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Application of the Ultramicro Forward-Mutation Assay to the Monitoring of Indoor and Outdoor Air Mutagenicity-Examples of Chengdu City and Tokyo

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1. Introduction

Various chemicals are emitted into the air from factories, offices, incinerators, and vehicles by industrial activity (Alfhei & Mψller, 1981; Mψller et al, 1982; Watanabe & Hirayama, 1997). In addition, indoor air is polluted by chemicals used or exhausted intentionally or unintentionally during heating (use of town gas and fossil fuel), cooking, smoking and building materials (Endo et al, 2000; Koyano et al, 1999; Takagi et al, 1997). People are concerned about the health impacts of the hazardous chemicals contained in those materials. The carcinogens and mutagens detected in air can be classified into two groups: particulate matter and gas/vapor matter. Regarding particulate matter, various substances including inorganic ones such as heavy metals and organic compounds such as polycyclic aromatic hydrocarbons are detected. Such substances and compounds include polycyclic aromatic hydrocarbons (PAHs) like benzo[a]pyrene, which is now attracting attention as a carcinogenic/mutagenic substance, and dinitropyrene, which shows extremely high mutagenicity for *Salmonella* TA98 strain (Watanabe & Hirayama, 1997). It is widely known that PAHs are formed during the incomplete combustion of organic matter (Somenath & Wilson, 1992). PAHs are also formed when fossil fuel is burned by industry and in daily life and so the health hazards of PAHs contained in the environment cannot be avoided. People spend 80 to 90% of their time indoors, and especially infants, elderly people and sick people with a weak immune system spend even longer hours indoors. Therefore, it is important to keep the indoor environment clean because human health is greatly influenced by the environment. However, there have been few studies on the mutagenicity of indoor air because the amount of such substances that can be sampled is limited. A new mutagenicity test that is more sensitive than the existing widely-usedAmes method: “plate incorporation method” and “preincubation method” needs to be developed. We studied use of the ultramicro forward mutation Assay,

which is is more sensitive than conventional methods. In this method, a mini pump was used to collect suspended particulate matters (SPMs) onto quartz fiber filters at 1 L/min for 24 hours and the filters were then subjected to sonication. The extracts were used as samples for mutagenicity measurement. We used the ultramicro forward mutation assay with *Salmonella typhimurium* TM677 strain in our mutagenicity test and confirmed that the new test method is sufficiently sensitive to measure indoor air samples. Therefore, we compared the mutagenicity of indoor and outdoor SPMs and that of air samples obtained in Chengdu, China and Tokyo, and report on the measurements of PAHs in those samples.

2. Materials and methods

2.1 Reagents and instruments

We used dichloromethane (Wako Chemical Industries, Ltd.) for the residual pesticides test, methanol (Wako Chemical Industries, Ltd.) for high-performance liquid chromatography, and dimethyl sulfoxide (Dojindo Laboratories) for fluorescence analysis. We used agar (BBL, Nutrient Broth, Difco), D-biotin (Merck), 8-azaguanine (Sigma Chemical), and other commercially available reagents. For counting colonies, we used a Bio Multi Scanner (BMS-400, Ipros Corporation and CA-7, System Science).

2.2 Collection of samples and method

We asked residents in Chengdu and Tokyo (ten families in each) to collect air samples from their kitchen, bedroom, living room and outdoors. Mini pumps (MP-15CF, Sibata Scientific Technology Ltd.) were used to collect the samples onto quartz fiber filters (2500 QAT-UP ϕ25 mm, Pallflex) at the flow rate of about 1 L/min for 24 hours. The samples were collected in Chengdu in summer (July 2000) and winter (Dec. 2000 – Jan. 2001) and in Tokyo in winter (Jan. – Feb. 2001). In each case, indoor and outdoor conditions were recorded when the air samples were collected. The collected quartz filters were stored in deep freezer.

2.3 Extraction of organic matter

To extract organic matter in indoor and outdoor SPMs collected with the mini pumps, the quartz fiber filters were removed from the freezer, cut into small pieces, put into stoppered test tubes and subjected to extraction by sonication. After the test tubes containing the samples with 9 mL of dichloromethane were sealed, they were dipped in an sonication generator and exposed to ultrasonic waves for 15 minutes. Subsequently, 3 mL of the supernatant extract was dispensed into a screw cap test tube for HPLC analysis and a further 6 mL was dispensed into another screw cap test tube for the mutation test. For HPLC analysis, the solvent was distilled away under nitrogen flow, then the dried up extract was redissolved in 1.5 mL of acetonitrile and centrifuged. Four hundred micro liter of the obtained supernatant was used for analysis of PAHs. For the mutagenicity test, the solvent was distilled away from 6 mL of extract and then the supernatant was kept in a freezer at −80^{o}C until the test.

2.4 Preparation of samples

It was necessary to minimize the amount of DMSO, which was used to dissolve the samples in the ultramicro forward mutation assay, because the toxicity of DMSO to bacteria should be restricted as much as possible. Therefore, the medium-exchange procedure was used to

prepare solutions of respective concentrations. Namely, 0.2 μL of DMSO was dispensed into sterilized micro vials for test samples in advance and then the solutions of test substances in different additive amounts were added. Nitrogen gas was blown into the mixed solutions in the vials to distill away the solvent and obtain the DMSO solutions of respective concentrations, which were then used for the mutation test. We used the extracts of outdoor SPMs as a positive reference material in the mutation test.

2.5 Mutagenicity test

We conducted this test using the ultramicro forward mutation assay with *Salmonella typhimurium* TM677 strain. The frozen bacteria of the TM677 strain, which had been stored at −80°C, were dissolved at room temperature, the bacteria suspension was stirred well, then 0.3 mL of the suspension was inoculated into an L-shaped test tube with 2.7 mL of minimal E medium (ME). The L-shaped test tube was subjected to culture shake in an incubator shaker (37°C) for 3 hours. (The composition of 1 L of ME was as follows: 20 g of glucose, 2 g of magnesium sulfate heptahydrate, 20 g of citric acid, 100 g of dibasic potassium phosphate, 19.2 g of dibasic potassium phosphate, 6.6 g of sodium chloride, 12.2 mg of D-biotin, and 1000 mL of ion-exchange water.) This culture solution was diluted with ME again ten times. The obtained solution was used as a test bacteria liquid without S9mix. The mutation test in this study was conducted using the ultramicro forward mutation assay without adding S9mix. Namely, 10 μL of bacteria liquid was put into a micro vial that contained the analyte solution and the bacteria were pre-incubated in an incubator at 37°C. In this pre-incubation, a rotator was used to turn the micro vial and to thoroughly mix the analyte solution and the bacteria liquid. After incubation, 90 μL of 0.15 M sterilized phosphate buffer (pH 7.4, PBS) was added into the micro vial and mixed well. Eighty micro liter of the obtained solution was dispensed into each aluminium-capped test tube cap. Then, 2.5 mL of agar containing 8-azaguanine was put into each of these small test tubes, mixed and overlaid on a culture medium (agar plate) to use the plates for measurement of mutagenicity. Ten micro liter of the remaining sample in the micro vial was dispensed into a screw cap bottle, which contained 10 mL of PBS, mixed well, then 33.5 μL of this sample was dispensed into each of three test tubes with an aluminum cap. Different from measuring the mutation, 2.5 mL of soft agar without 8-azaguanine was put into each of these small test tubes, mixed well and overlaid on a culture medium (agar plate) to be used for counting viable bacteria. After incubation, the formed colonies were counted with a colony counter, and with the obtained numbers of mutated colonies and living bacteria colonies, the following formula was used to calculate the mutation frequency.

$$\text{Mutation frequency} = \text{Number of mutated colonies} / (\text{Number of living bacteria colonies} \times 2400)$$

In this study, for the air samples found to be positive or false-positive, the primary regression equation was obtained from the direct part of the dose-response relationship by the least-squares method. The mutation frequency was then obtained per m^3 of air (converted from the extract solution) in the primary regression analysis. The control value (spontaneous mutation frequency) was subtracted from this value to obtain the mutagen-specific activity. In addition, the fluctuation of bacteria activity depending on the test date was corrected with the results of mutation tests of the positive reference material, which were conducted simultaneously on the same test dates.

