Environment Protection Engineering

Vol. 32 2006 No. 4

KATARZYNA PIEKARSKA*,
JOANNA KARPIŃSKA-SMULIKOWSKA*

EFFECT OF MICROSOMAL FRACTION INDUCTION ON THE DETECTABILITY OF MUTAGENIC AIR POLLUTANTS BY MEANS OF THE AMES BACTERIAL MUTAGENICITY TEST

It was found that pollutants adsorbed by atmospheric particulate matters being collected during the summer and winter seasons in the centre of the city of Wrocław, Poland, are mutagenic. The research was based on a short-term test of *Salmonella* (the Ames test). The samples tested indicated the presence of pollutants which might affect directly or indirectly the genetic material. In order to activate metabolically the indirect mutagens, two types of rat liver microsomal fraction were used, namely the fraction activated by widely used Aroclor 1254 and the fraction activated by Phenobarbital, rarely used in environmental sample tests. Comparative tests of both fractions show that it might be possible to replace highly toxic Aroclor 1254 with a less harmful Phenobarbital in order to obtain the microsomal fractions that can interact with air particulate pollutants.

1. INTRODUCTION

Atmospheric air is polluted with various substances, the amount and type of which depend on the source of emission. Polycyclic aromatic hydrocarbons (PAHs) and their nitro and amino acid derivatives are considered to be particularly harmful to human health because of their mutagenic and cancerogenic nature [1], [2]. The detection and identification of many pollutants based on chemical analyses are expensive, time-consuming and require modern analytical techniques. Many substances constituting pollutants act synergistically, thus, based on the results of chemical methods, it is not possible to collect a reliable information on biological effects caused by the mixture of pollutants. In the case of environmental samples, we deal with such mixtures. On the other hand, based on biological screening tests we can evaluate total effect of all substances contained in a given sample [3].

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

The tests conducted to evaluate the mutagenicity of environmental pollutants, including detecting mutagenic effect of organic extracts from atmospheric particulates, testify to the utility of a short-term *Salmonella* test (the Ames test), devised in the seventies of the twentieth century by the American professor Bruce Ames [4]. This test allows us to estimate the level of reverse mutations from auxotrophic cells of specially created mutants of the *Salmonella typhimurium* LT2 strains to histidine-independent prototrophic cells. In order to activate metabolically promutagens, the fraction S-9 is applied, which is composed of induced microsomal enzymes isolated from rat liver. The fraction S-9 makes it possible to extend the results obtained from the bacterial test to mammals.

The most frequently used inductor of liver enzymes is a mixture of polychlorinated biphenyls contained in Aroclor 1254, a strong and hardly decomposable environmental poison. A considerably less toxic Phenobarbital is a compound of a comparable induction activity. The fraction S-9 was induced by Phenobarbital and then used solely for the activation of isolated promutagens, but not applied to complex mixtures of substances, i.e. environmental samples [3], [5], [6].

The aim of this paper was to determine the seasonal changeability of the mutagenic effect of atmospheric particulates found in air samples collected in the Wrocław area and to compare the utility of the microsomatic fraction induced by Aroclor 1254 and Phenobarbital in the Ames test.

2. MATERIALS AND METHODS

The samples were collected in a continuous manner for 17–24 hours in the summer and winter seasons with the Staplex aspirator. A total number of hours in which atmospheric particulates were collected was 960 in winter, and 1669 in summer. The air flow rate ranged from 71.7 to 82.08 m^3/h. The air was aspirated through a Schleicher & Schull GF-9 glass filter with the pore size of 0.5–1.4 μm. A total content of air pollutants collected in winter samples was 7.07565 g, and in summer samples – 7.0419 g. Immediately upon collecting the samples, the filters were put into a refrigerator and stored there at the temperature of –20 °C until the extraction. Next, the pollutants were extracted with dichloromethane without access to light in Soxhlet apparatus for 8 hours with a 15-minute reflux [7], [8]. After the completion of the extraction, dichloromethane was evaporated from vacuum evaporator. The extract obtained was divided into two portions. The first of them was subjected to HPLC analysis, and the second one – to the Ames test once the solid residue was dissolved in a sterile DMSO in such a way that 1 cm^3 of the solution contained pollutants originating from 1000 m^3 of air. The extract was poured into the Petri dishes in such a way that its quantity used per plate corresponded to 100; 50; 25; 12.5; 6.25; 3.125; 1.56; 0.78; 0.39; 0.195; 0.097 and 0.049 m^3 of the air tested. The test procedure described by MARON and AMES was applied here [9]. All samples were tested in five repetitions. The samples were incubated at 37 °C for 72

