Mutagenicity of Suspended Particulate Matter Divided in Three Sizes Indoors

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To clarify indoor air pollution, indoor and outdoor air samples were collected from 22 houses in Tokyo using a low-volume cascade impactor with a quartz fiber filter. Suspended particulate matter (SPM) was classified into three sizes: ≥ 10 μm, 2.5–10 μm and ≤ 2.5 μm. The mutagenic compounds were extracted by the dichloromethane/sonication method. The solution was concentrated by N_2 gas. Mutagenicity was determined by the microsuspension method, employing the *Salmonella typhimurium* (*S. typhimurium*) YG 1024 strain in the presence and absence of S9 mix. The samples showed generally higher mutagenicity in the absence of S9 mix than in its presence. Outdoor air tended to have higher or similar mutagenicity to indoor air. The smallest SPM (≤ 2.5 μm) fraction showed the highest mutagenicity (revertants/m^3· air). These results suggest that one of the main sources of high mutagenic SPM indoors is air entering from outdoors.

Key words —— suspended particulate matter, cascade impactor, mutagenicity

INTRODUCTION

Since much of daily life is spent indoors, the extent of indoor air pollution by carcinogens and mutagens should be evaluated. Indoor pollution of suspended particulate matter (SPM) by polycyclic aromatic hydrocarbons (PAH) and mutagens has been reported to be due to air entering from the outside,[1] smoking indoors,[2] cooking such as grilling of meat and fish,[3] heating by using kerosene heaters,[4,5] and burning of incense sticks.[6] SPM contains trace amounts of various carcinogens and mutagens. Various SPM are present in the air, and particles with smaller diameters contain larger amounts of carcinogens and mutagens, and their rate of deposition in the alveoli and peripheral bronchi generally increases with decreasing particle size.[7]

Therefore, it is important to evaluate environmental carcinogens and mutagens in collected SPM by particle diameter in order to clarify the causes of lung cancer and genetic diseases due to long-term SPM exposure, and to prevent injury to health.

In this study, we planned to measure the mutagenicity of the SPM, which were classified into 3 sizes. However, it is a problem that there are very few amounts of samples. Therefore, it was necessary to use highly sensitive mutagenicity test such as microsuspension method, which was developed by Kado *et al*. In this method, the concentration of tester strain at preincubation period is about 30 times higher than that of the Ames method and the preincubation method. Moreover, preincubation time is prolonged, and total volume is also reduced. As for the tester strain (YG1024) used this time, the acetyltransferase quantity productivity plasmid (pYG219) is introduced into the TA98 strain. The YG1024 strain shows high sensitivity to amines, nitroarenes,[8] as well as environmental samples such as SPM.[9] It is well known that this strain has excellent ability to detect the mutagenicity of SPM, so we used the strain in this study.

To clarify the status of air pollution by mutagens in the metropolitan area, we collected indoor and outdoor air samples from houses in the 22 wards of Tokyo using low-volume, small-size cascade impac-

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Table 1. Sampling Conditions of Indoor Air

Sample code	Group	Sampling date	Weather[1] Start	Weather[1] End	Distance from road	House Style[2]	Sampling point	No. of cigarettes/day[3]	Heating[4]	Cooking[5]	Ventilation[6]
A	I	09/11/00	C	C	< 1 km	a	Living	5	—	+	—
B	I	11/28/00	C	F	< 50 m	a	Living	2	AC	+	—
C	I	11/09/00	C	C	< 200 m	a	Living	1	—	+	++
D	I	10/13/00	C	C	facing the street	a	Living	5	AC	+	++
E	I	10/20/00	C	R	< 50 m	a	Living	20	—	—	—
F	I	09/21/00	F	F	facing the street	a	Living	10	AC	—	—
G	I	12/05/00	C	C	< 200 m	a	Living	10	AC	—	—
H	I	09/24/00	C	F	< 1 km	b	Living	5	—	—	—
I	II	08/26/00	F	F	< 1 km	c	Kitchen	—	—	+	+++
J	II	09/25/00	R	R	facing the street	b	Kitchen	—	—	+	+++
K	II	10/28/00	R	R	facing the street	a	Living	—	—	+	+++
L	II	10/29/00	F	F	< 1 km	c	Living	—	AC	+	+++
M	II	12/09/00	F	F	< 1 km	a	Living	—	—	+	++
N	II	12/11/00	F	F	< 200 m	a	Living	—	KH	+	++
O-1	III	09/03/00	F	F	facing the street	a	Kitchen	—	—	—	—
O-2	III	12/22/00	F	F	facing the street	a	Kitchen	—	—	—	—
P	III	09/07/00	C	C	< 200 m	c	Living	—	—	—	—
Q	III	09/17/00	C	F	< 50 m	c	Living	—	—	—	—
R	III	10/17/00	C	C	< 50 m	a	Living	—	—	—	—
S	III	10/23/00	C	C	< 1 km	a	Kitchen	—	—	—	—
T	III	10/29/00	R	R	< 1 km	d	Living	—	—	—	—
U	III	11/27/00	F	F	< 200 m	d	Kitchen	—	EH	—	+
V	III	12/21/00	F	F	< 1 km	c	Living	—	—	—	—