2.6 Analysis of PAHs

The following eight substances were examined in the PAH analysis: pyrene (Py), benz[a]anthracene (BaA), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenz[a,h]anthracene (dBahA), benzo[b]chrysene (BbC), benzo[ghi]perylene (BghiP), and dibenzo[a,e]pyrene (dBaeP). An HPLC provided with a concentrating column/spectral fluorescence detector was used for the analysis. A hundred micro liter of the sample solution was injected into the HPLC and separated into acetonitrile and aqueous mobile phase. After separation, fluorescence was detected at suitable wavelengths for the respective PAHs, and a quantitative determination was conducted from the peak heights of the respective chromatograms.

3. Results

3.1 Mutagenicity of indoor and outdoor SPMs in ordinary family homes in Chengdu in summer and winter

We measured the mutagenicity of the extracts obtained from the samples collected by our collaborators living in Chengdu, and compared the results. Figure 1 shows one example relation between the mutation frequencies and the respective sample amounts for the mutagenicity of the obtained samples. The test results show a good dose-response relationship between the air sample amounts and the mutation frequencies, and mutagenicity was detected in the air samples of Chengdu. As shown, we could detect the

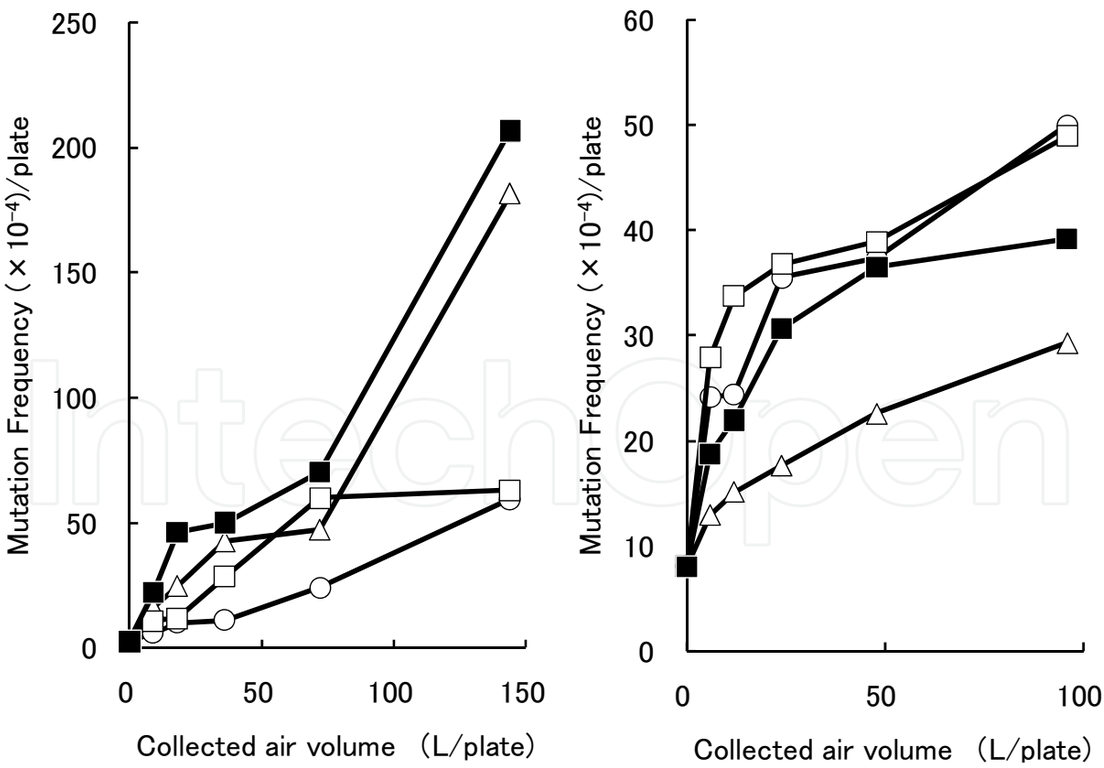


Fig. 1. Mutation test results of indoor and outdoor SPMs in Chengdu (A) winter, (B) summer, circle: kitchen, triangle: living room, open square: bedroom, closed square: outdoors

mutagenicity of indoor air by using the ultramicro forward mutation assay. Figures 2 and 3 show the measurement results of mutagenicity of the SPMs in the kitchen, bedroom, living room and outdoors of the respective collaborators. The results are shown as mutagen-specific activity obtained per m^3 of air converted from the extract solution in two seasons (Fig. 2 – winter and Fig. 3 – summer). Table 1 shows the values in the respective sampling places in both seasons. As shown in Figures 2 and 3 and Table 1, the average values of mutagen-specific

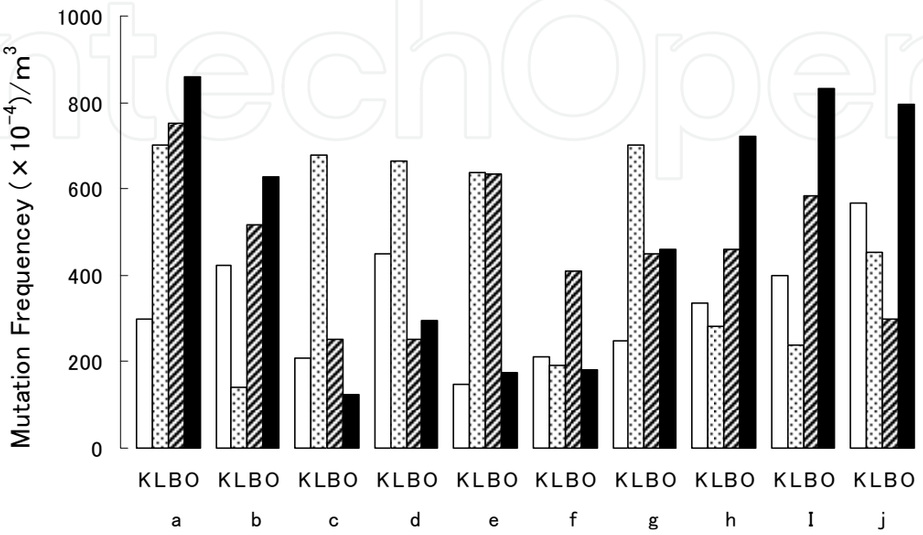


Fig. 2. Mutagenicity of indoor and outdoor SPMs in Chengdu (winter) K: kitchen, L: living room, B: bedroom, O: outdoors

