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Mutagenicity and Nitropyrene Concentration of Indoor Air Particulates Exhausted from a Kerosene Heater*1

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The particulates in a room warmed with a radiant kerosene heater were collected, extracted and fractionated into diethyl ether-soluble neutral, acidic and basic fractions. The mutagenicity of these fractions was measured with *Salmonella typhimurium* strains TA98, TA98NR, TA98/1,8-DNP$_6$ and TA100 in the presence and absence of S9 mix. Room air without the heater showed very low mutagenicity. However, a sample from a room at the beginning of the burning period showed very high mutagenicity (237 His$^+$ revertants/plate/m^3 of air in strain TA98 in the absence of S9 mix). In contrast, emissions from the heater after it was burning stably showed low mutagenicity (9 His$^+$ revertants/plate/m^3). The crude extract of particulates from the heater at the beginning of the burning period was analyzed by high-pressure liquid chromatography (HPLC) and showed a considerable amount of nitropyrenes (NPs); the concentrations of 1-NP and 1,6-diNP were 1.62 ng and 0.149 ng/m^3 of air, respectively, and accounted for 1.2% and 17.6%, respectively, of the mutagenicity in strain TA98 in the absence of S9 mix. In addition, an HPLC-Ames histogram showed that peaks of mutagenicity corresponding to 1-NP and diNPs accounted for 75.7% (1-NP, 4.9%; 1,6-diNP, 17.1%; 1,8-diNP, 46.3%; 1,3-diNP, 7.4%) of the HPLC-recovered mutagenicity for strain TA98 without S9 mix. These results suggest that kerosene heaters, especially immediately after ignition, create mutagenic substances such as NPs.

Key words: Nitropyrene — Mutagenicity — Indoor air pollution — Kerosene heater — Nitrogen dioxide

Lung cancer mortality has been increasing in Japan. Although epidemiologic studies have not provided definitive evidence that air pollution is a cause of lung cancer, they tend to suggest that the incidence of lung cancer is elevated in urban environments and that carcinogens found in air particulates may be contributing factors.[1,2] Mutagenic activity was observed in ambient particulate samples from industrial and urban areas[3-6] and particulate matter from special sources such as automobile and diesel exhaust[7-10] and fly ash from various combustion processes.[11] Recently, Walker *et al.*[12] reported that mutagenicity present in air particulates has a strong association with lung cancer mortality. The high correlation also supports the hypothesis that mutagenicity of air particulates may be useful as an indicator of the carcinogenic potential of air pollution.

Until recently, air pollution was associated with air contaminants of industrial workplaces and with urban air including automobile exhaust. However, most people spend considerable amounts of time, in many instances 80 to 90% of each day, indoors in homes, offices or other workplaces, and commercial and public buildings.[13-15] Furthermore, employed men and women spend about 50 to 63% of their lives in their homes and homeworkers spend about 82 to 90% of their lives in their homes.[15] Therefore, exposure to indoor air pollutants, especially in houses, may be important for human health.

A number of indoor pollutants, including cigarette smoke, radon and radon decay prod-

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ucts, and combustion by-products, such as polycyclic aromatic hydrocarbons (PAHs),[*4] nitrogen dioxide (NO_2), carbon monoxide (CO) and sulfur dioxide (SO_2), have been reported to be associated with adverse health effects.[13-17] van Houdt *et al.*[18] reported that, with respect to the mutagenic activity of particulate matters, cigarette smoke is the most important indoor air pollutant. A major portion of mutagenic activity in air samples, excluding that contributed by cigarette smoke, originates from various combustion sources.[19,20] Combustion of fuel can produce CO, CO_2, SO_2, NO_2, formaldehyde, hydrocarbons and various particulates[13,21,22] containing pyrene, benzo[*a*]pyrene and other PAHs.[19,23] Such fuels are consumed mainly in heating and cooking in homes. We have measured the mutagenicity of indoor air particulates in a kitchen where city gas is used for cooking, and showed that when chicken was grilled over a city gas flame, the mutagenicity increased. In addition, the mutagenicity decreased when the grilling was stopped or when the area was ventilated.[24] These results confirmed previous observations[25,26] and suggested that mutagenic compounds volatilized during cooking. Kerosene heaters, convective and radiant, are widely used in Japanese residences because of their efficiency and low cost. The combustion products from these heaters are important indoor pollutants.