1: C) cloudy, F) fine, R) rain, 2: a) reinforcing apartment, b) reinforcing detached house, c) wooden detached house, d) wooden apartment, 3: number of cigarettes smoked per day in sampling room, 4: AC: air conditioner, KH: kerosene heater, EH: electric heater 5: +: cooked during sampling period, —: no cooking, 6: +: ventilated 1 time during sampling period, ++: 2 times, +++: 3 times or more.

tors[10] and measured the mutagenicity of their extracts obtained with a solvent. We also investigated the factors associated with indoor and outdoor pollution.

MATERIALS AND METHODS

SPM Sampling ——With the cooperation of 22 inhabitants in the 22 wards of Tokyo, we collected indoor and outdoor air samples using low-flow cascade impactors (Tokyo Dyrec, Shinjuku Tokyo, Japan). Using this impactor, SPM were classified into three particle diameters ($\geq 10\ \mu$m, 2.5–10 μm, $\leq 2.5\ \mu$m) with a 50% cut-off value, and collected on a quartz fiber filter (Pallflex, 2500QAT) at a flow volume of 3 l/min for 24 hr.[10] Before sampling, filters were washed with dichloromethane (DCM, Wako Pure Chemical Industries, Ltd., Japan) and dried. The sampling period was August to December, 2000. In addition, indoor and outdoor factors (cooking, smoking, heating, ventilation, weather, and traffic in the neighborhood) were investigated. Table 1 shows the results. The filters after sampling were folded in two with the sampling side facing inward, light-shielded with aluminum foil, placed in zip-lock plastic bags, and stored in a freezer (–80ºC) until extraction.

Preparation of Samples —— Each filter after sampling was cut into 4 or 5 pieces and placed in test tubes. After addition of 10 ml DCM, organic components were extracted by 15-min sonication, followed by deaeration/mixture and 15-min sonication again. Of the extract, 8 ml was filtered using filter paper (ADVANTEC Toyo No. 5C), and the solvent was removed under an N_2 gas flow. The obtained extracts were stored in a refrigerator (–30ºC) until the mutagenicity tests.

Mutagenicity Tests —— Mutagenicity was measured by the microsuspension method described by Kado *et al.*,[11] which is the same as the Ames method but with increased sensitivity.[12] Tests were performed in both the presence and absence of metabolic active enzymes (S9 mix) prepared from rat liver