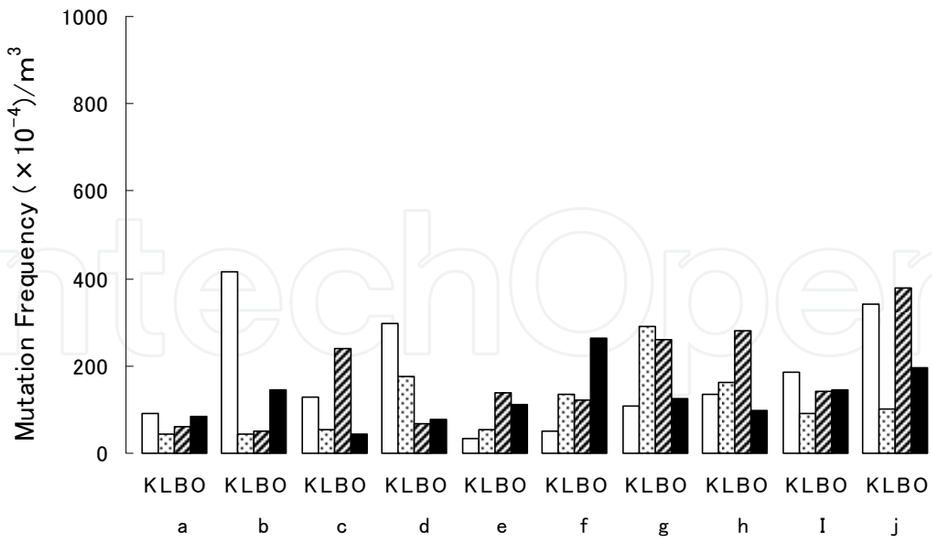


Fig. 3. Mutagenicity of indoor and outdoor SPMs in Chengdu (summer)K: kitchen, L: living room, B: bedroom, O: outdoors

activity in winter were 329 x 10-4/m^3 in the kitchen, 469 x 10-4/m^3 in the living room, 461 x 10-4/m^3 in the bedroom and 507 x 10^{-4}/m^3outdoors, and those in summer were 179 x 10-

$^{4}/m^3$ in the kitchen, 115 x $10^{-4}/m^3$ in the living room, 174 x $10^{-4}/m^3$ in the bedroom, and 129 x $10^{-4}/m^3$ outdoors. Thus, the mutagen-specific activity was higher in winter than in summer. In winter, the values were higher in outdoor air than in indoor air, and in summer were higher in indoor air than in outdoor air. For the sampling places, the value in the kitchen was the lowest in winter, but the highest in summer. There was no specific difference in the values between the different floor levels.

Season	Sampling place	Mutation frequency ($\times 10^{-4}$) /m^3										
		a	b	c	d	e	f	g	h	I	j	Average value
Winter	Kitchen	299	423	209	450	149	211	249	336	401	568	329
	Living	702	141	678	664	638	191	702	283	237	452	469
	Bedroom	752	518	250	252	635	411	448	461	583	298	461
	Outdoors	860	626	125	294	173	180	458	722	832	797	507
Summer	Kitchen	91	414	129	299	35	51	110	136	186	342	179
	Living	44	44	54	175	53	137	291	164	90	101	115
	Bedroom	62	50	241	68	137	121	261	282	143	379	174
	Outdoors	83	146	44	79	110	265	126	97	144	197	129
Remarks		7F	6F With smoker	3F	4F	3F	6F	6F	16F With smoker	3F	3F	

Table 1. Mutagen specific activity by sampling places in Chengdu

3.2 Comparison of mutagenicity of indoor and outdoor SPMs in ordinary family homes in Chengdu and Tokyo in winter

We measured the mutagenicity of the SPMs collected by our collaborators living in Tokyo, and compared the results with those in Chengdu. Figure 4 shows one example result of the air sample amounts and the mutation frequencies for the mutagenicity of the obtained SPMs. The results show a good dose-response relationship between the air sample amounts and the mutation frequencies, and mutagenicity was detected in the samples collected in Tokyo. With the dose-response relationship obtained as in Figure 4, Figure 5 shows the mutagen-specific activity by respective collaborators living in Tokyo, which were calculated from the measurement results of the samples collected in the kitchen, bedroom, living room and outdoors. Table 2 shows the mutagen-specific activity for the respective sampling places. Figures 2 and 5 show that the mutagen-specific activity was generally higher in Chengdu than in Tokyo. Tables 1 and 2 show that the average values of mutagen-specific activity per unit air amount (m^3) in Tokyo were77 x $10^{-4}/m^3$ in the kitchen, 127 x $10^{-4}/m^3$ in the living room, 96 x $10^{-4}/m^3$ in the bedroom, and 66 x $10^{-4}/m^3$ in outdoor, which were lower than those in Chengdu. Regarding outdoors and indoors, the values outdoors were higher than indoors in Chengdu while those outdoors were lower than indoors in Tokyo. Regarding the mutagen-specific activity in terms of building structure, generally the values were lower in detached houses than in collective housing. In Tokyo, generally the indoor values in smokers' houses were higher than in non-smokers' houses.

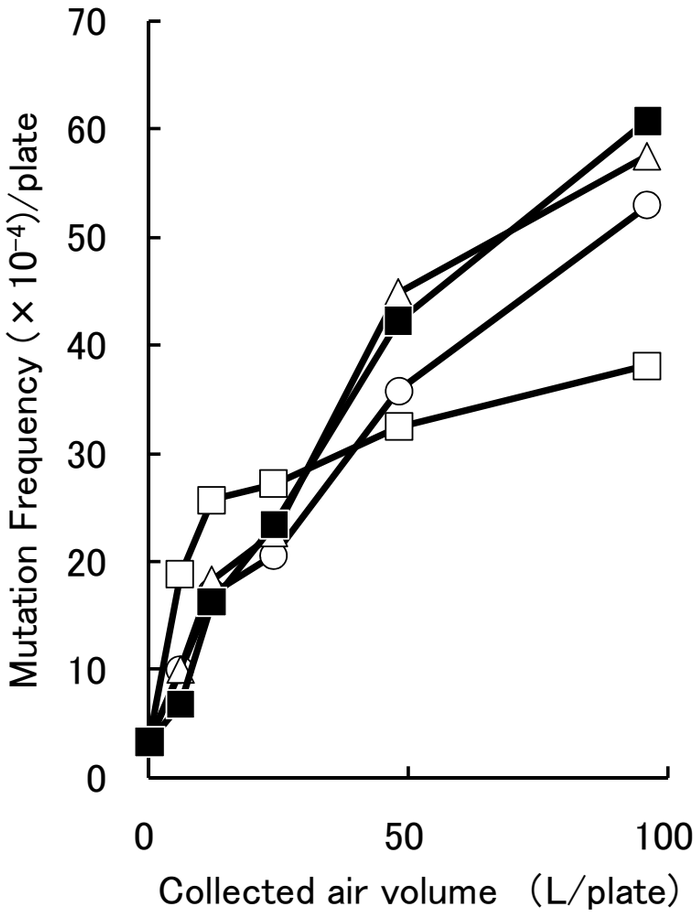


Fig. 4. Mutation test results of indoor and outdoor SPMs in Tokyo (winter) circle: kitchen, triangle: living room, open square: bedroom, closed square: outdoors

