Mutagenicity of Size-Fractioned Airborne Particles Collected with Andersen Low Pressure Impactor

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Mutagenicity of size-fractioned airborne particles collected with a multi-stage fractioned sampler, an Andersen low pressure impactor, was measured by microsuspension assay using Salmonella typhimurium YG1024 strain (× 20 conc. bacterial solution). Fine particle samples showed mutagenicity with a good dose–response relationship under the conditions of both with and without a metabolic activation system (S9 mix). In generally, the activities without S9 mix were higher than those with S9 mix, and the highest activity per unit air volume was observed in the sample of 0.52 μm in diameter. Most of the negatives were observed in coarse particle samples. The size distribution of mutagenic activity per unit air volume was skewed to the smaller size range and had one peak at 0.52 μm, although that of mass concentration was bimodal, having two peaks bounded around 1–2 μm in diameter. The highest mutagenic activity per unit mass of particles in a series of fractioned samples was observed in the sample of less than 0.22 μm in diameter. It was suggested that ultrafine particles were more mutagenic than fine particles (PM 2.5–0.1), as if the mass concentrations of ultrafine particles were 10% or less than those of fine particles. It was also shown the Andersen low pressure impactor is very useful for studies of carcinogens and mutagens in suspended particles in ambient air.

Key words —— Andersen low pressure impactor, fine particles, mutagenicity

INTRODUCTION

Ambient air contains various chemical substances, some of which are carcinogens and/or mutagens. Humans continually take these chemical substances into the body by breathing, raising concerns over chronic effects such as lung cancer caused by long-term exposure to such substances. Of these chemical substances, polycyclic aromatic hydrocarbons and nitroarenes, many of which are carcinogens and/or mutagens, are known to exist in airborne particles. Therefore, it is very important when considering countermeasures against lung cancer to clarify the actual existence of and exposure to carcinogens and mutagens in suspended particles in ambient air. Recent epidemiological reports have shown that associations exist between mortality due to respiratory diseases and air pollution, especially fine particles such as PM 2.5.[1–3] It is therefore very important to evaluate the biological effects of fine particles in ambient air by using bioassays such as mutagenicity tests. Although the mutagenicity of different size fractions of airborne particles has been reported, most of such studies collected the particles using Andersen high volume samplers, dichotomous samplers, and so on.[4–7] Very few experimental studies have been reported on the mutagenicity of multi-stage fractioned fine particles of less than 1 μm in diameter because of the lack of suitable sampling devices and the sensitivity of the mutagenicity test.[8] Recently, a multi-stage fractioned sampler, the Andersen low pressure impactor (ALPI), which is able to collect ultrafine particles of less than 0.43 μm in diameter at lower pressure than the former Andersen sampler, has been developed.[9] Also, highly sensitive methods, such as a modified Salmonella liquid incubation assay and newly developed tester strains that are highly sensitive to environmental amino and nitro mutagenic groups, have been reported.[10–12]

In the present paper, the mutagenicity of size-fractioned airborne particles collected with ALPI was

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measured by using a combination of sensitive methods, microsuspension assay and Salmonella YG1024 strain.

MATERIALS AND METHODS

Reagents ——

Solvents: Pesticide residue analysis grade dichloromethane (DCM; Kokusan Chemical Works Ltd., Japan) and fluorometric analysis grade dimethylsulfoxide (DMSO; Dojin Chemical Laboratory Ltd., Japan) were used.

Metabolic Activation System: S9/Cofactor A set for Ames test (S9 mix; Oriental Yeast Co. Ltd., Japan) was used.