hours. After that time revertant colonies (his $^{+}$ revertants) growing in the Petri dishes were counted. The tests were conducted in the presence of two tester strains of *Salmonella typhimurium:* TA98 and its derivative YG 1041. The strain TA 98 is susceptible to the activity of mutagens causing mutations such as a change of the frameshifts, whereas the strain YG 1041 is characterized by the susceptibility to nitro-, amino- and hydroxylamine derivatives of PAHs due to its higher nitroreductase and O-acetyltransferase content encoded in plasmids (table 1) [10], [11]. The *Salmonella* tester strains were donated to us by Dr. T. Nohmi from the Division of Genetics and Mutagenesis in the National Institute of Hygienic Sciences, Tokyo, Japan. Prior to starting the tests, we checked each time the genetic markers of the tester strains, as well as their spontaneous reverse mutations and susceptibility to diagnostic mutagens (without the metabolic activation with the fraction S9: 0.2 μg of 2,4,7-trinitro-9-fluorene per plate for the strain TA 98, and 50 μg of 2,6-dinitrotoluene per plate for the strain YG 1041; and with metabolic activation: 2.5 μg of 2-aminofluorene per plate for both strains). Direct mutagens found in the extracts of atmospheric particulates were detected using a buffer solution, whereas promutagens were tested for their occurrence in extracts using a microsomal (S9) fraction of Wistar rat liver induced with Aroclor 1254 (A) or Phenobarbital (B). The fraction was obtained according to the methods invented by MARON and ONG [5], [9]. Of numerous methods the biuret method described in [7] was chosen. This was the test for protein content used in order to mark the protein capacity in a fraction. This method, which is based on detecting peptide bonds which are characteristic of proteins, is strongly recommended for researches into microsomal fraction. The protein content determined by the biuret method [7], [12] was 64.44 mg/cm^3 in the fraction A, and 33.64 mg/cm^3 in the fraction B. The content of proteins in the fraction B was assumed to be 100 per cent. The concentration of the rat liver homogenate in the fraction S-9 amounted to 10 per cent.

Table 1

The *Salmonella typhimurium* strains used

Strain	Description
TA 98	TA 1538 his D3052 (pKM101)
YG 1041	TA 98 (pYG233): a nitroreductase and O-acetyltransferase-overproducing strain

The mutagenicity of the extracts tested was expressed by a mutagenicity ratio (MR), the quotient of an average number of revertants induced by the samples being tested by an average number of spontaneous revertants. The sample was considered mutagenic when, at least twice, it caused as high an increase in the mutation level (the MR ratio) as the natural spontaneous mutation level for the strains being tested that demonstrated a linear correlation between the mutagen dosage and the response [3], [4].

3. RESULTS

The average concentration of the pollutants collected amounted to 57.8428 mg/1000 m^3 of air in winter, and 43.737 mg/1000 m^3 of air in summer (table 2). Instrumental analysis revealed six PAHs (pyrene, benzo(a)anthracene, benzo(b)fluoranthene, benzo-(k)fluoranthene, benzeo(a)pyrene and indeno(1,2,3,c,d)pyrene), whose total amount reached 11.3822 μg/1000 m^3 in the sample collected in winter, and 0.5348 μg/1000 m^3 in the sample collected in summer (table 3). Based on the concentration of PAHs determined in samples we can conclude that the concentration of these compounds in the winter samples is over 20 times higher than in summer samples.

