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# SEASONAL VARIABILITY OF THE MUTAGENICITY OF AIRBORNE PARTICLES FOR SALMONELLA TYPHIMURIUM TA 98 AND SACCHAROMYCES CEREVISIAE XV185-14C

The Ames test and the yeast assay were used to investigate the mutagenicity of airborne particles found at the center of Wrocław (SW Poland) and collected over the period of one year, i.e. from August 1997 to July 1998. In the Ames test the strain of *Salmonella typhimurium* TA 98 was used, and in the yeast assay – the haploid strain of *Saccharomyces cerevisiae* XV185-14C. All the samples tested proved to be mutagenic in the Ames test. The mutagenicity of the particulates was the highest in winter, and the lowest in summer. The volume of the air corresponding to the dose of particulates producing the mutagenic effect was dependent on the concentration of suspended particles and the compounds adsorbed in the ambient air. The majority of the samples did not have the mutagenic effect on the yeast. The response of one sample only was dubious. In the samples studied, PAHs and their derivatives as well as heterocyclic compounds were found.

# 1. INTRODUCTION

Mutagenicity of airborne particles determined in different biotests on pro- and eukaryotic organisms has been reported many times [1]. The studies of the mutagenicity of particulate air pollutants were carried out mainly in highly industrialized countries of Western Europe [2], North America [3] and Japan [4]. In Poland, such studies were so far undertaken in the Upper Silesia [1] and Warsaw [5]. Recently they have also been started in Wrocław [6], [7]. Those studies not only proved that mutagenicity of particulates evaluated in the Ames test was higher in winter than in summer, but they also allowed us to determine the chemical nature of the compounds responsible for the biological activity of particulates, particularly with respect to nitro and amino derivatives of PAHs. However, the variability of the mutagenicity of airborne particles in the full annual cycle was not described. Such studies are also very

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rarely reported in the available literature [2], [8]–[10]. The majority of authors usually confine themselves to testing at most several samples of particulates.

The aim of this study was to investigate the seasonal variability of mutagenic potential of airborne particles in the center of Wrocław (SW Poland) and to determine the groups of mutagens responsible for this process. The Ames test was chosen because it is widely used for studying the mutagenicity of air pollutants[2], [4], [10], [11] and allows an accurate prediction of the carcinogenicity of mutagenic compounds [12]. To reveal the activity of promutagenic compounds, 10% microsomal fraction S9 of rat liver was used. The yeast assay has been so far only once used in the studies of the mutagenicity of airborne particles [2]. It enabled the statement that in the case of *Saccharomyces cerevisiae* D7 these pollutants had low ability to cause the mutation of mitochondrial DNA, the mitotic gene conversion in the *locus* trp5 and the reversion of the mutation in the *locus* ade2 in the strain of *Saccharomyces cerevisiae* XV 185-14C.

# 2. MATERIALS AND METODS

# 2.1. THE STRAIN OF SALMONELLA TYPHIMURIUM

The strain of *Salmonella typhimurium* TA 98 was donated to us by Professor B. Ames (Laboratory Department of Biochemistry, University of California). It has the following genetic markers: his<sup>-</sup>, rfa,  $\Delta$ uvrB, +R.

# 2.2. THE STRAIN OF SACCHAROMYCES CEREVISIAE

The haploid strain of *Saccharomyces cerevisiae* XV185-14C [13], which allowed us to study the reversion of mutations, was donated by Dr. R. C. von Borstel (Department of Genetics, University of Alberta, Edmonton, Canada). It has the following genetic markers: MAT a, trp5-48, arg4-17, lys1-1, ade2-1, his1-7, hom3-10. They are responsible for its inability to produce tryptophane, arginine, adenine, histidine and homoserine. The ade2-1 mutation is the ochre stop codon (5'-TAA-3') and can be reversed mainly by <sup>tyr</sup>tRNA suppressors.

# 2.3. COLLECTION AND PREPARATION OF THE SAMPLES

Airborne particles formed at the center of Wrocław over the period from August 1997 to July 1998 were studied. The particulates were collected on glass filters by the pump delivering 67.8 m<sup>3</sup>/h. The filters were changed every 24 hours. The particulates collected on approximately 20 filters each month were combined as one sample. The filters with collected particulates were extracted for 8 hours with dichloromethane (Lab-Scan) in Soxhlet apparatus.