Season	Sampling place	Mutation frequency ($\times 10^{-4}$) /m^3										
		a	b	c	d	e	f	g	h	I	j	Average value
Winter	Kitchen	8	135	55	33	115	134	172	47	56	16	77
	Living room	45	110	37	55	147	540	169	50	63	54	127
	Bedroom	12	122	41	25	115	319	208	80	11	30	96
	Outdoors	38	110	72	55	54	72	132	24	46	53	66
Remarks		Detached house	Collective housing 14F With smoker	Detached house	Detached house	Collective housing 7F With smoker	Detached house	Detached house With smoker	Collective housing 4F	Detached house	Detached house	

Table 2. Mutagen specific activity by sampling places in Tokyo

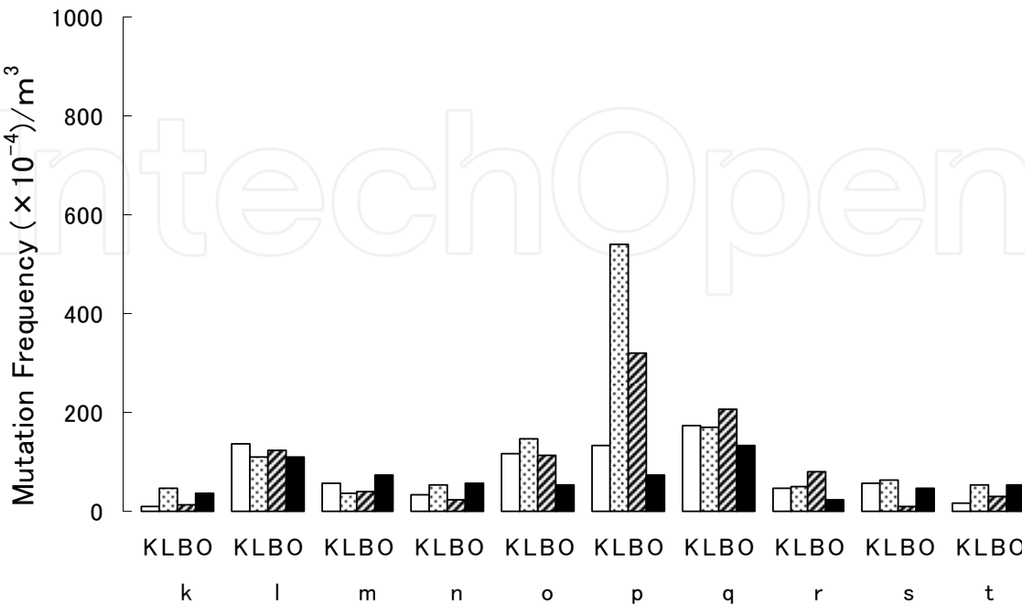


Fig. 5. Mutagenicity of indoor and outdoor SPMs in Tokyo (winter) K: kitchen, L: living room, B: bedroom, O: outdoors

3.3 Comparison of concentrations of polycyclic aromatic hydrocarbons contained in indoor and outdoor SPMs in Tokyo and Chengdu in winter

Figures 6, 7 and 8 show the concentration of BaP, which is a typical carcinogen in PAHs, detected in the kitchen, bedroom, living room and outdoors in Tokyo in winter and in Chengdu in both winter and summer. Tables 3, 4 and 5 show the concentration of the eight target substances (Py, BaA, BaP, dBahA, BdC, BghiP and dBaeP) detected in the respective houses of our collaborators. These figures and tables show the average concentrations of BaP per unit air (m^3) as follows: 10.98 ng/m^3 in the kitchen, 17.06 ng/m^3 in the living room, 16.95 ng/m^3 in the bedroom and 16.01 ng/m^3 outdoors in Chengdu in winter; 3.60 ng/m^3 in the kitchen, 2.06 ng/m^3 in the living room, 3.09 ng/m^3 in the bedroom and 8.39 ng/m^3 in Chengdu in summer; 0.34 ng/m^3 in the kitchen, 0.38 ng/m^3 in the living room, 0.33 ng/m^3 in the bedroom, and 0.52 ng/m^3 in Tokyo. In Chengdu, the values were higher in winter than in summer, whereas those in Tokyo were lower than in Chengdu in any season. The average concentrations of other PAHs showed the same trend. In Chengdu, the living room in summer and the kitchen in winter showed slightly lower values than other rooms in the same seasons. In Tokyo in winter, the value in the bedroom was lower than in other rooms in the same season. In Chengdu in winter, there was only a slight difference in the values between indoors and outdoors. Even in summer in Chengdu and in winter in Tokyo when the concentration of outdoor PAHs was comparatively low, generally the concentration of indoor PAHs in smokers' houses was higher than that of outdoor PAHs.

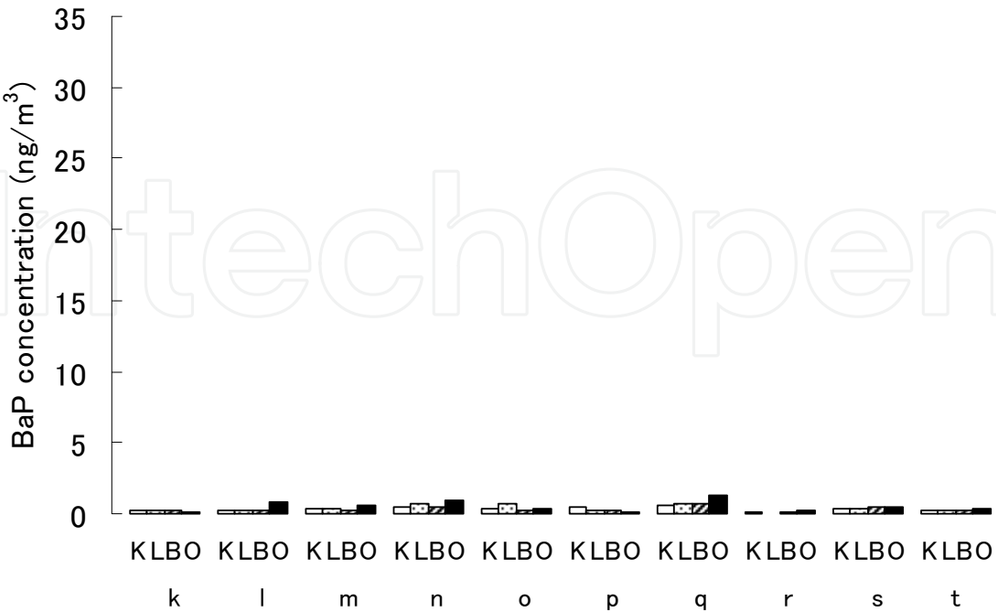


Fig. 6. BaP concentration in indoor and outdoor SPMs in Tokyo (winter) K: kitchen, L: living room, B: bedroom, O: outdoors