Table 2

Comparison of the air samples collected in winter and summer

Type of sample	Capacity of air sample [m^3]	Mass of pollutants [mg/1000 m^3]	Mass of tar substances [mg/1000 m^3]
Winter	122326	57.8428	2.1496
Summer	161004	43.737	1.2172

Table 3

Content of PAHs in extracts of air pollutants collected from 1000 m^3 of air

PAHs	Air sample			
	Winter		Summer	
	μg	%	μg	%
Pyrene	2.398	21.00	0.1276	23.80
Benzo(a)antrhacene	1.9872	17.46	0.1076	20.12
Benzo(b) fluoranthene	1.6674	14.65	0.0776	14.51
Benzo(k)fluoranthene	1.9858	17.45	0.059	11.03
Benzo(a)pyrene	2.228	19.57	0.039	7.29
Indeno(1,2,3,c,d)pyrene	1.1158	9.80	0.124	23.19
Total	11.3822	100	0.5348	100

The samples of air pollutants being tested proved to be mutagenic for *Salmonella typhimurium* tester strains TA 98 and YG 1041 (figure 1). A wide range of the concentrations tested allowed us to determine the concentrations that had both mutagenic and toxic effect on the strain YG 1041. In the case of the strain TA 98, even the concentration of pollutants contained in 100 m^3 of air did not enable any determination of the toxicity threshold for the samples tested.

The highest MR values were obtained for the strain YG 1041 (figure 3). In the case of this strain, a high linear correlation was found between the concentration of the sample and the number of the revertants obtained. The maximum value of MR for

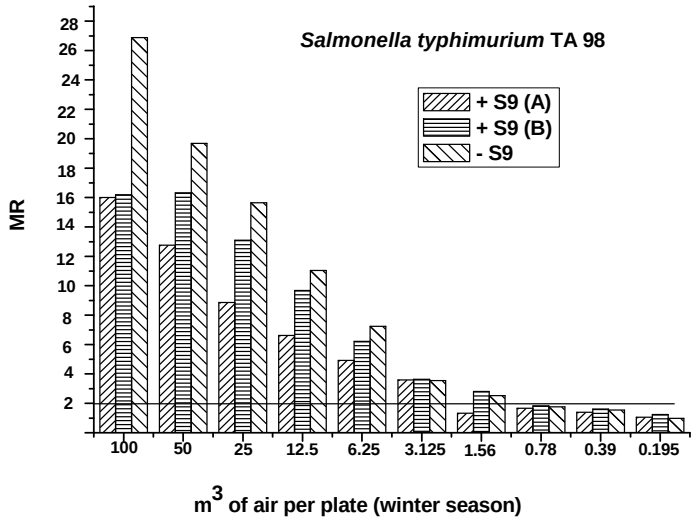

Fig. 1. MR values for the samples of the air collected during the winter season

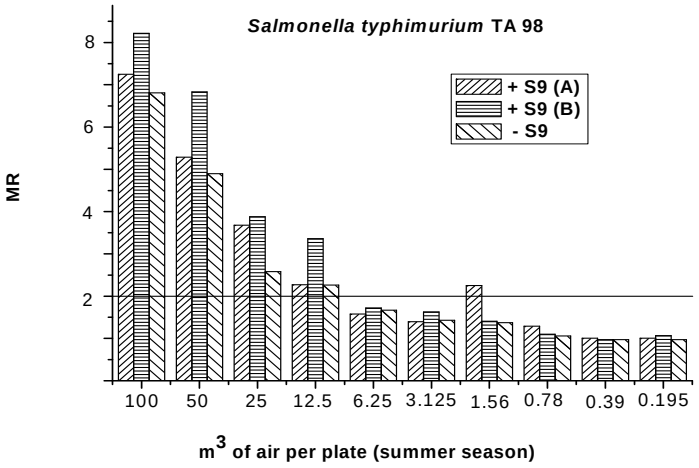

Fig. 2. MR values for the samples of the air collected during the summer season

this strain (the samples collected in winter, figure 3) tested without the fraction, upon involving the pollutants comprised in 6.25 m^3 of air in the test, was 35.63. In the case of the tests with Aroclor-induced fraction, the highest MR value equal to 46.97 was obtained after applying 12.5 m^3 of the air to the test, whereas in the case of the tests conducted with Phenobarbital-induced fraction, the highest MR value equal to 89.26 was reached for the pollutants contained in 6.25 m^3 of air. These highest MR values were obtained at the number of pollutants involved in the test that were lower compared to

these in the samples collected in summer (figure 4). In the case of this sample, the highest MR values equal to 43.16 were reached in the test conducted without the Aroclor-induced fraction in the presence of pollutants contained in 12.5 m^3 of air. The highest values of the mutagenicity ratio for the pollutants from 25 m^3 of air were obtained in the tests conducted with Aroclor- and Phenobarbital-induced fractions. They were 62.72 and 55.32, respectively. The lowest MR values, on the level of spontaneous mutations, were obtained at the concentration of 0.0485 m^3 of the air per plate.