The extracts were evaporated in order to obtain a solid residue which was subsequently dissolved in DMSO (Fluka) in such a way that  $0.1 \text{ cm}^3$  contained the extract from 3.2, 1.6, 0.8, 0.4, 0.2 and 0.1 mg of particulates. The samples prepared in this way were used for the Ames test and for the yeast assay.

### 2.4. PHYSICOCHEMICAL ANALYSIS BY GC-MS

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

#### 2.5. THE AMES TEST

The procedure described by MARON and AMES [14] was used.  $0.5 \text{ cm}^3$  of phosphate buffer or the same volume of the fraction S9 was added to a sterile test tube. Next, the components were added as follows:  $0.1 \text{ cm}^3$  of the tested sample,  $0.1 \text{ cm}^3$  of the overnight broth culture of the test strain and  $2 \text{ cm}^3$  of TOP-agar at 45 °C containing 0.2 cm<sup>3</sup> of 0.5 mM histidine and biotine. The content of the test tube was mixed and during 20 seconds poured onto a Petri dish containing the minimal Vogel–Bonner medium. All samples were tested in 5 repetitions. The dishes were incubated at 37 °C for 48 hours. After this time, the number of revertants growing on the dishes were counted.

Average numbers of spontaneous revertants were close to those reported by MARON and AMES [14]. For metabolic activation of promutagens present in the samples, 10% fraction S9 was used.

Sensitivity of bacteria to control mutagens was tested by the positive controls (without the metabolic activation:  $0.2 \ \mu g$  of 2,4,7-trinitro-9-fluorenone per a dish, with the metabolic activation:  $10 \ \mu g$  of 2-aminofluorene per a dish). Mutagenic activity of the solvent and the filter material were also checked.

### 2.6. THE YEAST ASSAY

The procedure of the yeast assay described by ZIMMERMANN [15] was used. After initial incubation yeast cultures with high frequency of spontaneous reversion were rejected. Yeast cells were suspended in distilled water in such a way as to obtain the density of  $2 \cdot 10^8$  cells/cm<sup>3</sup>. To the test tubes containing  $4.5 \text{ cm}^3$  of liquid YPG medium, we added 0.5 cm<sup>3</sup> of cell suspension in distilled water ( $2 \cdot 10^8$  cells/cm<sup>3</sup>) and extracts from 0.4, 0.8, 1.6 and 3.2 mg of particulates dissolved in 0.1 cm<sup>3</sup> of DMSO. Controls included also the solvent and the extract from a clean glass filter. Control

mutagen was 10  $\mu l$  of EMS. The exposure lasted 24 hours at 30 °C with agitation of the samples.

The exposure was terminated by centrifugation of the suspension at 4 °C. The supernatant was discarded and the cells were resuspended in 5 cm<sup>3</sup> of distilled water. The suspension was diluted in order to obtain the solutions whose dilutions were as follows:  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$ . 0.1 cm<sup>3</sup> of each solution was plated on YPG medium with agar (in 5 repetitions). After 5–6 days of incubation white and red colonies on the dishes were counted.

#### 2.7. PRESENTATION OF THE RESULTS

The results are expressed as the mutagenicity ratio (MR), which is the ratio of the average number of revertants induced by the sample per a dish to the average number of spontaneous revertants. According to the procedure, the sample was considered mutagenic when MR  $\geq 2$  and when it showed linear dose–response dependence [14,] [15].

## 3. RESULTS AND DISCUSSION

#### 3.1. RESULTS OF PHYSICOCHEMICAL ANALYSES

Seasonal variability of suspended particulates concentration and composition. Concentration of suspended particulates in the atmosphere changed during the year and was the highest in winter (in the middle of the heating season), and the lowest in summer (figure 1). A similar trend was observed towards organic compounds

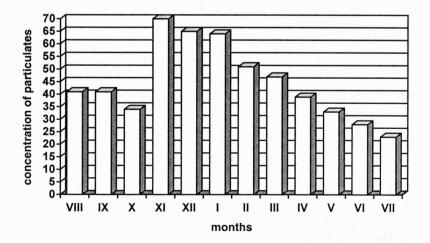


Fig. 1. Seasonal variability of the concentration of suspended particulates in the atmosphere  $[\mu g/m^3]$ . Based on the data from the Regional Inspectorate of Environmental Protection in Wrocław