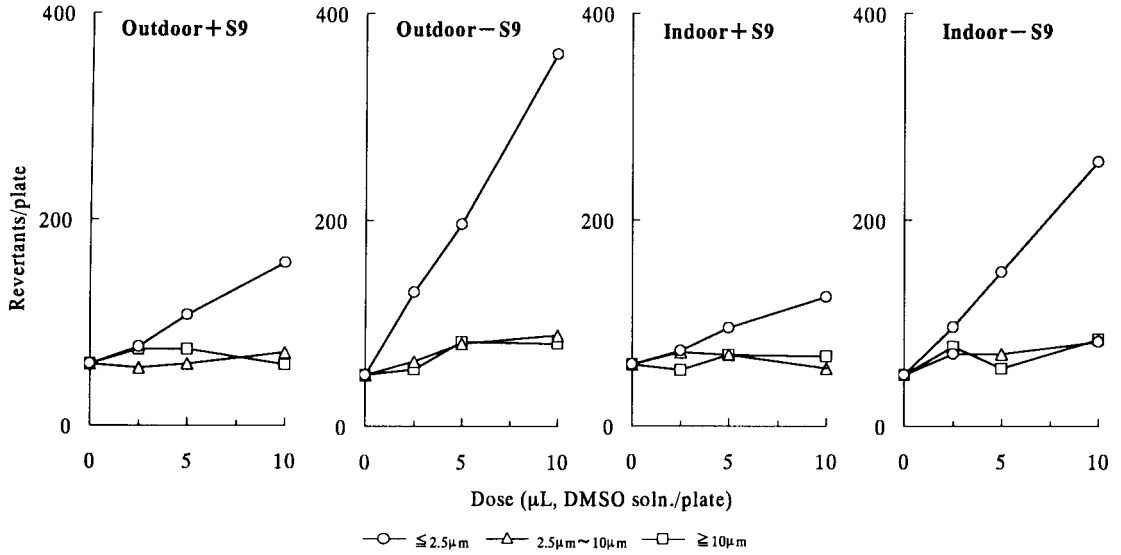

Fig. 1. Examples of Results of Mutagenicity Test for Extracts from Indoor SPM Collected at T

homogenate using *Salmonella typhimurium* (*S. typhimurium*) YG 1024 strain.[13] Extract samples were re-dissolved in 100 μl DMSO, and 10, 5, and 2.5 μl were placed in sterile test tubes and subjected to tests. Mutagenicity was regarded as positive when there was a concentration-response relationship between the sample dose and the number of revertant colonies that appeared on the plate (response), and the number of revertant colonies was twice the solvent control value or more. Mutagenicity was regarded as false-positive when the number of revertant colonies was 1.5–2 times the control value and as negative [not detected (n.d.)] when the number of revertant colonies was less than 1.5 times the control value. For positive and false-positive samples, a linear regression equation was obtained from the linear portion of the concentration-response relationship by the least squares method, and the specific mutagenic activity (revertants/m^3) was obtained from its slope.

RESULTS

Figure 1 shows the results of the mutagenicity tests. Though differences were observed between the presence or absence of S9 mix and among the particle diameters, a relatively good concentration-response relationship was observed. This suggested sensitive detection of SPM mutagenicity according to the particle diameter.

Therefore, the specific mutagenic activity of each SPM sample collected inside and outside the house according to the particle diameter and the total value in all samples in each particle diameter category were calculated. Mutagenicity was observed in most SPM samples obtained from the investigated houses. Of the 22 houses, only two samples (N and O-1) showed no mutagenicity in any SPM sample irrespective of indoor/outdoor samples, particle diameter, or presence or absence of S9 mix.

Figures 2 and 3 show specific mutagenic activity according to the particle diameter. Here, based on records written by the participants at the time of SPM sampling, the houses were classified into those with smokers (Group I: A-H), those without smokers where cooking was performed during sampling (Group II, I-N), and those without smokers where no cooking was performed during sampling (Group III, O-V).

High mutagenicity was often observed in the absence of S9 mix. As shown in Fig. 3, when the indoor air and outdoor air were compared, the mutagenicity (without S9 mix) in the indoor air was higher than or similar to that in the outdoor air in six of the eight houses in Groups I and 2 of the six houses in Group II. However, in Group III, the outdoor air tended to show higher mutagenicity in all houses except O-1 that showed no mutagenicity in both indoor and outdoor samples.

