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MUTAGENICITY OF POLLUTANTS AND THEIR FRACTIONS ADSORBED ON AIRBORNE PARTICULATE IN THE CENTRE OF WROCŁAW (POLAND)

Mutagenicity of pollutants and their fractions adsorbed on suspended particulate matter < 10 μm (PM10) in the centre of Wrocław was studied using the Ames test (SW Poland, 642 000 inhabitants). Particulate matter was sampled by the method of high-volume aspirometry in summer and in winter. Samples were extracted with dichloromethane. Extract was separated into fractions with sequential elution by solvent chromatography. In the Ames test there was used *Salmonella typhimurium* TA 98 strain and YG 1021 and YG 1024 strains of increased sensitivity to mutagenic activity of nitro PAHs. In order to perform activation of promutagenes, S9 fraction obtained from rat liver was used. Pollutants adsorbed on particulate matter displayed greater mutagenicity in winter than in summer. In summer, mutagenic activity was displayed exclusively by polar pollutants, in winter by PAHs and polar compounds. Among them there were direct and indirect mutagenes. Significant role in mutagenicity of pollutants adsorbed on particulate matter played nitro PAHs. Mutagenic effect found due to the Ames test was the result of total activity of many mutagenes displaying synergistic and antagonistic actions in the mixture.

1. INTRODUCTION

Many pollutants adsorbed on suspended particulate matter are genotoxic. Most commonly known genotoxicity is that of polynuclear aromatic hydrocarbons (PAHs) [5], [17]. Many of them are promutagenes requiring metabolic activation in mammal organism. During combustion of fuels and as the result of reactions of organic pollutants in the atmosphere there are also created other genotoxic compounds: aromatic polar compounds, heterocyclic compounds and phenols [13]. Significant mutagenicity is displayed by nitro, amine and hydroxyloamine derivatives of PAHs [22].

Mutagenic activity of organic pollutants adsorbed on suspended particulate matter in the centre of Wrocław had already been found [1], [8]–[11], [25]. It was proved that

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the mutagenicity is greater in winter than in summer. It depends, among many other factors, on the intensity of traffic. Nitro, amine and hydroxyloamine derivatives of PAHs have a significant share in mutagenicity of pollutants adsorbed on suspended particulate matter.

Results of hitherto research on mutagenicity of chromatographic fractions of pollutants adsorbed on suspended particulate matter in Wrocław indicate significant participation of polar pollutants in their mutagenicity. However as yet there had not been determined a qualitative composition of either chromatographic fractions of pollutants responsible for this mutagenicity or participation of nitro PAHs in mutagenicity of those fractions (JADCZYK [8]).

The purpose of this work was to assay mutagenicity of the group of organic pollutants adsorbed on suspended particulate matter in the centre of Wrocław (SW Poland, 642 000 inhabitants) in summer and in winter. Extract of pollutants was separated by means of sequential elution chromatography (SESC). Qualitative composition of the obtained fractions of pollutants was determined by GC-MS method. For assaying mutagenic fraction of pollutants the Ames test was used. In the tests, there was taken into consideration metabolic activation of promutagenes under the influence of microsomal enzymes; fraction S9 obtained from rat liver was used [12]. Application of *Salmonella typhimurium* strains, YG 1021 and YG 1024, of increased nitroreductase and *O*-acetyltranferase activity allowed determination of participation of nitro PAHs in mutagenicity of the given fractions. There were also performed further separation of fractions of polar pollutants and tests on mutagenicity of subfractions. Results of earlier tests suggested significant participation of this group of chemical compounds in mutagenicity of pollutants adsorbed on suspended particulate matter.

2. MATERIALS AND METHODS

Sampling and sample preparation. The material for study was particulate matter > 10 μm (PM10) from the centre of Wrocław. Samples of particulate matter were collected on glass fibre filters (Staplex) with a pump of 67.8 m^3/h throughput. All the particulate matter collected in August and September of 1997 and May, June and July of 1998 on 122 filters (198518.4 m^3 of air) was gathered into one sample. Particulate matter collected in November and December 1997 and in January, February and March 1998 on 106 filters (172483.2 m^3 of air) was gathered into another sample. Filters with the particulate matter collected were subjected to 8-hour extraction with dichloromethane in Soxhlet apparatus. From 6550.7 mg of suspended particulate matter collected in summer there was obtained 696.8 mg of extract (10.6%), and from 10047.8 mg of particulate matter collected in winter there was obtained 2047.2 mg of extract (20.4%).

1/4 of the extracts obtained was separated into fractions with SESC using a sequence of solvents of gradually increased polarity [4], [7]. The sediment obtained was set on glass beads, each 106 μm in diameter, which were placed in a glass precolumn (2 cm in diameter and 30 cm in length.) Underneath there was placed a glass column (2 cm in diameter and 1 m in length) filled with silica gel (0.075–0.15 mm). Hexane was used for elution of aliphatic hydrocarbons, and 15% toluene in hexane for elution of aromatic hydrocarbons (table 1). The residue containing polar compounds was eluted with dichloromethane. Part of this fraction was subjected to further separation leading to obtaining subfractions (table 2).