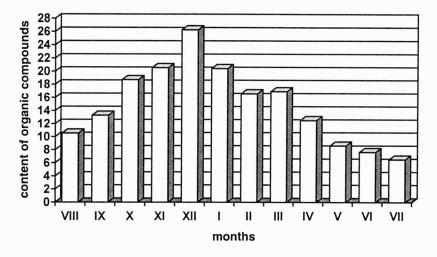


Fig. 2. Seasonal variability of the content of organic compounds in suspended particulates [%]

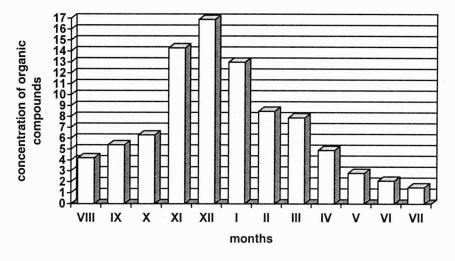


Fig. 3. Seasonal variability of the concentration of organic compounds adsorbed on suspended particulates [µg/m<sup>3</sup>]

adsorbed on the particulates tested (figure 2). The concentration of airborne particles and their composition influenced the concentration of organic compounds in ambient air, including mutagenic and carcinogenic agents (figure 3).

*Results of GC-MS analyses.* The samples tested were found to contain a number of compounds known for their promutagenic and mutagenic activity (tables 1–3). They included PAHs from the EPA list, other PAHs and their nitro and amino derivatives, and other cyclic compounds including those with nitrogen atoms in aromatic rings.

Compound	Month											
	VIII	IX	x	XI	XII	I	II	III	IV	v	VI	VII
Phenanthrene					+		+					
Anthracene			+	+		+		+				
Fluoranthene	+	+	+	+	+	+	+	+	+	+	+	+
Pyrene	+		+	+	+	+	+	+	+	+		
Benzo(a)anthracene					+	+	+					
Chrysene						+	+		+	+		
Benzo(k)fluoranthene					+	+			+	+	+	
Benzo(a)pyrene	+		+		+	+	+	+	+	+		
Benzo(ghi)perylene	+			+	+						+	
Indeno(1,2,3-cd)pyrene			+	+							+	

PAHs from the EPA list detected in the tested samples of airborne particles

The other PAHs detected in the tested samples of airborne particles

				1								
Compound	Month											
	VIII	IX	х	XI	ХП	Ι	II	III	IV	v	VI	VII
Methylpyrene		54		12	1	+	+					
Benzo(e)pyrene	+		+		+				+	+		
Cyclopentapyrene					+	+	+	+	+			
Methylchrysene					+		+		+			
Benzochrysene	+			+	+					+	+	
Benzofluorene			+		+	+ '	+	+	+		+	
Methylenefluorene		+										
Benzofluoranthene			+		+	+			+	+		
Methylanthracene				+		+	+					
Methylbenzoanthracene				+	+							
Benzophenanthrene				+		+						
Cyclopentaphenan-							+					
threne												
Terfenyl									+			
Perylene		+				+			+			
Triphenylene		+	+	+	+		+	+			+	
Methyltriphenylene				+			+					
Benzoacephenantrylene											+	
Phenylnaphthalene					+							

Seasonal variability of the mutagenicity of airborne particles

Table 3

Compound	Month											
	VIII	IX	Х	XI	XII	I	Π	III	IV	v	VI	VII
Derivatives of pyridine		+	+	+	+	+	+		+	+	+	+
Derivatives of quinoline	+	+		+			+					
Ethydibenzotiophene				+		+	+					
Nitroderivatives of									+		+	
phenol												
Dimethylethylphenol											+	
Phenetrol				+								
Benzoanthracenone				+		+	+		+	+	+	
Naphtydioxineone											+	
Fluorenone				+			+				•	
Phenalenone				+		+	+					
Anthracenodione		+			+	+	+	+	+	+	+	
Phenindione							+					
Nitroimidazole	+											
Aromatic amines	+			+						+		+
Acridine				+			+					•
Benzocarbazole							+					
Dihydrodibenzoazepine							+					
Cotinine									+		+	
Naphtaleneanhydrine									+			
Anthracenecarbonitryle									+			
Benzonaphtofurane						+						

The other compounds in the tested samples of airborne particles

Those compounds were present in the samples in very low concentrations, while phthalates dominated; plasticizers were also widespread.

