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Mutagenicity of Particulate Air Pollutants Collected around Tokyo, Japan

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Many industrial plants are located in and around Tokyo, the capital of one of the most heavily industrialized countries, Japan. The daily life of more than ten million people and the industrial activities in and around the city consume huge amounts of fossil fuels. It is known that burning of fossil fuels releases chemical mutagens and carcinogens. Thus, to investigate the present status of air pollution, particularly as regards chemical mutagens, and to obtain basic data for future monitoring of air pollution, particulate air pollutants were collected and assayed for mutagenicity with bacterial detection systems. The results are reported here.

MATERIALS AND METHODS

Chemicals. D-glucose-6-phosphate (G-6-P) and ATP were products of Sigma Chemical Co., St. Louis, Mo. NADH and NADPH were purchased from Oriental Yeast Co., Ltd., Tokyo and dimethylsulfoxide (DMSO), cyclohexane, methanol, and polychlorinated biphenyl (Tetra), from Wako Pure Chemical Industries Ltd.

Sampling of pollutants. Particulates were collected on glass fiber filters (GB-100, Toyo Roshi Kaisha Ltd., Tokyo) with a vacuum sampler (HCV-1000, Shibata Chemical App. Mfg. Co., Ltd., Tokyo). Eight samples were obtained from four points. Sample No. 1 was obtained from 1,180 m^3 of air on the west coast of the Izu Peninsula (Ashiho, Numazu, Shizuoka) on the 6th and 7th of January, 1979, and weighed 25 mg. Izu is about 130 km west of Tokyo. Sample No. 2 weighed 461 mg and was collected from 1,440 m^3 of air at a crossroad carrying heavy traffic, that is, the intersection of Setagaya Ave. and Fuchu Ave. (1600 Noborito, Tama, Kawasaki) on the 10th and 11th of January, 1979. Sample No. 3 weighed 164 mg and was taken from 1,440 m^3 of air within a factory site (Kotobuki Iron Works Co., Ltd., 3-1-3 Fujisaki, Kawasaki). This site is just separated from Tokyo by the Tama River. Sampling was carried out on the 11th and 12th of January, 1979. Sample No. 4 weighed 52 mg and was obtained from 1,440 m^3 of air in an office at Ishida Precision Machine Co., 2053 Nakanoshima, Tama, Kawasaki. Sample No. 4 was collected on the 13th and 14th of January, 1979, and no kerosine stove was in operation. Sample No. 5 weighed 146 mg and was obtained while a kerosine stove was on (Corona Stove, Uchida Seisakusho, Ltd., Niigata); 3.5 L of kerosine were consumed during the 24 h sampling period. The other conditions were the same as for No. 4. The following three samples were obtained at the

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same place as No. 4 and No. 5. No. 6 weighed 66 mg and was obtained before using a kerosine stove on the 23rd and 24th of February, 1979. No. 7 weighed 49 mg and was collected on the 27th and 28th of the same month after using the stove. No. 8 weighed 68 mg and was collected during heating with the stove on the 1st and 2nd of March, 1979. Each of the three samples was obtained from 1,440 m^3 of air.

Extraction of mutagens. Particulate samples were extracted with 250 mL of methanol in Soxhlet extractors for 16 h. Residues after methanol extraction were subjected to further extraction with 250 mL of cyclohexane by the same procedure. Extracts were concentrated to dryness and dissolved in DMSO.

Mutagenicity tests. *Salmonella typhimurium* TA 100 and TA 98 were kindly supplied by Dr. V. Simmon of SRI International. S-9 was prepared from livers of Sprague Dawley rats by the method of AMES et al. (1975). The composition of S-9 Mix followed that of NAGAO et al. (1977); briefly, the mix consisted of S-9 plus NADPH, NADH, ATP, and G-6-P. Testing was carried out as described by McCANN et al. (1975). Duplicate plates were made at each dose level, and the average number of colonies was obtained.

RESULTS AND DISCUSSION

The results of mutagenicity tests using *S. typhimurium* TA 98 are shown in Fig. 1 and Fig. 2 on the basis of sample weight. The number of revertants in the control was subtracted from each value, so that the net increase of revertants is shown in the figures; negative values are depicted as unity. Regardless of the presence of S-9 Mix, all samples showed mutagenicity. However, their activities were enhanced by the addition of S-9 Mix (Fig. 2). Except for No. 4, which showed unusually high activation, as will be discussed later, the mutagenic activities were by and large similar to each other. For comparison, Fig. 1 and Fig. 2 are replotted in Fig. 3 and Fig. 4 on the basis of volume of air. As expected from their similar specific activities on the basis of weight, with the exception of No. 4 (Fig. 2), the volume specific activities largely depended on the quantity of pollutants per unit volume. The quantity decreased in the following order: No. 2, No. 3, No. 5 and No. 1, while the mutagenic activity decreased in the order: No. 2, No. 5, No. 3 and No. 1. The inversion of No. 3 and No. 5 might be attributable to the production of a potent mutagen by the kerosine stove (No. 5) or to some ingredient of the heavy contamination in the sample from the iron works (No. 3).