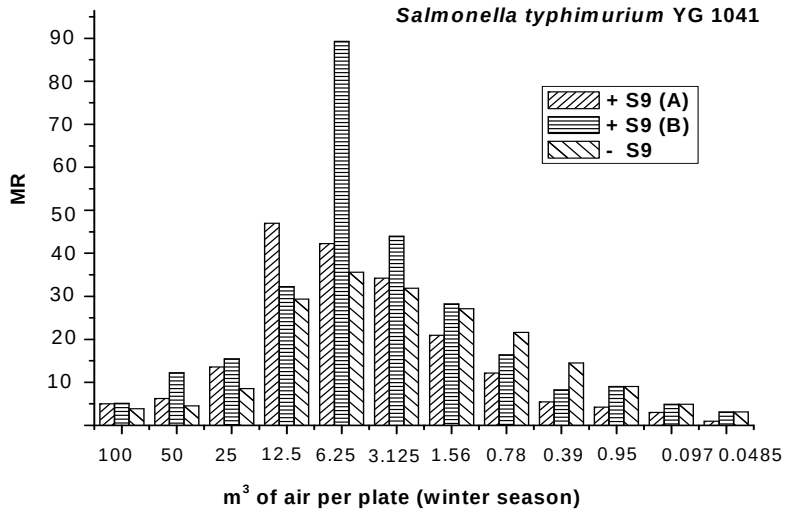

Fig. 3. MR values for the samples of the air collected during the winter season

In the strain TA 98, the MR values lower were compared with the strain YG 1041 (figure 2). The pollutants comprised in 100 m^3 of air were responsible for the highest MR values. The test on winter samples (figure 1) gave the MR of 26.88 without the Aroclor-induced fraction, whereas in the test with this fraction the MR was equal to 16.01. In the test with the Phenobarbital-induced fraction, MR reached 16.19. For the samples collected in the summer (figure 2), the MR equalled 6.81, 7.25 and 8.22 respectively. The lowest MR values, on the level of spontaneous mutations, were obtained at the volume of 0.195 m^3 of air per plate.

The MR values obtained for specific strains and activators of microsomal fraction demonstrate a large utility of the Phenobarbital-induced fraction in converting promutagens that constitute components of air pollutant particulates into active mutagens. The correlation ratio between the MR values obtained for the fractions A and B in the tests with the strain TA 98 was 0.97 for the winter sample, and 0.98 for the summer sample. The correlation ratios obtained in the tests with the strain YG 1041 were 0.83 and 0.90, respectively. This proves that Phenobarbital may be used as an alternative to Aroclor in inducing microsomal fraction.

