 m^3/plate and MR_{+F} = 46.21 at 12.5 m^3/plate) were obtained from the assays wherein was introduced the extract of winter-collected particulates. Being a TA98 derivative, the YG1041 strain shows an increased sensitivity to nitro-, amino- and hydroxylamino PAH derivatives, due to the presence of plasmids in its cells, which is connected with the overproduction of nitroreductase and *O*-acetyltransferase [20]. Within the tested range of air pollutant concentrations a clear dose–response relationship was observed for this strain, fully explaining the dependence of the pollutants present in the samples and their concentration on their biological effect. Moreover, a maximum number of revertants were obtained at lower concentration of the extracts tested compared with the number of revertants obtained at the same con-

centrations but in experiments with the TA98 strain. In the assays carried out with metabolic activation as well as without it, the mutagenic effect was observed at the extract concentration derived from 0.097 m^3 of air. Then, the MR values obtained in the presence of separate fractions of the particulates sampled were far lower than the MR values obtained in the case of unfractionated extract, still greater than those obtained when using the TA98 strain in the assays. The maximal MR value (5.93) for the sample of the particulates collected in the summer was obtained in the experiment carried out without S9, and the relevant extract was derived from 6.25 m^3 of air; if the metabolic activation was applied, the maximum MR value (MR = 4.95) was reached with the extract derived from 25 m^3 of air. Also in the case of that sample, its toxic effect was observed in all experiments. Mutagenic effects were observed at higher extract concentrations than those in the winter-collected sample. In the experiments carried out without S9 and with metabolic activation, the amounts of pollutants derived respectively from 1.56 m^3 and 12.5 m^3 of air were found to be mutagenic. The experiments with metabolic activation conducted on separate fractions of particulates collected in summer, just as the experiments with the TA98 strain, did not reveal any compounds of mutagenic nature. However, in the experiments without metabolic activation the mutagenic effect was observed in the case of all fractions, and the MR values relating to the PAH and nitro-PAH fractions appeared to be greater than those corresponding to the unfractionated extract.

Investigation results in table 4 show the effect of the extracts of airborne particulates on the neoplastic cells A549 of lung carcinoma. Owing to large active surface of lungs, air pollutants are resorbed at high rate in alveoli for the most part. Delicate nature of this tissue makes it especially susceptible to absorption of different chemical compounds and to direct damage by such compounds. The lung epithelial cells constitute the body's first protective barrier enabling removal of particulates and bacteria from the respiratory system. In vitro and in vivo studies on the mechanism of airborne particulate matter effect on living organisms have proved that the airborne particulate matter is to blame for the inflammatory condition in epithelial cells of the respiratory system and for oxidative stress [21]. The oxidative stress can be a cause of many dangerous illnesses. It consists in that certain chemical reactions are catalysed, which leads to the generation of active forms of oxygen (oxygen radicals). The oxygen radicals cause serious damage to bioparticles, including damage to DNA molecules. The damaged cells, if not eliminated in an early phase by the immune system, start to proliferate, which in consequence can lead to neoplasm formation.

The investigations confirmed that organic pollutants adsorbed on particulate matter sampled in winter and in summer alike are toxic to A549 line cells under in vitro conditions. Toxic effects were observed at different air concentrations, depending on sampling season and the examined fraction of pollutants (table 4). In the case of experiments carried out on whole extracts, stronger toxic effect was caused by the extract from winter-collected particulates compared to the effect of the extract from of

particulates collected in summer. Each time, after 24, 48 and 72 hours of observation, the lethal effect in 50% of the cells tested was caused by smaller doses of winter-sampled air (12.5; 6.25; 6.25 m^3) compared with the doses of air that had been collected in summer (no toxic effect; 25; 25 m^3, respectively). A similar seasonal character of the cytotoxic effect was observed in many towns [22], [23]. However, different

Table 4

Effect of dust pollutant extracts on A549 line human lung cells under in vitro condition

Contact time (h)	Sampling season	Sample type	Toxic effect							
			50	25	12.5	6.25	3.125	1.56	0.78	0.39
			(m^3)							
24	winter	C	t	t	t	n	n	n	n	n
		II	t	t	n	n	n	n	n	n
		III	t	t	n	n	n	n	n	n
		IV	t	t	n	n	n	n	n	n
	summer	C	n	n	n	n	n	n	n	n
		II	t	t	n	n	n	n	n	n
		III	t	t	n	n	n	n	n	n
		IV	t	t	t	n	n	n	n	n
	A549 control		n	n	n	n	n	n	n	n
48	winter	C	t	t	t	t	n	n	n	n
		II	t	t	n	n	n	n	n	n
		III	t	t	n	n	n	n	n	n
		IV	t	t	n	n	n	n	n	n
	summer	C	t	t	n	n	n	n	n	n
		II	t	t	t	n	n	n	n	n
		III	t	t	t	n	n	n	n	n
		IV	t	t	t	n	n	n	n	n
	A549 control		n	n	n	n	n	n	n	n
72	winter	C	t	t	t	t	n	n	n	n
		II	t	t	t	n	n	n	n	n
		III	t	t	t	t	n	n	n	n
		IV	t	t	t	n	n	n	n	n
	summer	C	t	t	n	n	n	n	n	n
		II	t	t	t	n	n	n	n	n
		III	t	t	t	t	n	n	n	n
		IV	t	t	t	t	n	n	n	n
	A549 control		n	n	n	n	n	n	n	n