Since no kerosine stove was used during sampling No. 4, reduced mutagenic activity compared to No. 5 was expected. This was so for the experiments without S-9 Mix (Fig. 1 and 3), but inversion of the order of activity between No. 4 and No. 5 was observed on addition of S-9 Mix (Figs. 2 and 4). Sample No. 4 was collected first and then No. 5 was collected 10 days later. As the floor was carpeted with a synthetic fiber material, it is possible that potent mutagens absorbed and accumulated on the carpet might have

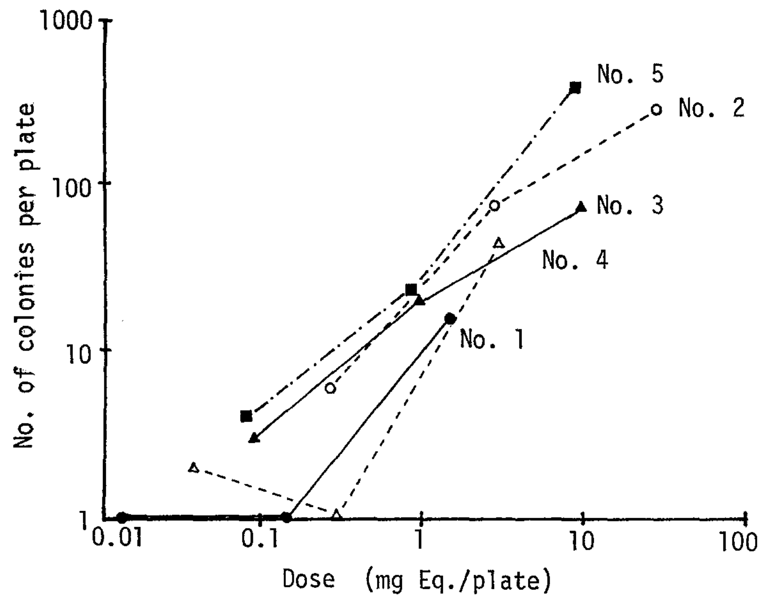

Fig. 1. Mutagenicity of 5 Samples Tested with S.t.TA 98 in the Absence of S-9 Mix

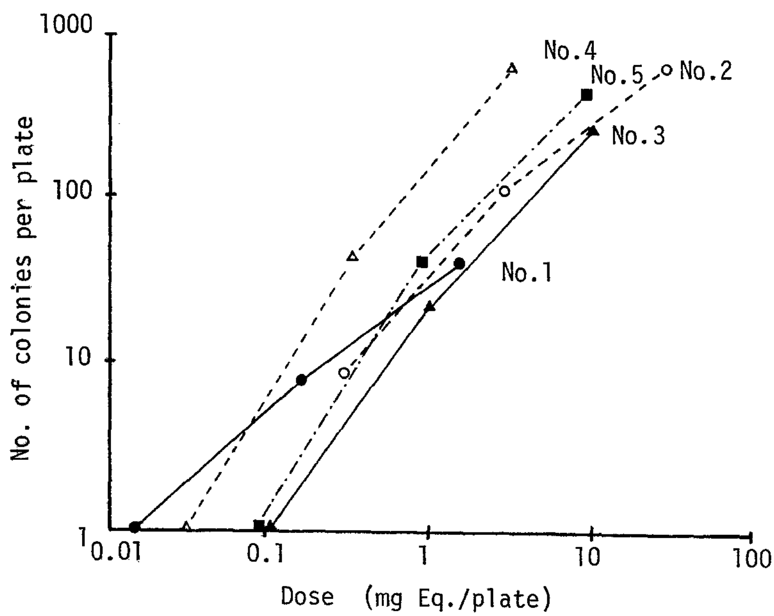

Fig. 2. Mutagenicity of 5 Samples Tested with S.t.TA 98 in the Presence of S-9 Mix