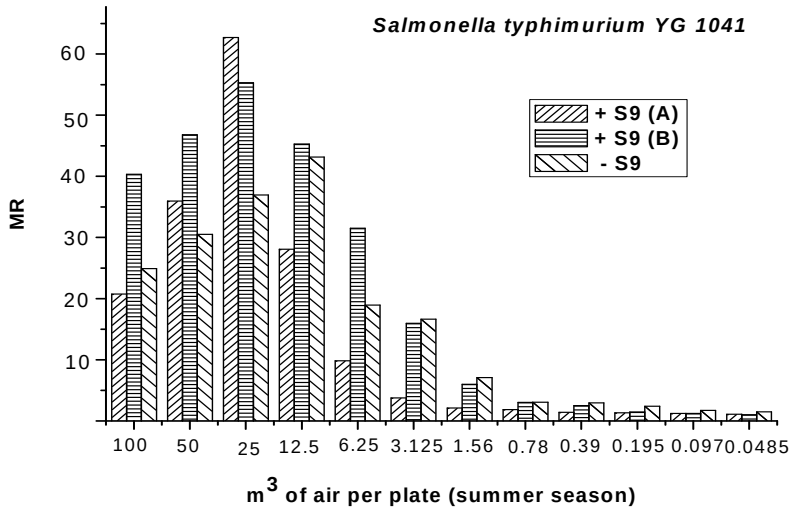

Fig. 4. MR values for the samples of the air collected during the summer season

4. SUMMARY

The pollutants in the samples collected in winter and in summer differ significantly in their concentration. The content of pollutants collected in winter constituted 132 percent compared with the samples collected in summer. Similar relationships were observed by other researchers [3]. These differences are due to an elevated emission of pollutants in urban agglomerations in the winter (house heating and automotive vehicles).

The results obtained indicate that a number of revertants (and thus the MR ratio) in the winter samples is higher than in the summer samples, which is consistent with the studies of other researchers [3], [8], [13]–[17]. A maximum number of revertants in specific tester strains were obtained at different concentrations of pollutants involved in the test, which testifies to their varied susceptibility to the compounds studied.

The lowest mutagenicity ratio (MR) values were obtained for pollutants present in air particulate extracts collected in summer, in the case of introducing the pollutant whose concentration corresponded to 0.0485 and 0.195 m^3 of air per plate with the strains YG 1041 and TA 98, respectively. The high mutagenicity ratios (MR) obtained both in the tests carried out with the microsomal fraction S9 and without it testify to the presence of the pollutants in the test samples, which might affect the genetic material indirectly (promutagens) and directly (direct mutagens). The highest MR values for the strain of *Salmonella typhimurium* YG 1041 were obtained in the tests carried out with the fraction S9 induced both with Aroclor and Phenobarbital. The strain was

then most susceptible to the promutagens present in the samples. Furthermore, very high MR values for this strain suggest the presence of nitro- and amino-PAH derivatives, as the strain shows a higher nitroreductase and O-acetyltransferase activity. In the case of the strain of *Salmonella typhimurium* TA 98, the highest MR values were obtained for the most concentrated samples, that is in the tests conducted for the sample collected in winter which were carried out without the microsomal fraction. This proves that the strain TA 98 responded strongly to direct mutagens.

The tests conducted with the fraction S-9 induced with Aroclor or Phenobarbital led to the similar values of mutagenicity ratio, which is demonstrated by high positive correlation indicators (0.83–0.97) between the MR values obtained for these both fractions. The results obtained may be the basis for replacing highly toxic Aroclor 1254 with Phenobarbital in order to produce the microsomal fraction that can interact with air particulate pollutants.

ACKNOWLEDGEMENTS

The research was done within the grant No. 3 PO4G07524 of the Ministry of Scientific Research and Information Technology.

REFERENCES

[1] FEILBERG A., NIELSEN T., BINDERUP M.L., SKOV H., POULSEN M.W.B., *Observations of the effect of atmospheric processes on the genotoxic potency of airborne particulte matter*, Atmospheric Environment, 2002, Vol. 36, 4617–4625.

[2] FOLINSBEE L., *Human health effects of air pollution*, Environ. Health Perspect., 1992, 100, 45.

[3] CLAXTON L.D., MATTHEWS P.P., WARREN S.H., *The genotoxicity of ambient outdoor air, a review: Salmonella mutagenicity*, Mutation Research, 2004, 567, 347–399.

[4] MORTELMANS K., ZEIGER E., *The Ames Salmonella/microsome mutagenicity test*, Mutation Research, 2000, 455, 29–60.

[5] ONG T., MUKHTAR M., WOLF C.R., ZEIGER E., *Differential effects of cytochrome P450- inducers on promutagen activation capabilities and enzymatic activities of S-9 from rat liver*, Journal of Environmental Pathology and Toxicology, 1980, 4, 55–65.

[6] ZEIGER E., ANDERSON B., HAWORTH S., LAWLOR T., MORTELMANS K., *Salmonella mutagenicity tests:* V. *Results from the testing of 311 chemicals*, Environ. Mol. Mutagen., 1992, 19(21), 2–141.