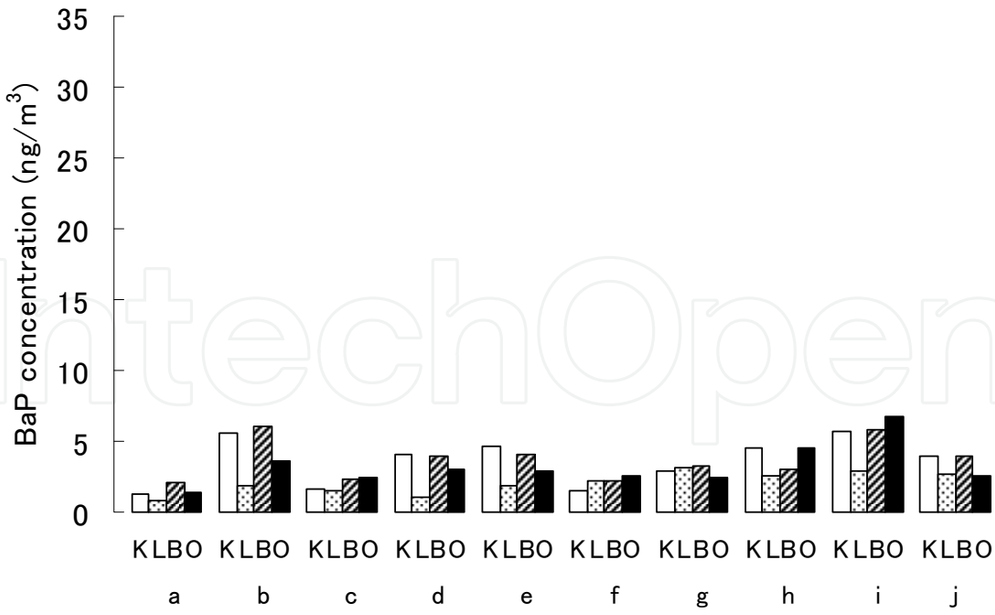


Fig. 7. BaP concentration in indoor and outdoor SPMs in Chengdu (summer) K: kitchen, L: living room, B: bedroom, O: outdoors

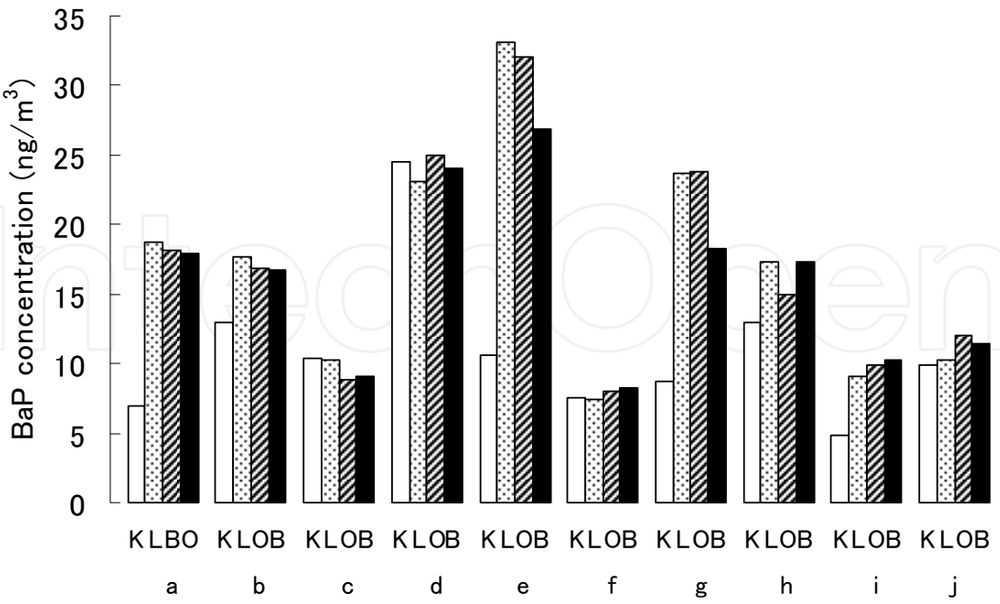


Fig. 8. BaP concentration in indoor and outdoor SPMs in Chengdu (winter) K: Kitchen, L: living room, B: bedroom, O: outdoors

4. Discussion

In the environment including indoor and outdoor air, soil and river water, there are various hazardous chemical substances such as carcinogens and mutagens (Goto et al, 1982; Iwamoto et al, 1990; Kasahara, 1990; Urano et al, 1992; Yamamoto, 1977). Automobiles, factories, offices, heating, cooking, etc. emit hazardous chemicals including benzo[a]pyrene (BaP), PAHs, heavy metals, NOx, nitrosamine, chlorinated hydrocarbons such vinyl chloride, SOx, etc. These substances are now attracting attention as major causes of human respiratory diseases and lung cancer. Especially in city areas, a close relationship has been found between the high incidence of lung cancer and outdoor air pollution. Substances attached to SPMs are inhaled by humans into the lungs. The area of the lung where such particles are deposited varies depending on the particle diameter: the smaller they are, the more hazardous (Broddin, 1977, Pierce & Katz, 1975; Sugita et al, 1996). Especially, particles smaller than 2.5 μm penetrate deep into human lungs (Heyder et al, 1986), and some substances cause asthma and cancer and have immunotoxicity and genetic toxicity. Recently, some substances have been found to be endocrine disruptors, and thus chemical substances can damage the human body in various ways. The amount of SPMs caused directly by incineration is decreasing in advanced countries that are switching energy sources from coal to oil and modifying their facilities. On the other hand, in China and other countries that are growingly rapidly, large amounts of hazardous substances such as SOx are continuously emitted because coal is still a major energy source. Coal, including charcoal and artificial coal, is also used for cooking and heating in ordinary family homes and so indoor and outdoor air pollution caused by gas emissions cannot be avoided. Since people spend most of their time indoors, indoor air pollution can be very hazardous for human