Extracts were evaporated to dryness and solubilized in a small quantity of dichloromethane for physicochemical tests and for biological tests in DMSO. Solution in dimethyl sulfoxide (DMSO) was diluted in such a way that 0.1 cm^3 corresponded to 3.2, 1.6, 0.8, 0.4, 0.2 and 0.1 mg of suspended particulate matter. The dose of 0.1 mg of particulate matter sampled in summer corresponded to 3.0 m^3, and in winter, to 1.7 m^3 of air pumped through a glass filter. Samples prepared in such a way were subjected to the Ames test.

Physicochemical analysis by GC-MS. Qualitative analysis of the extracts was carried out using a Hewlett-Packard gas chromatograph (HP 5890) coupled with a mass detector (HP 5972), with an HP 5 column (50 m long, with an internal diameter of 0.2 mm). Helium was used as the mobile gaseous phase (0.6 cm^3/min.). Qualitative identification was based on comparison of mass spectra of chromatographic peaks with standard spectra from the NIST-NBS75K data base.

The strain Salmonella typhimurium. The strain *Salmonella typhimurium* TA 98 was donated by Professor B. Ames (Laboratory Department of Biochemistry, University of California). The strains YG 1021 and YG 1024 were donated by Professor M. Watanabe (Division of Mutagenesis, National Institute of Hygienic Science in Tokyo).

The strain TA 98 has the following genetic markers: his^-, rfa, ΔuvrB, +R. The strains YG 1021, YG 1024 were constructed by introducing plasmids carrying genes coding for nitroreductase (pYG216) or *O*-acetyltransferase (pYG219) to the genome of the strain TA 98 [3], [21]–[23]: YG 1021 = TA 98 (pYG216), YG 1024 = TA 98 (pYG219).

The Ames test. The procedure described by MARON and AMES [12] was used. A sample volume of 0.5 cm^3 of phosphate buffer or the same volume of the S9 fraction was added to a sterile test tube. Other components were: 0.1 cm^3 of the sample tested, 0.1 cm^3 of the overnight broth culture of the test strain and 2 cm^3 of TOP-agar at 45 °C containing 0.2 cm^3 of 0.5 mM histidine and biotine. The content of the test tube was mixed and during 20 seconds poured into a Petri plate containing minimal Vogel–Bonner medium. All samples were tested in 5 repetitions. The plates were incubated at 37 °C for 48 hours. After this time, the number of revertants growing on the plates were counted. Average numbers of spontaneous revertants were close to those

reported by MARON and AMES [12], WATANABE et al. [21] and HAGIWARA et al. [6]. For metabolic activation of promutagens present in the samples, a 10% S9-mix fraction (an Aroclor 1254 actived) was used.

The sensitivity of bacteria to control mutagens was tested by the positive controls (without the metabolic activation: 0.2 μg of 2,4,7-trinitro-9-fluorenone per plate, with the metabolic activation: 10 μg of 2-aminofluorene per plate). Mutagenic activity of the solvent and the filter material were also checked.

Presentation of the results. According to the procedure, the sample was considered mutagenic when its mutagenicity ratio satisfied MR≥2 and when it showed a linear dose-response [12]. The mutagenicity ratio is the ratio of the average number of revertants induced by the sample per plate to the average number of spontaneous revertants.

3. RESULTS AND DISCUSSION

3.1. RESULTS OF PHYSICOCHEMICAL ANALYSES

Percentage share of the given fractions obtained with SESC was similar in both samples with one reservation only, because in the sample collected in summer there were significantly less aromatic hydrocarbons (figure 1). Using GC-MS method we identified significantly more chemical compounds in chromatographic fractions of the particulate matter extract sampled in winter compared to summer. In aliphatic hydrocarbons fractions, predominated alkanes and alkenes (table 1). There were also present

