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Mutagenicity of Air Pollutants Collected at Industrial, Urban-residential and Rural Areas

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Epidemiological studies suggest a relationship between air pollution and the incidence of human lung cancer (Hitosugi 1968). The organic matter derived from airborne particulates has been found to be carcinogenic in experimental animals, however, carcinogenic bioassays are time-consuming, complex and expensive (Hoffman and Wynder 1968).

Ames et al. (1975) have developed a rapid and sensitive in vitro screening method for detecting mutagens [the Salmonella/mammalian microsome mutagenicity test] and a good corelation between mutagenicity and carcinogenicity has been demonstrated (McCann et al. 1975). Using the bacterial assay system, many investigators have shown the mutagenicity of the extracts of airborne particulate matter (Pitts et al. 1977 and Teranishi et al. 1978). Although it was shown that airborne particulates collected in an industrial city possessed higher mutagenic potential than those in a residential area did (Tokiwa et al. 1977), the comparative data of mutagenicity of samples from different areas have been limited (Pitts et al. 1977).

We report in this paper results showing the difference in mutagenic activity of the organic fraction of ambient atomospheric particulates collected simultaneously at 3 different areas; an industrial, an urban-residential and a rural area.

MATERIALS AND METHODS

Sampling stations were located in 3 different areas in Hyogo Prefecture (Japan); an industrial area (Amagasaki), an urban-residential area (Kobe) and a rural area (Hamasaka). Amagasaki, with a population of about 0.5 million, is in the south-eastern part of Hyogo Pref. and the heavy chemical and steel industry are prospering. This city is one of the areas where air-pollution is heaviest in Japan and the pulmonary disease mortality rate is the highest in the Prefecture (ANNUAL REPORT ON SANITARY STATICS OF HYOGO PREFECTURE 1979). Kobe has a population of 1.4 million with heavy traffic and is an econosocial center of the Prefecture. Hamasaka, where motor vehicle traffic is low, is in the northern part of Hyogo, having a population of 12 thousand. Fishery and agriculture are prospering.

Sampling was simultaneously performed at 3 areas form Oct. 5 to Nov. 1 (the farmers' slack season) in 1978 to exclude a possible effect of agricultural chemicals on the mutagenicity of air-samples collected at the rural area. Airborne particulate matter was collected on a glass fiber filter (Toyoroshi, GB100R, 18 x 23 cm) by a high volume air sampler (Kimoto Electric Co., Ltd., Osaka, Japan) for 24 h. About 2000 $_m^3$ of air was pulled through each filter. Before and after sample collection, the filter was allowed to equilibrate at 55% humidity for 24 h and the weight of the total suspended particulate matter (TSP) was determined. The particulate-laden filter was placed in a Soxlet extractor and the organic matter was extracted with benzene for 8 h. The solution was evaporated to dryness by a rotary evaporator at 40°C and the weight of the residue (benzene-soluble fraction: BSF) was determined. BSF was dissolved in an appropriate amount of dimethyl sulfoxide (DMSO) to give a solution of 5 mg BSF/ml. All the samples had been stored frozen at -20°C prior to mutagenic assay.

The mutagenicity test with Salmonella typhimurium was performed as described by Ames et al. (1975). A tester strain used was TA98 because sensitive and reproducible results have been obtained (Dehnen et al. 1977). A 0.1 ml aliquot of the DMSO solution was added to a test tube containing 2 ml of top agar and 0.1 ml of a fresh overnight culture of the tester strain, then 0.5 ml of S9 mix or 0.1 M phosphate buffer (pH 7.4) was added. The contents were mixed and poured onto a minimal glucose agar plate. A 9000 x g supernatant (S9) was prepared from the liver of male rats treated with both phenobarbital and dibenzo(ah)anthracene (Teranishi et al. 1978).