Nitropyrenes (NPs) are ubiquitous in the environment as a result of various incomplete combustion processes.[27-29] Their widespread occurrence is not surprising because NPs are readily formed by exposure of pyrene to nitrogen dioxide.[30] Furthermore, the tumorigenicity of NPs has been demonstrated in laboratory animals.[27-29] Therefore, we measured the mutagenicity and NP content of indoor air particulates in a room warmed with a radiant kerosene heater.

Materials and Methods

Collection, Extraction and Fractionation of Indoor Air Particulates The particulates in a 28-m^3 room, warmed with a radiant kerosene heater, were collected many times for 20-min periods on a Teflon-coated filter (Pallflex T60A20) by a high-volume sampler (Kimoto Electric Co., Ltd., Osaka, model HV-120) at a speed of 1.5 m^3/min. The amount of fuel consumption by the heater was 363 ml/hr. The particles on the filters were extracted with benzene-ethanol (4:1) by ultrasonication.[10] The extracts were then fractionated into diethyl ether-soluble neutral, acidic and basic fractions according to the procedure described previously.[32]

Mutagenicity Assay The bacterial strains used for mutation assays were *Salmonella typhimurium* strains TA98, TA100, TA98NR and TA98/1,8-DNP_6. Each sample to be tested was dissolved in dimethyl sulfoxide (DMSO) and the mutagenic activity was measured by the Ames mutagenicity test with preincubation at 37° for 20 min as described previously.[10,24]

Measurement of NP Concentration The procedure for measuring 1-NP and 1,6-diNP was described previously.[10,24,32]

Measurement of NO_2 Concentration NO_2 concentration was measured by the method of Saltzman.[33]

High-pressure Liquid Chromatography High-pressure liquid chromatography (HPLC) was performed with a Shimadzu LC-3A coupled to a Shimadzu SPD-2A variable-wavelength UV detector. The column temperature was maintained at 50°. HPLC was used for analysis of the crude extract of the indoor air particulates collected from a room containing emissions from a kerosene heater for the initial 20-min burning period. The sample was applied to a Chemcosorb 5-ODS-H column (4.6×250 mm) (Chemco Scientific Co., Ltd., Osaka) and eluted with 60% methanol at 2 ml/min. Fractions were collected in sterilized collection tubes every 30 sec. The solvent was evaporated off (Tomy concentrator CC-180, Tomy Seiko Co., Ltd., Tokyo) and the residue was dissolved in 0.1 ml of DMSO. These tubes were used for the mutagenesis assay (TA98, without S9 mix).

Chemicals 1-NP and 1,6-diNP were purified to 99.9% purity with 70% acetonitrile or 70% methanol by HPLC as described previously.[34,35] Glucose 6-phosphate (G-6-P) was obtained from Sigma Chemical Co., St. Louis, MO, and NADPH and G-6-P dehydrogenase were obtained from Oriental Yeast Co., Ltd., Tokyo. All other chemicals were of reagent grade or higher quality, and were purchased from Wako Chemical Industries, Osaka.

Results

Mutagenicity of Indoor Air Particulates We measured the mutagenicity of indoor air particulates and NO_2 concentrations in a room

[*4] Abbreviations: 1-NP, 1-nitropyrene; diNP, dinitopyrene; HPLC, high-pressure liquid chromatography; DMSO, dimethyl sulfoxide; PAH, polycyclic aromatic hydrocarbon; NF, nitrofluoranthene.