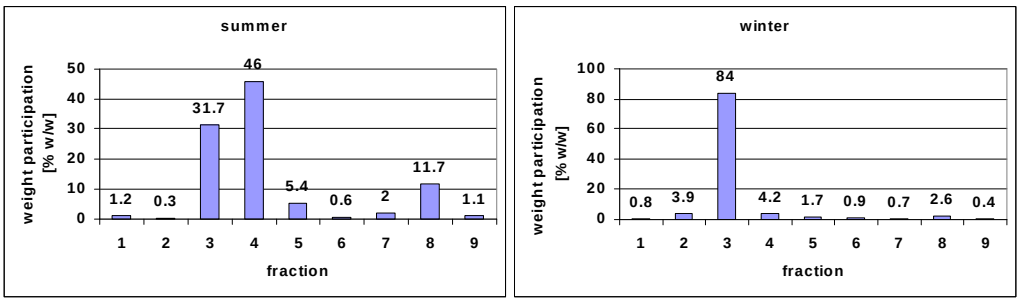


Fig. 1. Weight participatin of extracted fractions [% w/w]:
1 – aliphatic fraction, 2 – aromatic fraction, 3–9 – polar subfractions: soluble in chloroform (3), soluble in the mixture of 15% diethyl ether and 85% chloroform (4), soluble in the mixture of 3% ethanol and 97% diethyl ether (5), soluble in methanol (6), soluble in the mixture of 3% ethanol and 97% chloroform (7), soluble in the mixture of 3% ethanol and 97% tetrahydrofuran (8), soluble in dichloromethane (9)

PAHs and their halogen derivatives. In PAHs fraction of pollutants adsorbed on particulate matter sampled in summer, there was identified only one PAH, i.e. benzo(e)pyrene. In winter, there were more of them. In fractions, we found residue of aliphatic hydrocarbons, in winter also other compounds. Fractions of polar pollutants comprised compounds containing mainly heteroatoms in particles. There were also found organic acids and aliphatic and aromatic hydrocarbons. Composition of polar subfractions was presented in table 2.

3.2. RESULTS OF THE AMES TEST

Mutagenicity of complete extracts. Both tested samples of particulate matter induced mutations in all used *Salmonella typhimurium* strains (figure 2). Winter extract was more mutagenic than the summer one. Volume of polluted air inducing mutations in TA 98 strain without metabolic activation was 6.2 m^3 in winter and 21.2 m^3 in summer.

Metabolic activation of winter sample increased its mutagenic activity. Volume of polluted air resulting in mutagenic effect in TA 98 strain with metabolic activation was 1.7 m^3. This testifies to the presence of compounds which are promutagenes in winter particulate matter. Metabolic activation of summer sample resulted in a decrease in its mutagenic activity. Volume of polluted air resulting in mutagenic effect in the test with TA 98 strain with metabolic activation was 30.3 m^3. This indicates partial detoxication of mutagenes adsorbed on particulate matter sampled in summer.

Strains of increased nitroreductase and *O*-acetyltranferase activity proved to be more susceptible to mutagenic activity of the samples tested than TA 98 strain. Volume of polluted air resulting in mutagenic effect in YG 1021 strain was 1.3 m^3 in winter and 3.3 m^3 in summer. In the case of YG 1024 strain, the volumes were 0.4 m^3 in winter, and 2.5 m^3 in summer. This testifies to a significant participation of nitro PAHs in mutagenicity of pollutants adsorbed on suspended particulate matter.

Mutagenic ratio (MR) of complete extracts was lower than the total MR of the given fractions. Similar phenomenon in regards to complete extract of pollutants adsorbed on particulate matter and 8 fractions of those pollutants was encountered in Upper Silesia, S Poland [14]. There are many different biologically active pollutants adsorbed on suspended particulate matter. They can mutually reinforce or weaken their activity. The answer obtained from the test is a resultant of those interactivities.

Mutagenicity of fractions of aliphatic hydrocarbons. Fractions of aliphatic hydrocarbons (figure 2) from both samples were not mutagenic in the studied range of concentrations in all test strains. This is in agreement with the results obtained earlier in the study of mutagenic effect of particulate matter sampled in Upper Silesia on TA 100 strain [13]. This confirms also a previous information about particular sensitivity of YG 1021 and YG 1024 strains only to selected groups of mutagenes [3], [6]. Aliphatic

hydrocarbons are considered atmospheric pollutants of significantly lower activity than aromatic hydrocarbons and their derivatives [5].

Mutagenicity of PAH fractions. PAH fractions of particulate matter sampled in summer in the tested range of concentrations were not mutagenic in all test strains (figure 2). The sample, in comparison with the winter trial, comprised a very low content of chemical compounds. The GC-MS method allowed us to detect in this sample one compound from PAH group – benzo(e)pyrene. This constituted a 13 times smaller part of the whole sample in summer (0.3%) than in winter (3.9%).

PAH fraction of particulate matter sampled in winter was mutagenic in all *Salmonella* strains. Mutagenicity of this fraction increased under the influence of metabolic activation. Volumes of polluted air responsible for mutagenic effect in TA 98 strain were 31.0 m^3 without and 6.5 m^3 with metabolic activation. Multiple aromatic hydrocarbons are known promutagenes. During enzymatic transformations in vertebrate organisms, catalysed mainly by microsomal enzymes of liver, compounds of direct genotoxic activity are created. A classic example is benzo(a)pyrene undergoing enzymatic transformations to mutagenic and carcinogenic 7.8-dihydrodiol-9.10-*trans*-epoxide. It also undergoes oxidation to quinones, from which 6-phenoxyradicals are created which react with DNA [17]. YG 1021 and YG 1024 strains were more sensitive than TA 98 strain to mutagenic activity of PAHs adsorbed on particulate matter sampled in winter. Volume of polluted air responsible for mutagenic effect in YG 1021 strain was 3.3 m^3, and in YG 1024 strain, 3.7 m^3. This testifies to the presence of trace quantities of nitro PAHs in this fraction. Due to their trace presence they could be masked by other compounds and therefore they were not detected by GC-MS method.