RESULTS AND DISCUSSION

Concentration of the total suspended particulate matter (TSP) and of the benzene-soluble fraction (BSF) were determined. The result was shown in Table 1. The concentration range of TSP and BSF in air were 62.0 - 277.0 $\mu g/_m^3$ and 6.4 -35.6 $\mu g/_m^3$ in Amagasaki (an industrial area); 30.3 - 182.9 $\mu g/_m^3$ and 3.6 - 20.6 $\mu g/_m^3$ in Kobe (an urban-residential area); 29.2 - 75.4 $\mu g/_m^3$ and 1.6 - 5.4 $\mu g/_m^3$ in Hamasaka (a rural area), respectively. Mean values of TSP concentration in Amagasaki and in Kobe were 2.9 and 1.8 times higher than that in Hamasaka, and the similar, but more marked tendency was obtained for BSF concentration. BSF% in TSP in the rural area was significantly lower than those in the two urban areas. A comparison of TSP and BSF concentration between the different areas indicated that the atomospheric particulates in the urban areas (Amagasaki and Kobe) came mainly from human activities, i.e., combustion and industrial process, on the contrary, particulate matter of anthropogenic origin is not predominant in the rural area.

Mutagenic activities of the air samples were tested in the presence and absence of metabolic activation using Salmonella tester strain TA98. The result are tabulated as the mean number of revertant colonies per 500 µg BSF and per cu. meter air in Table 2. All the

Table 1. Concentration of TSP and BSF in air collected at 3 sampling stations in Hyogo Prefecture

Station (sample No.)	TSP/air ($\mu g/_m3$)	BSF/air ($\mu g/_m3$)	BSF/TSP (%)
Amagasaki(16)	125.00 ± 32.04*	15.14 ± 4.05*	12.11 ± 1.01*
Kobe (16)	80.32 ± 23.04*	8.93 ± 2.68*	11.16 ± 0.92#
Hamasaka (10)	42.53 ± 10.48*	2.69 ± 0.82*	7.34 ± 1.88*#

(Mean value ± 95% confidence limit)
(*,#) ; Significantly different by Student's t test ($P<0.05$)

Table 2. Mutagenic activity of air pollutants collected at the industrial, the urban-residential and the rural areas in Hyogo Prefecture

Station (area)	Sample No.	S9 mix	Revertant colonies per 500 ug BSF	per m^3 air
Amagasaki (industr.)	16	(-)	439.1 ± 63.7[a]	14.41 ± 5.35[d]
		(+)	623.1 ± 82.0[c]	20.42 ± 7.67[f]
Kobe (residen.)	16	(-)	478.9 ± 81.0[b]	9.41 ± 4.09[e]
		(+)	386.6 ± 90.1[c]	7.74 ± 3.77[f]
Hamasaka (rural)	10	(-)	210.5 ± 37.5[a,b]	1.22 ± 0.44[d,e]
		(+)	224.6 ± 59.3[c]	1.23 ± 0.47[f]

(Mean value ± 95% confidence limit)
(a-f) ; Significantly different by Student's t test ($P<0.05$)

samples from 3 areas exhibited mutagenicity both with and without S9 mix and the activity varied between consecutive days. Although meteorological parameters such as wind direction have not been evaluated, they may be a major reason for the day to day variations of the activity (Chrisp and Fisher 1980). The industrial air samples possessed the highest mutagenic activity, ranging from 6.0 to 59.0 revertants per cu. meter air without S9 mix. The lowest mutagenic activity was detected in the rural samples (0.4 - 2.3 rev./m^3 with S9 mix, 0.5 - 2.6 rev./m^3 without S9 mix). Mutagenic activity in the urban-residential samples ranged from 1.0 to 28.7 rev./m^3 with S9 mix and from 1.6 to 30.8 rev./m^3 without S9 mix.

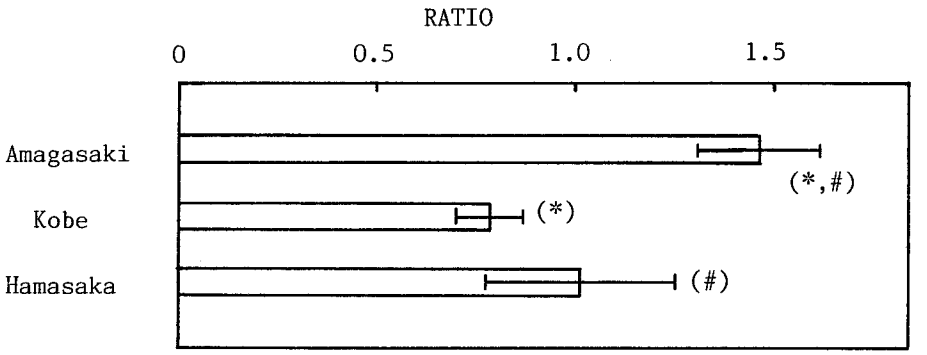

Fig. 1 Ratio of the mutagenic activity (S9[+]/S9[-])

(*,#) ; Significantly different by Student's t test (P 0.05)
The bars indicate the mean value ± 95% confidence limit.