Particle Collection with ALPI —— Particles were collected on the rooftop (6F) of the National Institute of Public Health, Shirokanedai 4–6–1, Minato-ku, Tokyo in the winter of 2001 using an ALPI sampler (LP-20, Tokyo Dylec Corp., Japan). Five series of particle samples (I, II, III, IV and V) were collected from November 19 to December 24, each fractioned into 13 stages (> 12.1, 8.5, 5.7, 3.9, 2.5, 1.25, 0.76, 0.52, 0.33, 0.22, 0.13, 0.06, and < 0.06 μm in 50% cut-off diameter). Each sample was collected over 7 days at the flow rate of 20 l/min on a fluorocarbon-coated glass fiber filter (T60A20, φ80 mm, Pallflex Products Corp., U.S.A., purchased from Tokyo Dylec Corp.). The filters were previously washed with DCM. No mutagenicity has been observed in blank filter. After sampling, the filters were weighed using an analytical balance (Mettler-Toledo AT 201) with 10 μg reading precision and stored at –20°C in darkness until extraction.

Extraction of Organic Substances —— Each filter was cut into small pieces and put into a centrifuge tube. 10 ml of DCM were added, then the samples were extracted by sonication for 10 min. The solutions were centrifuged at 3000 rpm for 10 min and 4 ml of supernatant was filtered with filter paper (No. 5C, Advantec Corp., Tokyo, Japan). After filtration, the solution was evaporated under a mild nitrogen stream. The organic extracts were stored at –80°C until tested.

Mutagenicity Test —— The mutagenicity test was conducted by a microsuspension procedure which was a slight modification of Kado's method[10,12] using Salmonella typhimurium YG1024 strain[11] (× 20 conc. bacterial solution) under the conditions of both with and without a metabolic activation system (S9 mix). The extracts were dissolved in DMSO and put into test tubes with 10, 5, and 2.5 μl in duplicates for each dose. After adding 100 μl of 0.1 M sodium phosphate buffer or S9 mix, and 100 μl of concentrated bacteria, the tubes were capped and preincubated at 37°C with shaking. After 90 min of preincubation, the tubes were removed and 2.5 ml of molten top agar containing both 0.5 mM biotin and 0.5 mM histidine were added. The molten suspensions were immediately mixed with a Vortex mixer and poured onto minimal glucose plates. The plates were incubated at 37°C in the dark for 48 hr and were counted using an automatic laser colony analyzer (Spiral System Instruments Inc., Model 500A).

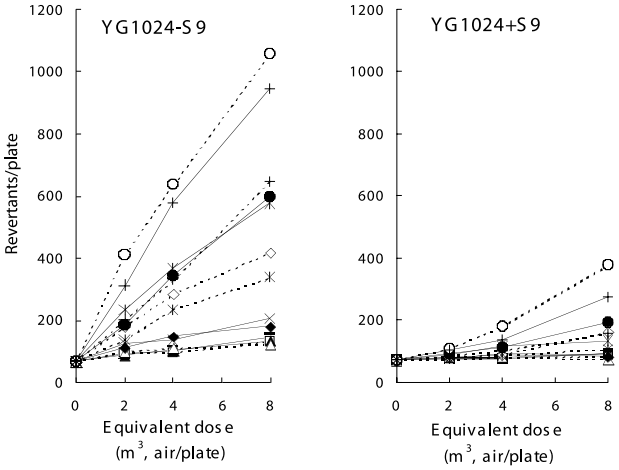

Fig. 1. Dose–response Curves of a Series of Size-Fractioned Particles Collected by ALPI
—■— > 12.1, ---□-- 8.5, —▲— 5.7, --△-- 3.9, —◆— 2.5, --◇-- 1.25, —●— 0.76, --○-- 0.52, —|— 0.33, ---+--- 0.22, —*— 0.13, --*-- 0.06, —×— 0.06 >.