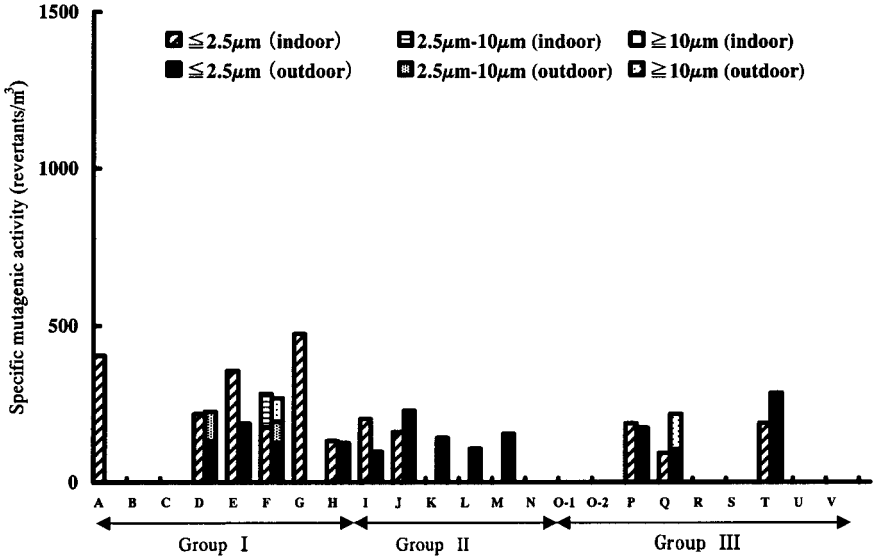

Fig. 2. Specific Mutagenic Activity (+S9 mix) of the Three SPM Fractions Collected Indoor and Outdoor

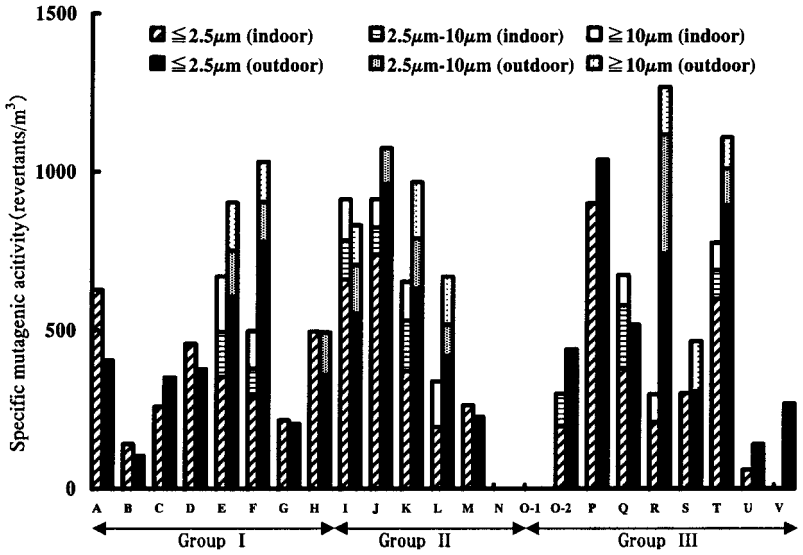

Fig. 3. Specific Mutagenic Activity (–S9 mix) of the Three SPM Fractions Collected Indoor and Outdoor

DISCUSSION

In Group III, it is possible that the mutagenicity of the indoor air increased with that of the outdoor air. This suggests that outdoor air influences indoor air pollution, when there are no other indoor pollution factors such as smoking and cooking.[1)]

At seven sites of Group III, outdoor air exhibited higher mutagenicity than indoor air. Smoking is well known to be associated with indoor air pollution, and the YG1024 strain easily detected such mutagenicity.[2,9)] On the other hand, in Group I, two (D and E) of the four sites (smoked and no cooking, D–G) showed similar or higher mutagenicity than indoor air. These results also suggested that smoking might be associated with indoor air pollution by mutagens. Further investigation in areas with clean outdoor air is required. In this study, we did not obtain detailed records of the contents of cooking or ventilation during cooking and thus did not examine the influence of cooking on indoor air pollution. However, in Group II, SPM collected in two kitchens (I and J) showed higher mutagenicity than that collected in the living room (K and M). Therefore,

the influence of indoor cooking can not be excluded. Since some studies have also suggested that the grilling of meat and fish may be a cause of indoor air pollution, more detailed investigation including evaluation of the contents and time of cooking and ventilation during cooking is necessary.

In conclusions, the mutagenicity of the SPM samples was generally higher in the absence of S9 mix than in its presence, in outdoor SPM samples than in indoor SPM samples, and for a small particle diameter (≤ 2.5 μm). Smoking was a factor causing indoor air pollution. When there was no major indoor pollution source such as smoking and cooking, it was suggested that outdoor air was the source of indoor air pollution. The higher mutagenicity in the absence of S9 mix observed in this study may be associated with the characteristics of the YG1024 strain used to obtain high mutagenicity detection ability. This strain may have been affected by nitroarene that shows high mutagenicity in the absence of S9 mix,[11] which reduced the mutagenicity of this strain. Therefore, highly sensitive detection methods using strains without such a bias should be developed in the future.

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