The samples from late autumn, winter and early spring were richer than those from late spring, summer and early autumn. This applies also to promutagenic and mutagenic compounds and may be associated with higher emission of pollutants in the heating season.

### 3.2. RESULTS OF THE AMES TEST

All extracts from airborne particulates collected in different seasons of the year were mutagenic for *Salmonella typhimurium* (figures 5, 6). This allows us to conclude that the samples contain mutagens causing frame-shift mutations, which can be detected in the strain TA 98.

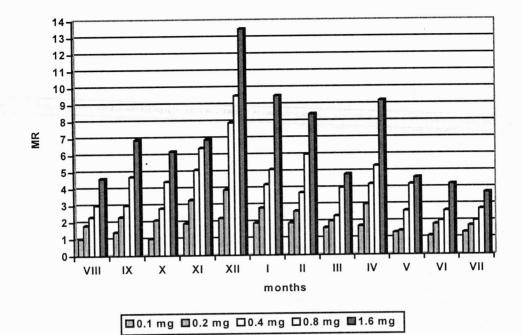


Fig. 4. Mutagenicity ratios (MR) in the Ames test without the activation by means of the fraction S9

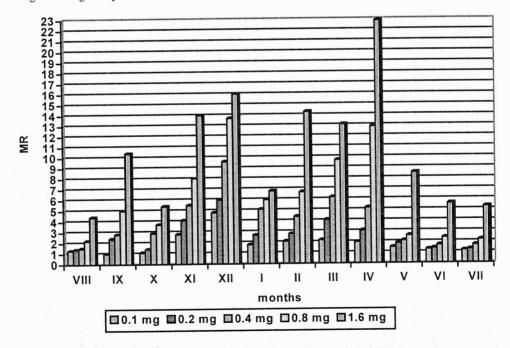


Fig. 5. Mutagenicity ratios (MR) in the Ames test with the activation by means of the fraction S9

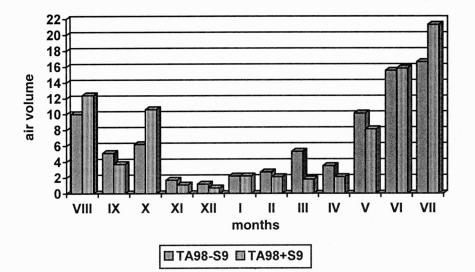


Fig. 6. Seasonal variability of the air volume  $[m^3]$  required to obtain the mutagenic effect in the Ames test (MR = 2.0)

Metabolic activation by means of the fraction S9 usually increased the mutagenicity of the extracts tested. This indicates that in the suspended particulates promutagens are present. These can belong to the PAHs group detected with the GC-MS method. The extracts tested were the mixtures of a number of chemical compounds. The fraction S9 is a mixture of many enzymes. The enzymes from rat liver present in this fraction probably catalyse both activation of promutagens and detoxification of mutagens. The response obtained in the test is the resultant of those processes taking place in different proportions in the samples with different chemical composition.

Similar results were obtained by other authors, who in their studies of the mutagenicity of airborne particles with the Ames test used the fraction S9 in order to induce metabolic activation. The fraction increased or decreased the mutagenicity of the samples for bacteria used in the Ames test, depending on the chemical composition of the samples [2], [4], [16].

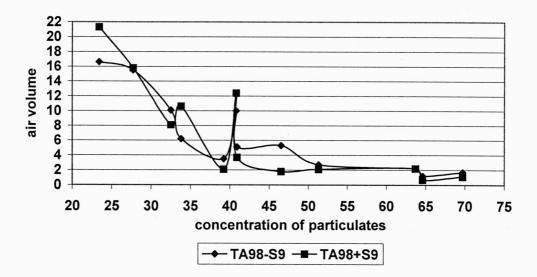
Air volumes containing the particulates required to double the reversion to prototrophy (MR = 2), calculated on the basis of the Ames test, varied considerably, depending on the sensitivity of the strain and on the time of sample collection (figure 6).