[7] KADO N.Y., WONG J.M., KUZMICKY P.A., WOODROW H.N., NING H., SEIBER J.N., HSIEH P.H., *Quantitative integration of the Salmonella microsuspension assay with supercritical fluid extraction of model airborne vapor-phase mutagens*, Mutation Research, 1992, 271, 253–260.

[8] KRISHNA G., NATH J., ONG T., *A simple method for extraction of mutagens from airborne particles*, Environm. Monit. Asses., 1985, 5, 393–398.

[9] MARON D.M., AMES B.N., *Revised methods for the Salmonella mutagenicity test*, Mutation Research, 1983, 113, 173–215.

[10] HAGIWARA Y., WATANABE M., ODA Y., SOFUNI T., NOHMI T., *Specificity and sensitivity of Salmonella typhimurium YG1041 and YG1042 strains possessing elevated levels of both nitroreductase and acetyltransferase activity*, Mutation Research, 1993, 291, 171–180.

[11] WATANABE M., ISHIDATE M., NOHMI T., *A sensitive method for detection of mutagenic nitroarenes: construction of nitroreductase-overproducing derivatives of Salmonella typhimurium strains TA 98 and TA 100*, Mutation Research, 1989, 216, 211–220.
[12] MEJBAUM-KATZENELLENBOGEN W., MOCHNACKA I., *Practical course of biochemistry* (in Polish), PWN, Warszawa, 1969.
[13] BINKOVA B., CERNA B., PASTORKOVA A., JELINEK R., BENES I., NOVAK J., SRAM R.J., *Biological activities of organic compounds adsorbed onto ambient air particles: comparison between the cities of Teplice and Prague during the summer–winter seasons 2000–2001*, Mutation Research, 2003, 525, 43–59.
[14] CASSONI F., BOCCHI C., MARTINO A., PINTO G., FONTANA F., BUSCHINI A., *The Salmonella mutagenicity of urban airborne particulate matter (PM 2,5) from eight sites of the Emilia-Romagna regional monitoring network (Italy)*, Science of the Total Environment, 2004, 324, 79–90.
[15] DUCAATTI A., VARGAS V.M.F., *Mutagenic activity of airborne particulate matter as an indicative measure of atmospheric pollution*, Mutation Research., 2003, 540, 67–77.
[16] KARPIŃSKA-SMULIKOWSKA J., PIEKARSKA K., MOSKAL J., CZARNY A., *Evaluation of air pollution by bioindication tests*, Acta Poloniae Toxicologica, 2000, Vol. 8, No. 1, 147–159.
[17] DU FOUR V.A., VAN LAREBEKE N., JANSSEN C.R., *Genotoxic and mutagenic activity of environmental air samples in Flanders, Belgium*, Mutation Research., 2004, 558, 155–167.

WPŁYW INDUKCJI FRAKCJI MIKROSOMALNEJ NA WYKRYWALNOŚĆ MUTAGENNYCH ZANIECZYSZCZEŃ POWIETRZA ATMOSFERYCZNEGO ZA POMOCĄ BAKTERYJNEGO TESTU AMESA

Stwierdzono aktywność mutagenną zanieczyszczeń zaadsorbowanych na cząsteczkach pyłu zawieszonego pobranego w sezonie letnim i zimowym w centrum Wrocławia. Badania prowadzono, używając krótkoterminowego testu bakteryjnego *Salmonella* (test Amesa). W badanych próbach stwierdzono obecność zanieczyszczeń mogących oddziaływać bezpośrednio i pośrednio z materiałem genetycznym. Aby metabolicznie aktywować mutageny pośrednie, użyto dwóch rodzajów wątrobowej frakcji mikrosomalnej aktywowanej powszechnie stosowanym Aroclorem 1254 oraz rzadko stosowanym w badaniach prób środowiskowych fenobarbitalem. Na podstawie badań porównawczych obu frakcji stwierdzono, że silnie toksyczny Aroclor 1254 można zastąpić mniej szkodliwym fenobarbitalem, co pozwoli otrzymać frakcję mikrosomalną aktywnie oddziałującą z pyłowymi zanieczyszczeniami powietrza.