C – whole sample, II – PAH fraction, III – nitro-PAH fraction, IV – dinitro-PAH fraction, n – non-toxic sample, t – toxic sample.

results were obtained from the assays with individual fractions of air pollutants. After 48 hours the toxic effect was caused equally by PAH, nitro-PAH and dinitro-PAH fractions dosed at 12.5 m^3, whereas analogical fractions obtained from winter extract

of particulates produced the same effect at 25 m^3 dose. The strongest toxic effect determined in A549 cells was observed after their 72-hour exposure to the unfractionated winter extract and its PAH fraction, and to summer-collected nitro-PAH and dinitro-PAH fractions, all derived from 6.25 m^3 of air.

4. CONCLUSIONS

1. The extracts from airborne particulate pollutants differed in the total content and percentage proportion of individual chemical compounds, depending on the sampling season.

2. Owing to the application of the *Salmonella* assay and the assay with A549 line of human cells obtained from neoplastic cells of pulmonary carcinoma, the mutagenic and cytotoxic activity of the airborne particulate pollutants collected in winter and summer in the urban area of Wrocław could be assessed.

3. The extracts from airborne particulate pollutants were subjected to the *Salmonella* assay using TA98 strain and its YG1041 derivative. High mutagenic effect was found in the experiments carried out both with and without metabolic activation. The mutagenic activity of airborne particulates was higher in winter than in summer.

4. The examinations proved that the YG1041 strain was highly useful for detection of mutagenic effect of nitro-derivatives of aromatic compounds.

5. In the majority of *Salmonella* assays, the mutagenic effect of the whole extracts of particulates was found to be greater compared with the effect of particular fractions (PAH, nitro-PAH and dinitro-PAH). In the sample of particulate matter collected in summer season, no indirect mutagens were found in fractions, although they were present in the whole extract.

6. A cytotoxic effect observed for the sample of particulate matter collected in winter season was slightly stronger compared with that of the sample collected in summer. However, the largest amount of the compounds producing such an effect was measured in the whole extract of particulate matter collected in winter as well as in its nitro-PAH fraction, and in the summer-collected nitro-PAH fraction and dinitro-PAH fraction.

7. Actual health hazard posed by organic pollutants adsorbed on suspended particulates can be established only in biological examinations, as those taking into account the resultant effect of the pollutants on living organisms. Therefore biological examinations and chemical analyses as well should be included in the standard monitoring of atmospheric air pollution.

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WŁAŚCIWOŚCI MUTAGENNE I CYTOTOKSYCZNE EKSTRAKTÓW PYŁU ZAWIESZONEGO POBRANEGO NA TERENIE WROCŁAWIA

Stosując test *Salmonella*, który prowadzono w obecności szczepów TA98 i YG1041, oraz test, któremu poddano linię komórek ludzkich raka płuc A549, stwierdzono mutagenność i cytotoksyczność zanieczyszczeń organicznych i ich frakcji zaadsorbowanych na cząstkach pyłu zawieszonego PM10 pobranego zimą i latem na terenie Wrocławia. Próbki pyłu pobierano na filtry szklane za pomocą wysokosprawnego aspiratora powietrza typu Staplex. Zanieczyszczenia ekstrahowano dichlorometanem w aparacie Soxhleta. Rozdziału na trzy frakcje: WWA, nitro-WWA i dinitro-WWA dokonano metodą chromatografii kolumnowej. Pyły pobrane w sezonie zimowym okazały się bardziej mutagenne i cytotoksyczne niż te zebrane w lecie. W badanych próbkach stwierdzono obecność zanieczyszczeń o charakterze mutagenów typu zmiany fazy odczytu i podstawienia pary zasad mogących oddziaływać bezpośrednio i pośrednio na materiał genetyczny. W większości testów uzyskano niższe wartości współczynnika mutagenności (MR) w obecności frakcji badanych zanieczyszczeń w porównaniu z wartościami MR uzyskanymi dla całkowitych ekstraktów. Efektu mutagennego nie zaobserwowano jedynie dla frakcji pochodzących z próbki pyłów pobranych latem i badanych z aktywacją metaboliczną. Próbka pobrana zimą, a także frakcja zimowa nitro-WWA oraz frakcja letnia nitro-WWA i dinitro-WWA zawierały najwięcej związków wywołujących efekt toksyczny.