In the tests without activation by means of the fraction S9, the volumes ranged between 1.1 m<sup>3</sup> in December 1997 and 16.6 m<sup>3</sup> in July 1998. The volumes were the smallest in winter, gradually increased in transition periods and reached the highest values in summer months. The reasons for this phenomenon were complex. The particulates collected in winter adsorbed more compounds soluble in dichloromethane (figure 2). The amount of extract obtained from particulates collected in transition

periods (spring, autumn) gradually decreased and reached its minimum in summer. The content of particles per unit of an air volume varied in a similar way (figure 1). It reached the highest values in winter, gradually decreased in spring and autumn and was the lowest in summer. Thus the particulates collected in winter were richer in organic compounds than those collected in summer. The dustiness of the air was greater in winter than in summer. This is probably caused by two reasons: emission of particulates with adsorbed mutagens increases in the heating season and is associated with increased use of fossil fuels; in winter consumption of fuel by cars increases by over a dozen percent.

The increase in the mutagenicity of the air in winter and its reduction in summer were also observed in the tests with metabolic activation by means of the fraction S9. Although enzymes belonging to the fraction S9 changed the activity of the samples by activation or detoxification of mutagens, this phenomenon did not influence the general seasonal variability of mutagenicity.

Similar seasonal variability of the mutagenicity of airborne particles was found in other European countries. The mutagenicity of particulates corresponding to the unit of air volume increases in winter (in the heating season), and decreases in summer. Such a trend was observed in not industrialized parts of Holland [8] and in the northern Italy [2], [9]. The concentration of PAHs also increases in winter, which has been found, among others, in the vicinity of a petrochemical plant in Taiwan [11].



Air volume corresponding to the dose of particulates causing double increase of the frequency of reversion to prototrophy (MR = 2) depended on the concentration of

suspended particulates (figure 7). The volumes were the largest in summer months when the concentrations of particulates were the lowest, decreased in transition months (spring and autumn) and reached the lowest values when the concentration of particulates in the air was the highest. August 1997 was an exception. The concentration of particulates in the atmosphere was similar to those in spring and autumn months. However, the mutagenicity of the air was as low as in other summer months. This regularity was observed for all used strains of *Salmonella*, both with and without the metabolic activation.

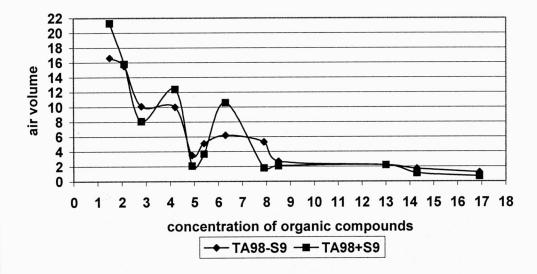


Fig. 8. The relationship between the concentration of organic compounds adsorbed on suspended particulates (the x-axis) and the air volume required to obtain the mutagenic effect in the Ames test -MR = 2.0 (the y-axis). The order of the months: VII, VI, V, VIII, IV, IX, X, III, II, I, XI, XII

The dependence of the mutagenicity upon the concentration of organic compounds adsorbed on suspended particulates present in the atmosphere was even more clear (figure 8). In winter, the concentration of suspended particulates was the highest, the content of adsorbed organic compounds was also the greatest. Therefore, in the heating season, the concentration of those compounds, including probably mutagens and carcinogens, increased more than the concentration of suspended particulates.

However, neither the concentration of suspended particulates nor the concentration of adsorbed organic compounds directly corresponded to the mutagenic activity of the air. This was probably caused by the differences in the composition of the samples. Such differences were found by the GC-MS method.

The mutagenicity of the air was not apparently correlated with the concentrations of gaseous pollutants, although other authors have reported their comutagenic activity [3], [17]–[20]. Thus, the main factor responsible for the mutagenicity of the air is the presence of mutagens adsorbed on airborne particles, and not gaseous pollutants

which react with organic compounds in the atmosphere giving product more or less mutagenic than the organic substrates.