Air samples in the industrial area possessed more than 10-fold greater mutagenic potential per cu. meter air with and without metabolic activation than that in the rural area, and several times greater than in the urban-residential area (Table 2). In addition, mutagenic activities per BSF of the samples from the rural area were about 1/3 - 1/2 of those from 2 urban areas, suggesting the quantitative and/or qualitative difference of the airborne mutagenic components between areas. Tokiwa et al. (1977) reported that the mutagenic activity in the air sample (methanol extracts) collected at an industrial city (Ohmuta, Japan) was approx. 3 times greater than that in a residential area (Fukuoka, Japan). Pitts et al. (1977) collected air particulates at rural and urban sites in California and showed that the sample collected from the rural site (elevation ca. 5000 ft.) was not mutagenic but all the materials from urban locations were mutagenic with and without S9 mix. These results clearly demonstrate that almost all the mutagenic compounds in the ambient atomosphere are originated from human activities and suggest that urban dwellers have a higher risk of lung cancer incidence than rural residents.

To evaluate the qualitative difference of airborne mutagens, the mutagenic activity of the samples in the presence of S9 mix was compared with that in the absence of S9 mix. An enhancement of the mutagenic activity by enzymatic activation was observed for the sample from the industrial area (Amagasaki) but not for those from the urban-residential (Kobe) and the rural area (Hamasaka) (Fig. 1). Talcott and Wei (1977) have reported that the mutagenic activity of air sample (acetone extracts) collected in Berkley, California, appeared to be lowered by the addition of S9 mix, on the contrary, the sample collected at a station located downwind from a steel mill (Buffalo, New York) was maximally active in the presence of S9 mix. Wang et al. (1978) and Ohnishi et al. (1980) indicated that automobile exhaust had more or less higher mutagenic activity

in the absence of S9 mix. These data suggest that the contribution of automobile exhaust to the mutagenicity of air particulates is great in the urban-residential and the rural areas in our study.

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REFERENCES

Ames BN, McCann J, Yamasaki E (1975) Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test. Mutation Res 31:347-363

Chrisp CE, Fisher GL (1980) Mutagenicity of airborne particles. Mutation Res 76:143-164

Dehnen W, Pitz N, Tomings R (1977) The mutagenicity of airborne particulate pollutants. Cancer Letters 4:5-12

Hitosugi M (1968) Epidemiological study of lung cancer with special reference to the effect of air pollution and smoking habits. Inst Public Health Bull 17:237-256

Hoffmann D, Wynder EL (1968) Chemical analysis and carcinogenic bioassays of organic particulate pollutants. In: Stern AC (ed) Air pollution Vol.2, Analysis, monitoring and surveying,2nd edn. Academic Press, New York, pp.187-242

McCann J, Choi E, Yamasaki E, Ames BN (1975) Detection of carcinogens as mutagens in the Salmonella/microsome test: assay of 300 chemicals. Proc Natl Acad Sci USA 72:5135-5139

Ohnishi Y, Kachi K, Sato K, Tahara I, Takahashi H, Tokiwa H (1980) Detection of mutagenic activity in automobile exhaust. Mutation Res 77:229-240

Pitts Jr. JN, Grosjean D, Mischke TM, Simmon VF, Poole D (1977) Mutagenic activity of airborne particulate organic pollutants. Toxicol Lett 1:65-70

Talcott R, Wei E (1977) Airborne mutagens bioassayed in Salmonella typhimurium. J Natl Cancer Inst 58:449-451

Teranishi K, Hamada K, Watanabe H (1978) Mutagenicity in Salmonella typhimurium mutants of the benzene-soluble organic matter derived from air-borne particulate matter and its five fractions. Mutation Res 56:273-280

Tokiwa H, Morita K, Takeyoshi H, Takahashi K, Ohnishi Y (1977) Detection of mutagenic activity in particulate air pollutants. Mutation Res 48:237-248

Wang YI Y, Rappaport SM, Sayer RF, Talcott RE, Wei ET (1978) Direct-acting mutagens in automobile exhaust. Cancer Letters 5:39-47

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