RESULTS AND DISCUSSION

Dose–response curves of the microsupension assay of a series of fractioned particles collected by ALPI are illustrated in Fig. 1 using sample (II) in Table 1 which has intermediate mass concentration among 5 samples. In Fig. 1, the sample dose as equivalent air volume is plotted on the horizontal axis, and revertants per plate on the vertical axis. As illustrated in Fig. 1, most of the samples show a good dose–response relationship of mutagenicity under the conditions of both with and without S9 mix and it is possible to quantitatively evaluate the activity. In generally, the activities without S9 mix were higher than those with S9 mix, and the highest activity per unit air volume was observed in the sample of

Table 1. Particle Concentration and Mutagenic Activity of Andersen Low Pressure Impactor Samples

Sample	Particle size range (μm)	Particle concentration (μg/m^3)	Mutagenic activity			
			Rev./m^3, air		Rev./mg, particle	
			−S9	+S9	−S9	+S9
(I)	> 12.1	3.16	17.23	0.00	5456	0
11/19–26	8.5–12.1	2.66	12.87	0.00	4843	0
199.5 m^3	5.7–8.5	5.21	14.35	0.00	2752	0
	3.9–5.7	5.26	21.10	4.56*	4008	866
	2.5–3.9	4.86	91.82	16.29	18884	3351
	1.25–2.5	7.52	53.70	20.65	7142	2747
	0.76–1.25	22.76	181.98	93.63	7997	4114
	0.52–0.76	10.58	150.18	61.54	14200	5819
	0.33–0.52	5.61	122.70	41.78	21857	7443
	0.22–0.33	2.16	80.01	20.28	37122	9408
	0.13–0.22	1.75	67.28	12.79	38349	7289
	0.06–0.13	0.70	39.67	4.70*	56536	6698
	0.06 >	0.50	16.80	0.00	33515	0
(II)	> 12.1	4.51	9.50	0.00	2106	0
11/26–12/3	8.5–12.1	2.96	7.76*	0.00	2623	0
199.5 m^3	5.7–8.5	4.71	8.43	0.00	1789	0
	3.9–5.7	4.41	6.92*	0.00	1570	0
	2.5–3.9	3.61	14.52	0.00	4023	0
	1.25–2.5	5.21	44.57	10.79	8551	2071
	0.76–1.25	5.06	66.71	14.93	13180	2950
	0.52–0.76	7.37	124.21	37.89	16861	5143
	0.33–0.52	3.36	111.18	24.61	33111	7330
	0.22–0.33	2.05	71.89	10.56	34989	5140
	0.13–0.22	1.70	64.10	8.28*	37620	4860
	0.06–0.13	1.00	34.53	4.55*	34455	4536
	0.06 >	0.65	16.67	0.00	25590	0
(III)	> 12.1	3.06	4.13*	0.00	1351	0
12/3–10	8.5–12.1	2.31	5.03*	0.00	2178	0
199.4 m^3	5.7–8.5	3.71	7.52	0.00	2027	0
	3.9–5.7	3.46	11.12	2.92*	3214	843
	2.5–3.9	3.31	12.12	2.88*	3660	870
	1.25–2.5	4.36	19.67	3.49*	4508	800
	0.76–1.25	7.87	45.55	14.38	5785	1826
	0.52–0.76	10.28	89.21	35.63	8677	3465
	0.33–0.52	5.12	65.34	17.22	12773	3367
	0.22–0.33	1.76	20.85	5.48*	11880	3119
	0.13–0.22	2.21	35.48	9.90	16077	4484
	0.06–0.13	1.30	38.49	4.62*	29513	3541
	0.06 >	0.85	20.34	3.83*	23856	4493

*: pseudopositive.

0.52 μm in diameter. The coarse particle samples such as > 12.1 μm were weakly or not mutagenic.