#### 3.3. RESULTS OF THE YEAST ASSAY

In the studied range of concentrations, nearly all extracts were not mutagenic for yeast (figure 9). The result given by the sample collected in July 1998 is regarded as dubious, because of the lack of linear dose–response dependence. The samples collected in spring and summer caused a slight increase in the reversion ratio. This ratio, however, was too low to consider those samples mutagenic for yeast. The samples from spring and summer were relatively the least mutagenic for *Salmonella*.

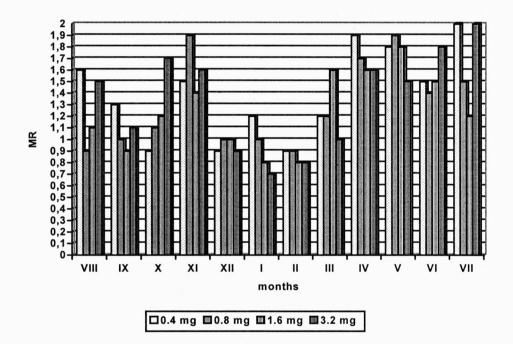


Fig. 9. Mutagenicity ratios (MR) in the yeast assay

So far only ROSSI et al. [2] tried to use the yeast assay to evaluate the mutagenic activity of airborne particles. They studied mitotic gene conversion in the *locus* trp5, reversion of the mutation in the *locus* ilv1-92 and mutation of mitochondrial DNA in the strain of *Saccharomyces cerevisiae* D7. The results of ROSSI et al. [2] were similar to those presented in this paper. The majority of samples tested by them were not mutagenic for yeast, and at the same time had a strong mutagenic effect on *Salmonella typhimurium* in the Ames test. Only a few samples inactive for *Salmonella typhimurium* in the Ames test were mutagenic for yeast. Our results and the results of

ROSSI et al. [2] indicate that the yeast assay should be used to complement the Ames test, to confirm the results negative in the test with *Salmonella typhimurium*. Other tests should be used to confirm positive results of the Ames test.

Earlier studies have proved that yeasts are insensitive to some compounds generally regarded as mutagenic, present in organic extracts from suspended particulates tested here: benzo[a]pyrene, benzo[e]pyrene, benzo[a]anthracene and chrysene. Tobacco smoke is not directly mutagenic for yeasts either [21]. The yeast assay is successfully used in the studies of environmental mutagenesis, often as a complementation of the Ames test. It was used to test several hundreds of different potentially mutagenic and carcinogenic compounds, including many pesticides and drugs.

The reason for a lower sensitivity of *Saccharomyces cerevisiae* in comparison with *Salmonella typhimurium* can be the presence of nuclear envelope in the eukaryotic yeast cells. The envelope protects better the genetic material from the mutagenic activity of xenobiotics. The structure of the cell wall is also different, therefore different compounds can enter the eukaryotic cell. Low level of natural nitroreductase activity in the yeast cells can be responsible for their low sensitivity to the mutagenic activity of nitro derivatives of PAHs. They have a considerable contribution to the total mutagenicity of air pollutants. Therefore efforts are made to introduce the bacterial gene coding nitroreductase to the yeasts genome [22].

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# SEZONOWA ZMIENNOŚĆ MUTAGENNOŚCI PYŁU ZAWIESZONEGO WOBEC SALMONELLA TYPHIMURIUM TA 98 I SACCHAROMYCES CEREVISIAE XV185-14C

Korzystając z testu Amesa i testu drożdżowego, badano mutagenność pyłu zawieszonego, który pobierano w centrum Wrocławia w okresie VIII 1997–VII 1998. W teście Amesa zastosowano szczep Salmonella typhimurium TA 98, w teście drożdżowym – haploidalny szczep Saccharomyces cerevisiae XV185-14C. Wszystkie badane próby pyłu okazały się mutagenne w teście Amesa. Mutagenność pyłu była największa zimą, a najmniejsza latem. Objętość powietrza, która odpowiadała dawce pyłu potrzebnej do uzyskania efektu mutagennego, zależała od stężenia pyłu i zaadsorbowanych na nim związków organicznych. Większość prób była niemutagenna wobec drożdży, jedna próba dała wynik wątpliwy. W badanych próbach stwierdzono występowanie WWA, pochodnych WWA i związków heterocyklicznych.

mutagennosé pylu pyl zavierowy test Amesa test droi oliowy