PAHs adsorbed on particulate matter polluting the atmosphere cause frame-shift mutations, which can be detected in Wrocław by application of *Salmonella typhimurium* TA 98, YG 1021 and YG 1024 strains, and mutations of base substitution, whose detection is effected by TA 100 strain used in Upper Silesia. As in the case of aromatic fraction of particulate matter sampled in winter in Wrocław, mutagenicity of aromatic fraction of particulate matter sampled in Upper Silesia increased after activation with S9 fraction [14]. Mutagenic activity also was displayed by PAH fractions isolated from particulate matter sampled by other researchers [2], [20]. In Upper Silesia, this concerned particulate matter sampled both in summer and in winter, with one reservation only, mutagenicity of PAHs adsorbed on particulate matter sampled in winter was greater [14]. Fraction of particulate matter sampled in Wrocław in summer was not mutagenic.

Mutagenicity of fractions of polar compounds. Mutagenic activity of polar fractions (figure 2) was similar to mutagenic activity of unfractionated extracts of suspended particulate matter. Volume of polluted air producing mutagenic effect in TA 98 strain without metabolic activation was 5.2 m^3 in winter and 28.8 m^3 in summer.

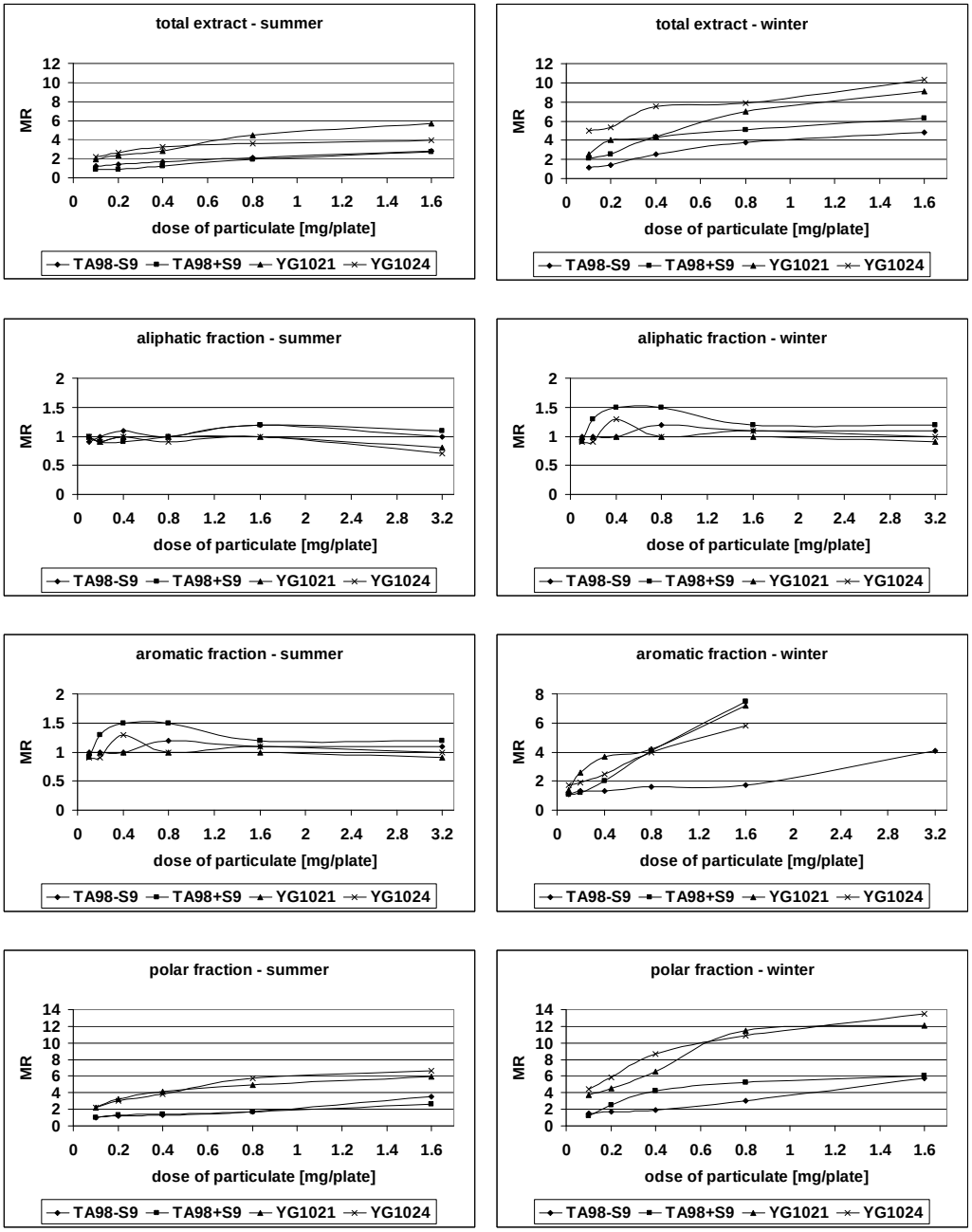
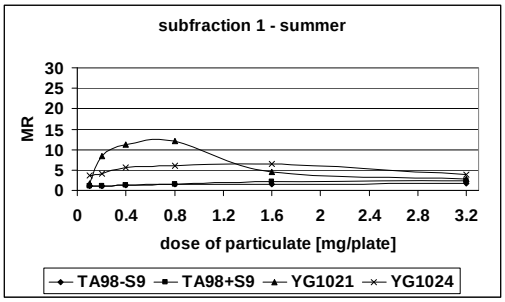
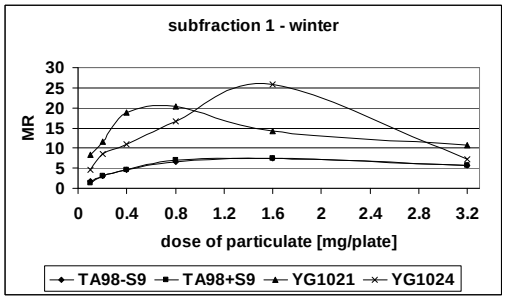
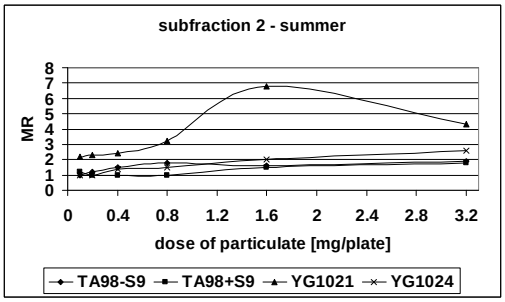