Sampling place		Date of starting sampling (yymmdd)	Quantity of airflow (m^3)	PAH concentration in air (ng/m^3)								Remarks
				Pyrene	BaA	BkF	BaP	dBahA	BdC	BghiP	dBaeP	
a	Kitchen	001218	1.504	6.88	6.08	2.99	6.99	1.29	1.45	9.34	1.15	7F
b		001219	1.539	11.18	10.84	5.42	13.02	2.47	2.69	18.14	2.26	6F
c		001220	1.758	9.73	8.13	3.83	10.38	2.16	2.18	15.56	1.88	3F
d		001222	1.669	23.76	23.80	9.15	24.55	4.19	4.91	32.44	3.87	4F
e		001225	1.680	7.41	7.70	4.70	10.66	2.22	2.52	15.95	1.92	3F
f		001231	1.655	7.30	6.14	3.45	7.60	1.93	1.80	11.78	1.34	6F
g		010101	1.673	6.06	6.67	3.43	8.73	2.02	1.72	11.74	1.26	6F
h		010104	1.639	11.41	12.29	5.55	13.02	3.11	2.74	17.60	2.06	16F
I		010114	1.644	5.69	4.37	2.39	4.87	1.25	1.10	7.70	0.85	3F
j		010115	1.598	7.42	8.08	4.67	9.94	2.41	2.06	14.79	1.70	3F
	Average value			9.68	9.41	4.56	10.98	2.31	2.32	15.50	1.83	
a	Living room	001218	1.483	13.10	15.29	7.81	18.78	3.60	3.92	25.51	3.10	7F
b		001219	1.517	14.18	15.22	7.68	17.64	3.41	3.65	24.58	2.96	6F
c		001220	1.758	8.21	7.90	3.80	10.31	2.04	2.18	15.44	1.96	3F
d		001222	1.656	18.93	21.04	8.97	23.07	3.91	4.87	31.16	3.64	4F
e		001225	1.656	23.17	24.73	15.24	33.17	8.62	7.52	50.21	5.59	3F
f		001231	1.655	5.70	6.12	3.55	7.44	2.11	1.81	11.83	1.36	6F
g		010104	1.673	12.45	17.07	9.06	23.64	5.43	4.86	30.98	3.33	6F
h		010104	1.650	14.47	17.12	7.31	17.28	4.15	3.63	23.53	2.76	16F
I		010114	1.621	5.67	8.15	4.00	9.08	2.11	1.93	13.60	1.37	3F
j		010115	1.621	8.40	8.61	4.90	10.23	2.51	2.17	15.59	1.63	3F
	Average value			12.43	14.13	7.23	17.06	3.79	3.65	24.24	2.77	
a	Bedroom	001218	1.473	12.57	14.62	7.61	18.10	3.44	3.71	24.86	2.95	7F
b		001219	1.512	13.58	14.33	7.25	16.86	3.34	3.54	23.34	2.81	6F
c		001220	1.764	6.99	5.46	2.90	8.84	1.16	2.02	13.07	1.64	3F
d		001222	1.669	24.16	23.97	9.75	24.93	4.22	5.08	32.68	3.85	4F
e		001225	1.680	20.80	20.65	13.32	32.03	6.44	7.48	47.07	5.56	3F
f		001231	1.644	8.52	7.41	3.82	8.07	2.19	1.89	12.36	1.47	6F
g		010104	1.673	14.94	20.46	9.21	23.79	5.36	4.90	30.92	3.44	6F
h		010104	1.627	11.11	13.29	6.36	14.98	3.96	3.11	20.58	2.41	16F
I		010114	1.644	9.79	8.62	4.29	9.86	2.26	2.03	14.40	1.56	3F
j		010115	1.621	10.30	10.70	5.75	12.03	2.88	2.48	17.67	1.98	3F
	Average value			13.28	13.95	7.03	16.95	3.53	3.62	23.70	2.77	
a	Outdoor	001218	1.473	14.26	15.67	7.44	17.91	3.39	3.68	23.90	2.95	7F
b		001219	1.517	13.25	13.58	7.17	16.69	3.25	3.47	22.93	2.78	6F
c		001220	1.796	7.13	5.63	3.11	9.05	1.42	2.02	13.70	1.78	3F
d		001222	1.645	19.80	23.09	9.30	24.07	4.15	4.82	31.88	3.68	4F
e		001225	1.656	17.63	18.12	11.61	26.83	5.20	6.46	39.83	4.79	3F
f		001231	1.668	9.49	8.19	3.95	8.20	2.50	1.90	12.51	1.39	6F
g		010104	1.673	15.13	18.38	7.21	18.31	3.90	3.66	23.26	2.64	6F
h		010104	1.650	14.28	16.68	7.36	17.33	4.21	3.64	23.84	2.79	16F
I		010114	1.644	11.26	9.39	4.49	10.30	2.39	2.15	15.04	1.55	3F
j		010115	1.621	10.88	10.49	5.47	11.45	2.80	2.37	16.97	1.76	3F
	Average value			13.31	13.92	6.71	16.01	3.32	3.42	22.39	2.61	

* With smoker in b and h

Table 3. Analysis of PAH in the outdoor air and indoor air in Chengdu (winter)

Sampling place		Date of starting sampling (yymmdd)	Quantity of airflow (m^3)	PAH concentration in air (ng/m^3)								Remarks
				Pyrene	BaA	BkF	BaP	dBahA	BdC	BghiP	dBaeP	
a	Kitchen	000710	1.423	1.89	1.03	1.29	1.26	0.68	0.67	4.48	0.42	7F
b		000713	1.489	2.95	3.03	2.57	5.61	1.19	1.53	11.20	0.99	6F
c		000706	1.545	1.94	1.07	0.87	1.68	0.54	0.55	4.29	0.35	3F
d		000707	1.418	3.22	2.32	1.88	4.11	0.91	1.02	7.44	0.60	4F
e		000712	1.437	3.56	3.18	2.47	4.60	1.21	1.32	10.01	0.78	3F
f		000714	1.623	1.80	0.97	1.20	1.54	0.73	0.78	6.20	0.55	6F
g		000715	1.673	2.51	1.51	1.89	2.91	1.33	1.24	9.74	0.95	6F
h		000705	1.531	2.69	2.03	2.18	4.57	1.19	1.30	9.94	0.88	16F
I		000719	1.661	3.38	2.67	3.05	5.74	1.79	1.83	14.46	1.29	3F
j		000720	1.562	3.37	3.02	2.11	3.96	1.64	1.43	11.20	1.06	3F
	Average value			2.73	2.08	1.95	3.60	1.12	1.17	8.90	0.79	
a	Living room	000710	1.504	1.26	0.55	0.45	0.81	0.30	0.26	1.88	0.20	7F
b		000713	1.510	1.32	1.49	0.81	1.86	0.39	0.49	3.70	0.33	6F
c		000706	1.534	1.95	1.29	0.77	1.51	0.40	0.47	3.52	0.30	3F
d		000707	1.418	1.23	0.70	0.57	1.06	0.24	0.28	1.97	0.36	4F
e		000712	1.429	1.89	1.84	0.79	1.84	0.49	0.59	4.08	0.43	3F
f		000714	1.646	2.01	1.57	1.22	2.24	0.75	0.81	6.24	0.51	6F
g		000715	1.611	2.36	1.88	1.86	3.10	1.13	1.15	8.80	0.81	6F
h		000705	1.531	1.97	1.26	1.10	2.53	0.58	0.66	4.99	0.40	16F
I		000719	1.673	1.79	1.30	1.70	2.89	0.96	0.97	7.76	0.79	3F
j		000720	1.562	2.41	2.16	1.57	2.73	1.13	1.01	7.77	0.69	3F
	Average value			1.82	1.40	1.08	2.06	0.64	0.67	5.07	0.48	
a	Bedroom	000710	1.504	1.76	1.05	0.85	1.38	0.43	0.43	3.29	0.34	7F
b		000713	1.489	2.34	2.62	1.64	3.64	0.81	1.00	7.29	0.56	6F
c		000706	1.523	2.83	1.67	1.22	2.46	0.61	0.68	5.43	0.57	3F
d		000707	1.418	3.31	1.86	1.42	3.07	0.69	0.70	5.45	0.45	4F
e		000712	1.437	2.28	1.75	1.43	2.85	0.82	0.84	6.53	0.61	3F
f		000714	1.635	2.50	1.72	1.37	2.51	0.76	0.87	6.62	0.40	6F
g		000715	1.685	1.90	1.39	1.45	2.41	0.77	0.83	6.44	0.58	6F
h		000705	1.542	2.47	1.60	1.30	3.03	0.75	0.74	5.61	0.55	16F
I		000719	1.673	3.69	3.04	3.73	6.70	2.85	2.38	18.82	1.74	3F
j		000720	1.562	2.15	1.85	1.37	2.53	0.93	0.93	5.94	0.63	3F
	Average value			2.52	1.86	1.58	3.06	0.94	0.94	7.14	0.64	
a	Outdoor	000710	1.483	2.24	1.41	1.26	2.07	0.60	0.65	4.98	0.51	7F
b		000713	1.500	3.36	3.47	2.59	6.03	1.23	1.51	11.48	0.97	6F
c		000706	1.523	2.60	1.66	1.09	2.28	0.48	5.05	0.64	0.59	3F
d		000707	1.418	3.22	2.42	1.90	3.92	0.90	1.00	7.14	0.67	4F
e		000712	1.458	2.92	2.43	2.03	4.05	1.10	1.21	8.29	0.89	3F
f		000714	1.623	2.15	1.52	1.23	2.23	0.80	0.77	6.36	1.14	6F
g		000715	1.661	2.42	1.81	1.84	3.25	1.10	1.16	9.02	0.86	6F
h		000705	1.509	3.17	2.45	2.06	4.48	1.15	1.23	9.46	0.89	16F
I		000719	1.685	2.85	2.44	3.13	5.76	1.90	1.94	15.17	1.37	3F
j		000720	1.562	3.04	2.58	2.10	3.92	1.64	1.55	11.07	1.11	3F
	Average value			2.80	2.22	1.92	3.80	1.09	1.61	8.36	0.90	