Mass concentration (μg per cubic meter) and mutagenic activities (revertants per unit air volume; rev./m^3 and per unit mass of particles; rev./mg) are shown in Table 1. Mutagenic activity was calculated from the slope of the linear portion of the dose–response curve using the statistical model of least squares linear regression. Negative data was described as 0. As shown in Table 1, most of the samples were mutagenic. In the condition without S9 mix, among 65 samples, 55 were clearly posi-

Table 1. Continued

Sample	Particle size range (μm)	Particle concentration ($\mu g/m^3$)	Mutagenic activity			
			Rev./m^3, air		Rev./mg, particle	
			−S9	+S9	−S9	+S9
(IV)	> 12.1	4.74	2.89*	0.00	609	0
12/10–17	8.5–12.1	2.45	4.89*	0.00	1998	0
200.2 m^3	5.7–8.5	2.80	9.44	0.00	3376	0
	3.9–5.7	2.75	8.43	0.00	3067	0
	2.5–3.9	2.60	6.78	0.00	2612	0
	1.25–2.5	2.55	13.92	3.41*	5463	1339
	0.76–1.25	3.95	24.78	11.59	6280	2936
	0.52–0.76	5.79	37.95	21.46	6550	3705
	0.33–0.52	3.30	33.05	13.51	10028	4098
	0.22–0.33	1.25	20.46	3.10*	16383	2486
	0.13–0.22	1.20	21.39	4.32*	17848	3607
	0.06–0.13	0.90	13.29	4.95*	14786	5507
	0.06 >	0.30	9.09	0.00	30325	0
(V)	> 12.1	2.71	3.17*	0.00	1171	0
12/17–24	8.5–12.1	1.60	0.00	0.00	0	0
199.6 m^3	5.7–8.5	2.76	3.43*	0.00	1245	0
	3.9–5.7	2.56	3.37*	3.90*	1320	1525
	2.5–3.9	2.31	6.03	0.00	2615	0
	1.25–2.5	2.61	10.34	7.34	3967	2817
	0.76–1.25	4.86	22.66	16.76	4663	3448
	0.52–0.76	6.76	29.08	25.98	4298	3840
	0.33–0.52	3.81	27.29	18.54	7164	4868
	0.22–0.33	1.45	16.02	3.96*	11022	2722
	0.13–0.22	1.65	20.36	12.40	12310	7499
	0.06–0.13	0.85	12.07	6.34	14169	7441
	0.06 >	2.66	6.39	3.20*	2406	1205

tives (twice or more revertants of spontaneous control), 9 were pseudopositives (one half to twice of spontaneous) and only one was negative (less than one half of spontaneous in present dose). On the other hand, 25 positives, 17 pseudopositives and 23 negatives were observed in the condition with S9 mix. Most of the negatives were observed in the coarse particle samples. Especially, samples of > 12.1, 8.5, and 5.7 μm in diameter were all negatives in the 5 weeks of testing. Negatives were also observed in three backup filter samples. One of the causes of the negative response was considered to be the smaller quantity of particles collected, because for these samples less than 0.13 mg of particles per filter was collected in 7 days.

Also as shown in Table 1, the total mass concentration of particles collected (sum of the 13 stages) showed a two-fold or larger difference from week to week (34.56–72.73 $\mu g/m^3$). The size distributions were bimodal, having two peaks bounded around particle diameter of 1 to 2 μm, and were similar in every series of samples tested from week to week, as reported by other investigators.[13,14] The size distribution patterns of mass concentration and mutagenic activities (rev./m^3 of air and rev./mg of particles) of a series of samples are shown in Fig. 2 using an application software Andersen Analyzer.[15] As illustrated, the size distribution of mutagenic activity per unit air volume (B) is skewed to the smaller size range and has only one peak at 0.52 μm, whereas that of mass concentration (A) was bimodal. Moreover, the mutagenic activity per unit particle mass (rev./mg) generally increased as the particle size became smaller (Table 1). The highest activity was observed in particles of less than 0.22 μm in diameter including ultrafine particles.