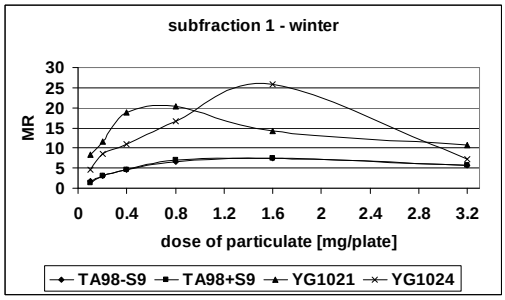
Fig. 2. Mutagenicity ratios (MR) of complete extract
and its fractions: aliphatic, aromatic and polar

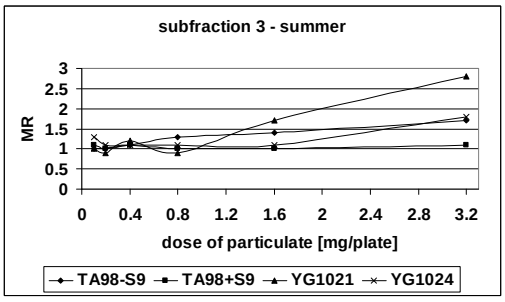
a)

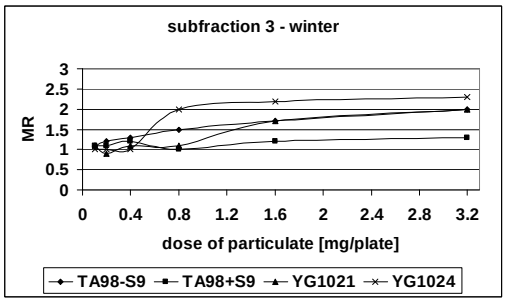

subfraction 1 - summer
MR
dose of particulate [mg/plate]
TA98-S9 TA98+S9 YG1021 YG1024

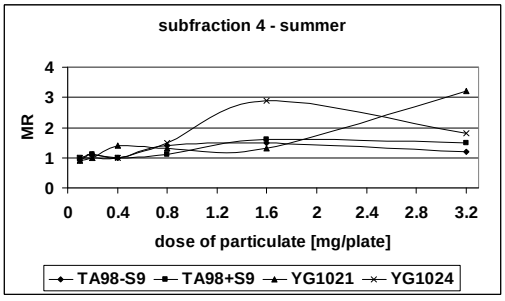

subfraction 1 - winter
MR
dose of particulate [mg/plate]
TA98-S9 TA98+S9 YG1021 YG1024