* With smoker in b and h

Table 4. Analysis of PAH in the outdoor air and indoor air in Chengdu (summer)

Sampling place		Date of starting sampling (yymmdd)	Quantity of airflow (m^3)	PAH concentration in air (ng/m^3)								Remarks
				Pyrene	BaA	BkF	BaP	dBahA	BdC	BghiP	dBaeP	
k	Kitchen	010203	1.99	0.37	0.18	0.10	0.21		0.04	0.37		Detached house
l		010122	1.98	0.26	0.14	0.11	0.27	0.08	0.08	0.54	0.09	14F
m		010119	1.96	0.37	0.28	0.20	0.40	0.10	0.10	1.24	0.09	Detached house
n		010116	2.07	0.55	0.29	0.14	0.44	0.06	0.07	1.13		Detached house
o		010206	1.70	0.46	0.25	0.14	0.34	0.05	0.03	0.52		7F
p		010107	2.06	4.31	1.42	0.29	0.48	0.14		1.29	0.11	Detached house
q		010113	1.70	0.39	0.39	0.16	0.53	0.05	0.08	0.67		Collective housing
r		010118	2.11	0.21	0.04	0.03	0.06		0.02	0.14		4F
s		010111	1.97	0.48	0.20	0.12	0.38	0.03	0.07	0.63	0.06	Detached house
t		010221	2.04	0.31	0.11	0.11	0.28	0.05	0.06	0.50		Detached house
	Average value			0.77	0.33	0.14	0.34	0.07	0.06	0.70	0.09	
k	Living room	010203	1.99	0.35	0.16	0.09	0.21	0.03	0.04	0.30		Detached house
l		010122	1.98	0.37	0.14	0.09	0.27	0.06	0.07	0.47		14F
m		010119	2.03	0.38	0.19	0.15	0.34	0.04	0.08	0.54	0.08	Detached house
n		010116	2.13	0.42	0.40	1.39	0.66	5.68	5.03	3.18	2.83	Detached house
o		010206	1.80	0.57	0.49	0.18	0.66	0.05	0.07	0.76		7F
p		010107	1.94	0.45	0.28	0.14	0.29	0.07	0.07	0.65	0.07	Detached house
q		010113	1.77	0.57	0.49	0.18	0.66	0.05	0.07	0.76		Collective housing
r		010118	2.30	0.16	0.03		0.05		0.01	0.14		4F
s		010111	1.92	0.30	0.15	0.13	0.37	0.04	0.06	0.99	0.06	Detached house
t		010221	1.91	0.57	0.24	0.19	0.26	0.07	0.07	0.60	0.11	Detached house
	Average value			0.41	0.26	0.28	0.38	0.68	0.56	0.84	0.63	
k	Bedroom	010203	2.03	0.30	0.16	0.11	0.22	0.04	0.05	0.37		Detached house
l		010122	1.98	0.37	0.10	0.08	0.19	0.05	0.06	0.34		14F
m		010119	2.09	0.33	0.16	0.11	0.29	0.06	0.05	0.65		Detached house
n		010116	2.13	0.47	0.29	0.17	0.50	0.09	0.12	0.80	0.13	Detached house
o		010206	1.76	0.38	0.15	0.09	0.24	0.05	0.05	0.42		7F
p		010107	0.51	0.51	0.27	0.12	0.29	0.06	0.07	0.66	0.06	Detached house
q		010113	1.77	0.48	0.45	0.21	0.65	0.06	0.10	0.78		Collective housing
r		010118	2.13	0.37	0.13	0.06	0.15		0.03	0.20		4F
s		010111	2.00	0.48	0.26	0.15	0.45	0.13	0.06	0.64	0.04	Detached house
t		010221	2.04	0.52	0.15	0.13	0.29	0.06	0.07	0.51	0.11	Detached house
	Average value			0.42	0.21	0.12	0.33	0.07	0.07	0.54	0.09	
k	Outdoor	010203	2.02	0.62	0.20	0.11	0.17	0.05	0.04	0.37		Detached house
l		010122	1.98	1.57	0.75	0.31	0.77	0.07	0.12	1.22	0.12	14F
m		010119	2.01	1.05	0.58	0.30	0.58	0.10	0.11	1.02	0.11	Detached house
n		010116	2.04	1.90	0.87	0.32	0.90	0.10	0.14	1.76	0.14	Detached house
o		010206	1.74	0.92	0.38	0.18	0.36	0.06	0.08	0.70		7F
p		010107	1.93	0.58	0.19	0.13	0.15	0.05	0.04	0.41		Detached house
q		010113	1.67	2.19	1.28	0.57	1.27	0.15	0.21	1.61	0.17	Collective housing
r		010118	1.93	0.69	0.22	0.09	0.21	0.04	0.03	0.34	0.07	4F
s		010111	1.97	1.09	0.46	0.21	0.45	0.06	0.07	0.73	0.05	Detached house
t		010221	2.01	1.11	0.12	0.13	0.32	0.04	0.06	0.50		Detached house
	Average value			1.17	0.50	0.24	0.52	0.07	0.09	0.87	0.11	

* With smoker in b and h

Table 5. Analysis of PAH in indoor air and outdoor air in Tokyo (winter)

health. Therefore, it is crucial to identify the amounts of various hazardous chemical substances such as carcinogens and mutagens in air, the sources of those substances and the exposure of the human body to them. However, there have been few reports on outdoor and indoor air pollution in China and the actual conditions of air pollution remain largely unknown. Therefore, in this study, we collected indoor and outdoor air samples in Chengdu, one of the biggest cities in China, and measured the mutagenicity of the carcinogens contained in the samples. By using a concentrating column, fast chromatography and spectral fluorescence detector, we simultaneously measured the concentration of PAHs. We used the ultramicro forward mutation assay for measurement of mutagenicity. Mutation tests, which use microbes, are widely conducted to measure environmental samples because the tests can easily measure mutagenicity. However, the Ames method, which is the typical method for mutation tests, is not suitable for measuring small amounts of samples because of insufficient detection sensitivity. Therefore, when measuring small amounts of samples, it is necessary to collect samples many times, accumulate and concentrate them to yield sufficient amounts of sample suited for the detection sensitivity. However, with this approach the time resolution decreases, more solvent is needed for extraction, and the work requires more time, cost and labor. In this study, we had to divide the obtained samples into small amounts because we had to measure and analyze the indoor SPMs at the same time. Therefore, we could only use smaller amounts of samples for measuring mutagenicity than usual. Taking these factors into account, we looked for a test method which had higher detection sensitivity and could detect mutagenicity with small amounts of samples, and decided to use the ultramicro forward mutation method which requires only about one-hundredth of the sample amount compared with the Ames method to measure the mutagenicity of all the samples.