From the reports of size distribution and elemental analysis of suspended particles, these size distributions were classified into three types:[13,14]

A bimodal distribution having two peaks bounded

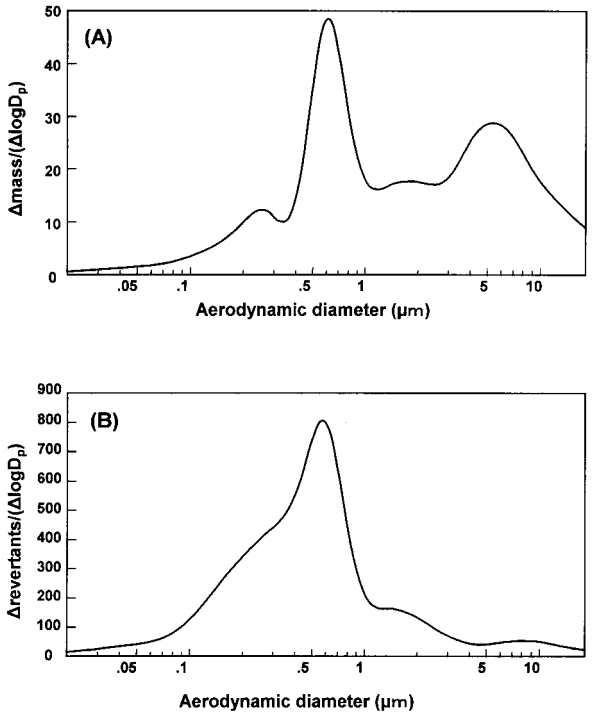

Fig. 2. Size Distribution Patterns of Mass Concentration (A) and Mutagenic Activities (B) of a Series of Particles Collected by ALPI

Mutagenicity test was conducted by a microsuspension procedure using Salmonella YG1024 strains without S9 mix. All data are cited from sample (II) in Table 1.

around particle diameter of 1 to 2 μm (for example, Mn, K, V, Cu, *etc*.)

Skewed to the smaller size range and having only one peak in the fine particle region (for example, Pb, Zn, S, *etc*.)

Skewed to the larger size range and having nearly one peak in the coarse particle region (for example, Si, Ca, Ti, *etc*.)

In the present results, the mass concentration showed a type-1 distribution and the mutagenicity showed a type-2 distribution. Fine particles consist of primary particles (mainly produced by combustion and so on) and secondary particles (chemical conversion of gases) and are thought to be more harmful than coarse particles.[14] From the present results, the mutagenic activity of fine particles contributed to 80–90% of that of all particles in total. This finding suggests that most mutagens are fine particles of less than 1 μm in diameter.

Recently, the biological effects of ultrafine particles of less than 0.1 μm in diameter have been reported.[16] Although the mechanism of biological effects of ultrafine particles has not been clarified, experimental studies on hamster showed that inhaled ultrafine particles of metal fumes can penetrate the blood vessels of the lung through alveolar epithelial cells.[17] It was also suggested that ultrafine particles would tend to induce adverse effects more than fine particles (PM 2.5–0.1) due to the numbers and surface areas of monodispersed particles of unit density of different sizes at mass concentration even if the mass concentration of ultrafine particles is extremely low.[16,18] In our present results, the highest mutagenic activity per unit mass of particles (revertants per mg, particles) in a series of fractioned samples was observed in particles of less than 0.22 μm in diameter. This suggested that samples including ultrafine particles induced mutagenicity more effectively, as if the mass concentrations of ultrafine particles were 10% or less than those of fine particles. These facts suggested that it is necessary to pay closer attention to the associations with cancers other than respiratory cancers because the carcinogens and mutagens in ultrafine particles can penetrate the body directly through the blood vessels.

As mentioned above, investigation of the mutagenicity of size-fractioned particles collected with ALPI is very useful for studies on the existence of and exposure to carcinogens and mutagens in suspended particles in ambient air, and also for studies on the properties of particles taken into the lungs. However, it has been suggested that impaction sampling involves some problems such as bouncing[19] and measuring precise weight of ultrafine particles. Also it is not so clear whether particle itself or chemical mutagens are more effective to the mutagenicity of ultrafine particles. Therefore, it is necessary to investigate better sampling methods and to detect the chemical substances that contribute to the mutagenicity of ultrafine particles.

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