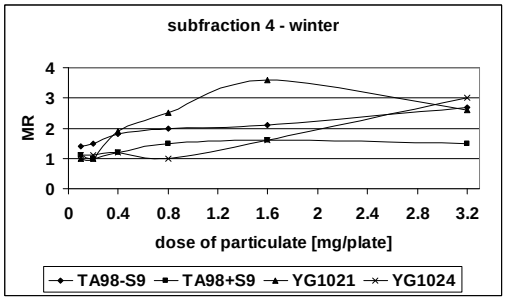

subfraction 2 - summer
MR
dose of particulate [mg/plate]
TA98-S9 TA98+S9 YG1021 YG1024


subfraction 1 - winter
MR
dose of particulate [mg/plate]
TA98-S9 TA98+S9 YG1021 YG1024


subfraction 3 - summer
MR
dose of particulate [mg/plate]
TA98-S9 TA98+S9 YG1021 YG1024


subfraction 3 - winter
MR
dose of particulate [mg/plate]
TA98-S9 TA98+S9 YG1021 YG1024


subfraction 4 - summer
MR
dose of particulate [mg/plate]
TA98-S9 TA98+S9 YG1021 YG1024


subfraction 4 - winter
MR
dose of particulate [mg/plate]
TA98-S9 TA98+S9 YG1021 YG1024

b)

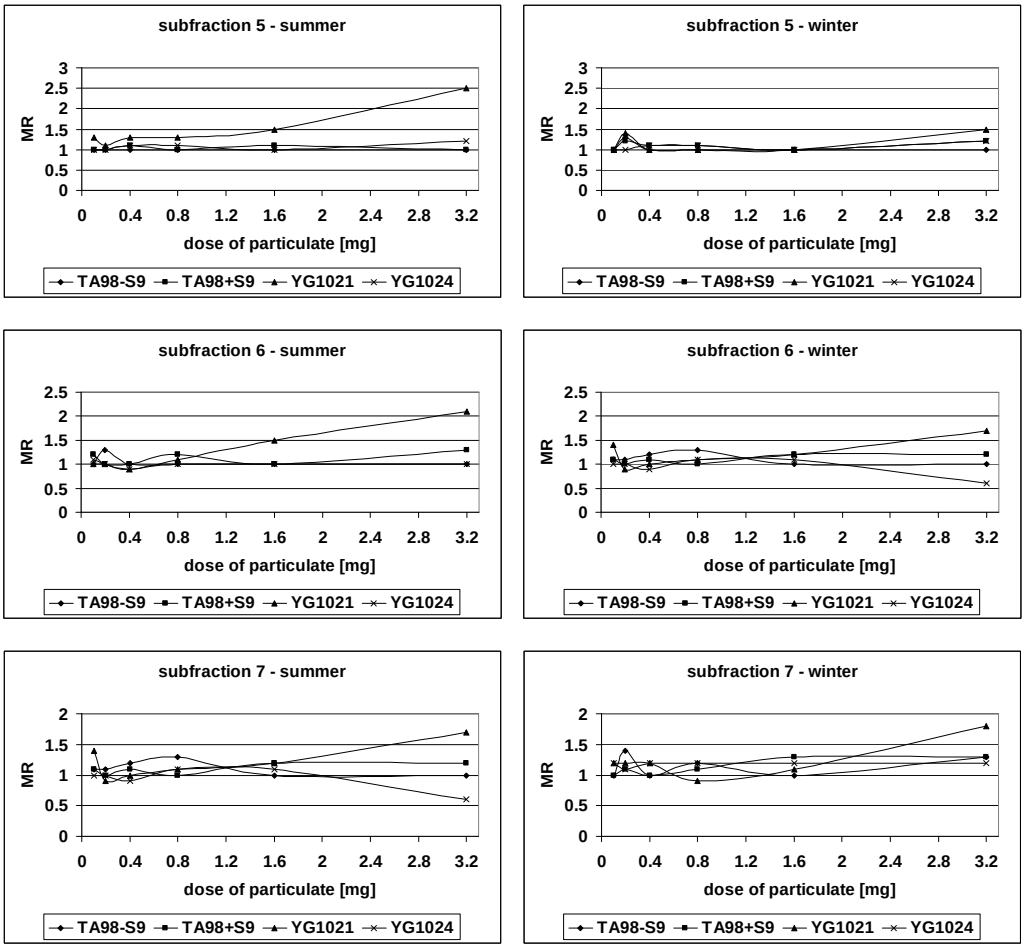


Fig. 3. Mutagenicity ratios (MR) of subfractions: a) soluble in chloroform (1), soluble in the mixture of 15% diethyl ether and 85% chloroform (2), soluble in the mixture of 3% ethanol and 97% diethyl ether (3), soluble in methanol (4); b) soluble in the mixture of 3% ethanol and 97% chloroform (5), soluble in the mixture of 3% ethanol and 97% tetrahydrofuran (6), soluble in dichloromethane (7)