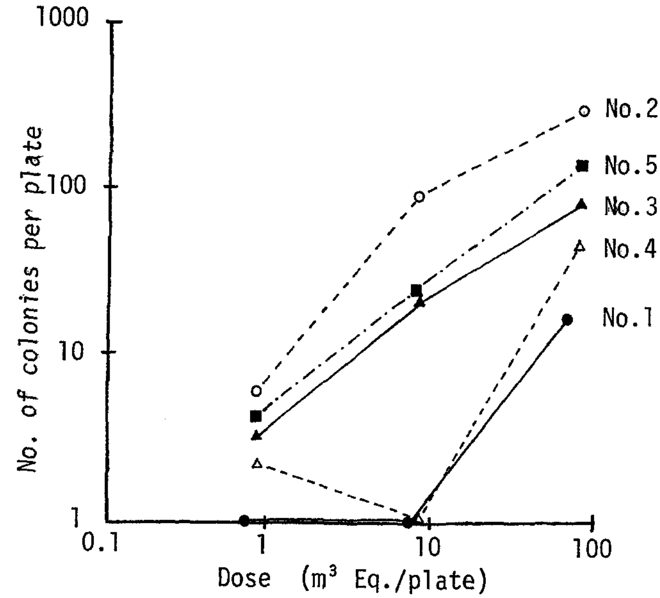

Fig. 4. Mutagenicity of 5 Samples Expressed on the Basis of Air Volume, Converted from Fig. 2.

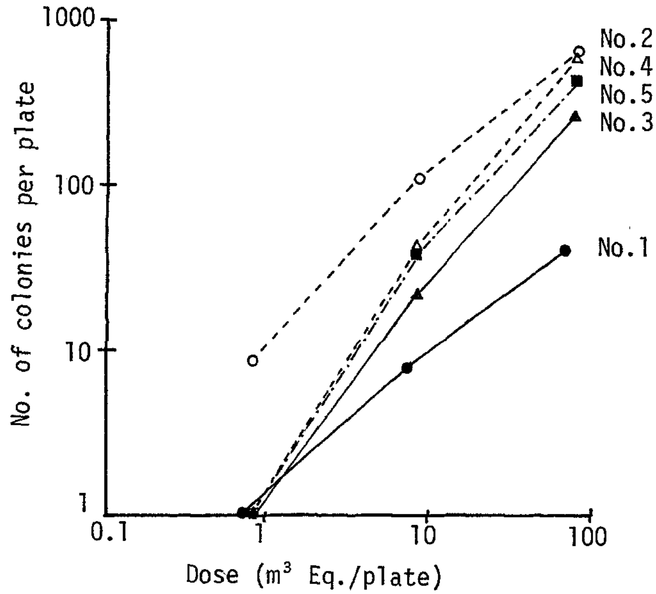

Fig. 3. Mutagenicity of 5 Samples Expressed on the Basis of Air Volume, Converted from Fig. 1.

TABLE 1

Mutagenicity of Samples No. 6, No. 7 and No. 8

Samples	Volume of air (m^3)	Amount of Sample obtained (mg)	mg Eq. per plate	m^3 Eq. per plate	No. of colonies per plate	Colonies per mg Eq.	mean**	Colonies per m^3 Eq.	mean**
No. 6	1,440	65.6	3.28	72.0	133	41	42	1.8	1.9
			0.66	14.4	28	43		1.9	
			0.13	2.9	2	15		0.7	
No. 7	1,440	48.4	2.42	72.0	134	55	74	1.9	2.5
			0.48	14.4	45	92		3.1	
			0.10	2.9	7	72		2.4	
No. 8	1,440	68.2	3.41	72.0	1,425	418	447	19.8	21.2
			0.68	14.4	325	477		22.6	
			0.14	2.9	149	1,104		51.7	

* Mean of duplicate plates after subtracting the control value (39 colonies per plate).

** The lowest dose level was omitted because of high fluctuation.

been collected in No. 4. Thus, another experiment was designed: Sample No. 6 was taken before burning fuel, No. 7 after burning, and No. 8 during burning. The experimental results are shown in Table 1. No. 8 showed extremely high mutagenic activity, compared with No. 6 and No. 7. This indicates that burning kerosine in a stove produces mutagens. No. 7 showed a slightly higher specific activity than No. 6, possibly due to residual mutagens from the burning kerosine. The results appear to support our hypothesis regarding the higher activity of No. 4 compared to No. 5. Experiments with No. 6, No. 7 and No. 8 showed little or no mutagenicity in the detection system of S. typhimurium TA 100 with or without S-9 Mix as well as in that of TA 98 without S-9 Mix.

Samples from No. 1 to No. 5 induced revertants above the control level in an S. typhimurium TA 100 system in the presence of S-9 Mix. However, the numbers of the colonies did not exceed twice the control value. None of the cyclohexane extracts from these five samples showed mutagenicity in the detection system of S. typhimurium TA 98 with or without S-9 Mix, showing that most mutagens able to induce frame shift mutations had been extracted with methanol.

No. 8 showed higher specific activity than No. 5. This might be attributable to possible differences in the condition of the kerosine-burning stove. No. 8 consisted of a smaller amount of material with higher activity compared to No. 5.

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