4.1 Measurement and comparison of mutagenicity in indoor and outdoor SPMs in ordinary family homes in Chengdu in summer and winter

In general, regarding measurement results obtained by the Ames method, it is well known that the mutagenicity of SPMs in the metropolitan area is highly influenced by direct mutagens (Matsushita et al, 1990; Takagi et al, 1988, 1994; Tamakawa et al, 1989). Our preliminary measurement results showed that the value was lower when S9mix was not added than when it was added, so we conducted the mutation tests without adding S9mix. It is known that the mutagenicity of outdoor SPMs is generally higher in autumn and winter than in spring and summer (Muramatsu et al, 1983; Ohtani et al, 1985; Watanabe & Hirayama, 1997). In the measurement this time, the mutagen-specific activity was two times higher in the kitchen and bedroom in winter than in summer, and four times higher in the living room and outdoors. We think that this phenomenon was due to the geographical conditions of Chengdu and the type of energy source. In 2000, China's dependence on coal for energy still exceeded 70%. Large trucks and tractors are widely used for distributing goods, and as the number of automobiles increases rapidly, the consumption of oil is increasing dramatically. However, China has no choice but to depend on its abundant coal resources rather than oil for its industrialization. At the time we collected the air samples, large amounts of coal were being used by industry and for producing coke (51.7%), power generation (28.6%), and living such as district heating, indoor heating and cooking (17.7%) . Coal is widely used for indoor heating and cooking, but some houses are not equipped with adequate ventilation systems, and the consumption of coal increases significantly in winter.

Therefore, large amounts of mutagens are formed and emitted into indoor and outdoor air. On the other hand, the value was high in the kitchen in summer when mutagen-specific activity was generally low. This phenomenon was likely caused by contaminants discharged during cooking. The influence of smokers in households was not clear, suggesting that air pollution was so serious that the influence of smokers alone could not be detected. However, the result may also have been influenced by the sampling method (Koyano et al, 1999) such as sampler positions and indoor airflow and so the cause remains unclear.

4.2 Measurement and comparison of mutagenicity of indoor and outdoor SPMs in ordinary family homes in Chengdu and Tokyo

The mutagen-specific activity in Chengdu was about four times higher in the kitchen and the bedroom, about three times higher in the living room and about seven times higher outdoors than in Tokyo. We believe the cause of this phenomenon was as follows. In Tokyo, people use gas heating which is not likely to discharge mutagenic SPMs, air conditioners in their houses (Takagi et al, 1997), and air ventilators in their kitchens, while sophisticated measures are taken to prevent outdoor air pollution. In Chengdu, the mutagen-specific activity was higher outdoors than indoors whereas in Tokyo it was lower outdoors than indoors. Houses in Tokyo are sealed more tightly than those in Chengdu, people ventilate their rooms less in winter and so the contaminants accumulate in their rooms. In Tokyo, the air samples collected in collective housing showed higher values than those in detached houses. The reason is not clear, but since collective housing has fewer windows structurally and is not well ventilated, contaminants are more likely to accumulate. However, more samples and detailed studies are needed. In Tokyo, smokers' houses generally showed high mutagenic activity, and smoking was the major cause of indoor air pollution whereas the influence of heating and cooking was small.

4.3 Measurement and comparison of the concentration of polycyclic aromatic hydrocarbons contained in indoor and outdoor SPMs in Chengdu and Tokyo in winter

In Chengdu, the concentration of BaP was three times higher in the kitchen, eight times higher in the living room, and five times higher in the bedroom and outdoors in winter than in summer. Meanwhile, the concentration of BaP was 40 times higher in the living room, 50 times higher in the bedroom, and 30 times higher in the kitchen and outdoors in Chengdu in winter than in Tokyo in winter. The concentration was five times higher in the living room, six times higher in outdoor air, and 10 times higher in the kitchen and bedroom in Chengdu in summer than in Tokyo even in winter. Similar results were found for PAHs. As stated above, we think that the major causes were the differences between the two cities in geographical conditions, energy sources, ventilation systems and measures for preventing outdoor air pollution. Especially, the PAH concentrations became high in Chengdu in winter due to the increased use of coal for heating, its unique climate and geographical conditions. Most of the collaborators for this study lived in the south of Chengdu and the northwest monsoon may carry polluted air from the city center to the area where they lived. In addition, Chengdu is located in the Sichuan basin where there are many cloudy days in winter and an inversion layer tends to form, which is likely to increase the PAH concentrations in winter. In Chengdu in winter when the outdoor PAH concentrations were high, the indoor PAH concentrations were also high. We think that the significant outdoor

air contamination influenced the indoor air due to air ventilation. Meanwhile, comparing the indoor and outdoor PAH concentrations in Chengdu, BghiP > BaP > pyrene in both winter and summer. In Tokyo in winter, BghiP > pyrene > BaP. Therefore, in general, the distributions of respective PAH concentrations are not always the same between areas where the PAH concentrations are high and other areas where the concentrations are low. This time, we measured and studied the mutagenicity of SPMs and concentration of PAHs, but could not measure gas/vapor matter because good standards have not been established for the sampling method. Recently, some studies have focused on gas/vapor matter as hazardous outdoor air contaminants (Watanabe & Hirayama, 1997), and so it is important to identify the actual conditions of indoor and outdoor air pollution caused by gas/vapor matter to better understand environmental pollution. We also must measure and study harmful substances to establish proper countermeasures against general environmental pollution.

5. Conclusion

We studied the evaluation of mutagens for small amounts of indoor air. By using the TM677 strain, which is much more sensitive than the commonly-used Ames method, we confirmed that we could measure the mutagenicity with only about 1440 L of samples (1 L/min × 24 hours). We measured air samples collected from ordinary family homes in Chengdu and Tokyo by using this new method and found that the mutagenicity was higher in Chengdu than in Tokyo and higher in winter than in summer.

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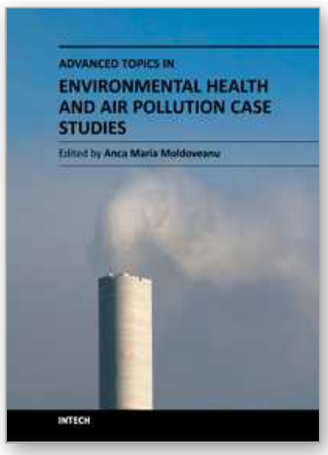
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The book describes the effects of air pollutants, from the indoor and outdoor spaces, on the human physiology. Air pollutants can influence inflammation biomarkers, can influence the pathogenesis of chronic cough, can influence reactive oxygen species (ROS) and can induce autonomic nervous system interactions that modulate cardiac oxidative stress and cardiac electrophysiological changes, can participate in the onset and exacerbation of upper respiratory and cardio-vascular diseases, can lead to the exacerbation of asthma and allergic diseases. The book also presents how the urban environment can influence and modify the impact of various pollutants on human health.

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