With metabolic activation the volume was 3.0 m^3 in winter and 31.8 m^3 in summer. A moderate increase in mutagenicity of polar fraction of particulate matter sampled in winter in TA 98 strain under the influence of metabolic activation proves that this fraction includes small quantities of promutagenes. This may be polar PAH, whose presence was found by GC-MS method. The fractions were composed, moreover, of many other compounds, which can be suspected of genotoxic activity. As in the case of unfractionable sample and the fractions composed primarily of PAHs described

above, the fraction of polar compounds of particulate matter sampled in winter proved to be richer in chemical content and more mutagenic. Greater sensitivity of YG 1021 and YG 1024 strains in comparison with TA 98 strain testifies to a significant participation of nitro PAHs in mutagenic activity of polar fraction of pollutants adsorbed on suspended particulate matter. Volume of polluted air provoking mutagenic effect in YG 1021strain was 0.4 m^3 in winter, and 2.4 m^3 in summer. In the case of YG 1024 strain, those volumes were 0.5 m^3 in winter and 2.5 m^3 in summer.

Significant mutagenicity is displayed also by fractions of polar compounds adsorbed on suspended particulate matter containing apart from PAHs also other mutagenes such as nitroarenes, aromatic amines, heterocyclic compounds, phenols, furans and dioxins [2], [14], [20]. Mutagenicity of those fractions can be even higher than that of fractions containing mainly PAHs [2], [14]. In Wrocław, polar fraction was responsible for the whole mutagenic activity of particulate matter sampled in summer. In winter, it displayed greater direct activity in TA 98 strain than aromatic fraction. High positive responses of YG 1021 and YG 1024 strains testify to the fact that just in this group of pollutants there were present, in particulate matter sampled in Wrocław, the greatest number of compounds whose natural groups are substituted for nitro groups. Increased mutagenic activity of those fractions of particulate matter under the influence of metabolic activation with S9 fraction sampled in winter in Wrocław, in Upper Silesia and in Duisburg (Germany) proves that those fractions comprise intermediate mutagenes [2], [14]. Mutagenicity of polar fraction is greater in winter than in summer. Such a regularity was found in Wrocław and in Upper Silesia [14].

Mutagenicity of subfractions of polar compounds. None of the subfractions obtained as the result of separation of polar compounds adsorbed on suspended particulate matter sampled in summer displayed mutagenic activity in TA 98 strain without activation with S9 fraction (figures 3a and 3b). The fraction displayed mutagenic activity before separation into subfractions. This shows that the mutagenic effect of this fraction as a whole is a result of a global activity of many chemical compounds possessing the character of direct mutagenes. After separation by the method of column chromatography they were probably shifted to different subfractions and they did not individually display a detectable mutagenic effect. In multicomponent mixtures, synergistic activity of mutagenes is also possible. Random mutagenic control tests of subfractions recombined after chromatographic separation suggested that lack of activity of subfractions was not effected by loss of mutagenes during fractionation and evaporation of solvents. In the case of particulate matter sampled in winter, the subfractions soluble in chloroform, i.e. mixture of 3% ethanol and 97% diethyl ether, and in methanol displayed mutagenic activity in TA 98 strain without activation with S9 fraction. This testifies to the presence of direct mutagenes in those subfractions.

In the tests with TA 98 strain being activated with S9 fraction, mutagenic activ-

ity was displayed only by subfractions soluble in chloroform. The subfraction was more mutagenic in the case of particulate matter sampled in winter than in summer. This testifies to the presence of polar promutagenes in that subfraction (figures 3a and 3b).

In the tests with YG 1021 strain, the mutagenic activity was displayed by the subfractions of particulate matter sampled in summer and soluble in chloroform, in the mixture of 3% diethyl ether and 97% chloroform, in the mixture of 3% ethanol and 97% diethyl ether, in methanol, in the mixture of 3% ethanol and 97% chloroform and in tetrahydrofuran. Mutagenic activity in YG 1021 strain was displayed also by subfractions of particulate matter sampled in winter and soluble in chloroform, in the mixture of 3% diethyl ether and 97% chloroform, in the mixture of 3% ethanol and 97% diethyl ether and in methanol (figures 3a and 3b). In the tests with of YG 1024 strain, mutagenic activity was displayed by fractions of particulate matter sampled in summer and soluble in chloroform, in the mixture of 3% diethyl ether and 97% chloroform and in methanol. The activity was also displayed by subfractions of particulate matter sampled in winter and soluble in chloroform, in the mixture of 3% ethanol and 97% diethyl ether and in methanol. Increased sensitivity of the tested subfractions of YG 1021 and YG 1024 strains to mutagenic activity in comparison with TA 98 strain testifies to a significant participation of nitro PAHs in their mutagenicity. Lack of sensitivity of TA 98 strain with a simultaneous sensitivity of strains of increased nitroreductase and *O*-acetyltranferase activity to mutagenicity of some subfractions proves that their mutagenic effect is caused exclusively by nitro PAHs. Mutagenic effect identified exclusively with YG 1021 and YG 1024 strains was higher in summer than in winter. An increased solar radiation conducive to nitration of organic pollutants in the atmosphere can be responsible for this. In the case of subfractions comprising other mutagenes, whose detection was allowed by TA 98 strain, this direction was generally opposite. More polar mutagenes were probably adsorbed on the particulate matter sampled in winter. They could partly mask an increase in the quantity of nitro PAHs in summer.

The results presented are close to the results of tests on mutagenic fractions of pollutants adsorbed on suspended particulate matter in Upper Silesia obtained in the same sequence of solvents [14]. Fraction soluble in chloroform displayed there indirect and direct mutagenic activity in *Salmonella typhimurium* TA 100 strain in summer and in winter. In winter, direct and indirect activity was also displayed by fractions soluble in the mixture of 10% diethyl ether and 90% chloroform, in the mixture of 3% ethanol and 97% diethyl ether and in methanol. In summer, those fractions displayed only direct mutagenic activity. Fraction soluble in the mixture of 10% diethyl ether and 90% chloroform induced also mitotic aberrations and mitotic arrest [15]. Clastogenic effect was caused by fractions soluble in chloroform, in the mixture of 10% diethyl ether and 90% chloroform and in the mixture of 3% ethanol and 97% diethyl ether [13].

Mutagenic ratio (MR) of fraction including all polar compounds adsorbed on suspended particulate matter was generally lower than the total of presented here MR of seven subfractions of organic compounds differing in polarity. An exception was mutagenicity of complete polar fraction of particulate matter sampled in Wrocław in summer in TA 98 strain without metabolic activation. Many different biologically active pollutants are adsorbed on suspended particulate matter PM 10. They can mutually reinforce or weaken their activity. An answer given by the Ames test is a resultant of those activities.

4. CONCLUSION

Pollutants adsorbed on suspended particulate matter PM10 in the centre of Wrocław displayed higher mutagenicity in winter than in summer. Mutagenic activity in summer was displayed exclusively by polar pollutants, in winter by PAHs and polar compounds. Among them there were direct and indirect mutagenes. In mutagenicity of pollutants adsorbed on suspended particulate matter PM10, nitroarenes are of a particular importance. Mutagenic effect caused by pollutants adsorbed on the particulate matter tested was a total of activities of many mutagenes. In multicomponent mixtures, such as the extracts and their fractions tested, mutagenes can display synergistic or antagonistic action. Mutagenic effect showed by the Ames test was a resultant of those interactivities.

Concentration of few indicator-pollutants can be determined by means of physicochemical methods. This allows only approximate evaluation of health hazards caused by mutagenes and carcinogens adsorbed on suspended particulate matter. Full evaluation of their combined activity can be provided only by biotests. The results presented above confirm legitimacy of the postulate of the Institute of Environmental Health in Stockholm to supplement physicochemical monitoring of atmospheric pollutants with mutagenicity tests of pollutants adsorbed on suspended particulate matter carried out using the Ames test [18]. Separation of these pollutants into fractions allows proving that the total effect of activity of multicomponent mixture is a total of activity of the individual components as regards their mutual reactivity.

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MUTAGENNOŚĆ ZANIECZYSZCZEŃ ZAADSORBOWANYCH NA PYLE ZAWIESZONYM W CENTRUM WROCŁAWIA (POLSKA) I ICH FRAKCJI

Korzystając z testu Amesa, zbadano mutagenność frakcji chromatograficznych zanieczyszczeń zaadsorbowanych na pyle zawieszonym PM10 w centrum Wrocławia (642 000 mieszkańców). Pył pobierano metodą wysokowydajnej aspirometrii latem i zimą. Próby ekstrahowano dichlorometanem. Rozdziału na frakcje dokonano metodą chromatografii kolumnowej. W teście Amesa zastosowano szczep *Salmonella typhimurium* TA 98 oraz szczepy YG 1021 i YG 1024 o podwyższonej wrażliwości na mutagenne działanie nitropochodnych WWA. Aby aktywować promutageny, stosowano frakcję S9 uzyskaną z wątroby szczurów. Zanieczyszczenia zaadsorbowane na pyle wykazywały większą mutagenność zimą niż latem. Latem aktywność mutagenną wykazywały wyłącznie zanieczyszczenia polarne, zimą WWA i związki polarne. Były wśród nich mutageny bezpośrednie i pośrednie. Znaczący udział w mutagenności zanieczyszczeń zaadsorbowanych na pyle miały nitropochodne WWA. Efekt mutagenny stwierdzany za pomocą testu Amesa był wynikiem łącznego działania wielu mutagenów wykazujących w mieszaninie działania synergistyczne i antagonistyczne.