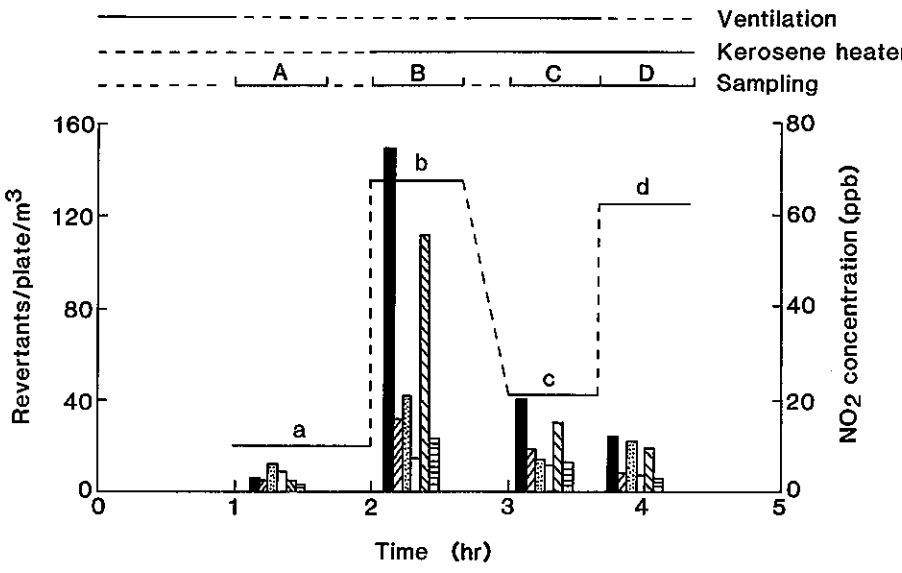

Fig. 1. Mutagenicity and NO_2 concentration of air in a room heated with a kerosene heater. Air particulates were collected on Teflon-coated filters by a high-volume sampler at a speed of 1.5 m^3/min. The filters were then extracted with benzene-ethanol and mutagenicity was assayed. Solid lines in the upper panel indicate the time schedule of ventilation, heating with the kerosene heater and sampling of air particulates in the room. Sampling time (solid line of the bottom line in the upper panel) for the air particulates of A (without heater), B (beginning of burning), C (after ventilation), D (burning stably) was 40 min (20 min × 2). During samplings B, C, and D, the kerosene heater was used. The NO_2 concentration was measured by the method of Saltzman.[33] Sampling times for room air of a (without heater), b (beginning of burning), c (after ventilation) and d (burning stably) were 60, 40, 40 and 40 min, respectively. The NO_2 concentrations of a, b, c, and d were 10.2, 68.1, 21.7, and 64.1 ppb, respectively. Symbols; ■, TA98 (−)S9; ▨, TA98 (+)S9; ▩, TA100 (−)S9; □, TA100 (+)S9; ▧, TA98NR (−)S9; ▤, TA98/1,8-DNP_6 (−)S9.

warmed with a kerosene heater. The results are shown in Fig. 1. The mutagenicity and NO_2 concentration of indoor air before heating were very low (Fig. 1, A and a). When a kerosene heater was ignited, mutagenic compounds and NO_2 were produced (Fig. 1, B and b). Both mutagenicity and the NO_2 concentration were decreased by ventilation (Fig. 1, C and c). The NO_2 concentration increased on stopping the ventilation; however, the mutagenicity did not increase (Fig. 1, D and d). These results suggest that the NO_2 was continuously produced during the combustion of kerosene in the kerosene heater but mutagens were produced only at the beginning of the burning period. The mutagenicity of air particulates from the kerosene heater was higher for *Salmonella typhimurium* strain TA 98 than for strain TA100 and was higher in the absence of S9 mix than in its presence. In addition, in the absence of S9 mix, the mutagenicity was greater in TA98 than in strains TA98NR and TA98/1,8-DNP_6 (Fig. 1). These results suggested that the indoor air particulates from the kerosene heater might contain nitroarenes. Therefore, in order to compare the mutagenicity with the amount of mutagenic compounds, we collected indoor air particulates from the room with the kerosene heater on a large scale. The sampling methodology is shown in Fig. 2. For convenience, control, continuous, initial and stable samples were designated as samples E, F, G, and H, respectively (Figs. 2 and 3 and Tables I and II). The indoor air particulates were produced to a greater extent at the beginning

of burning (0.2 mg/m^3) than after the heater was burning stably (0.027 mg/m^3) (Table I-Exp. I). The particulates were extracted and the mutagenicity was measured. Typical linear dose-responses were observed in strains TA98 and TA100, as shown in Fig. 3. Table I-Exp. I presents average values of mutagenic activities determined in Fig. 3. These results (Fig. 3 and Table I-Exp. I) show that the mutagenic activity, except in the control

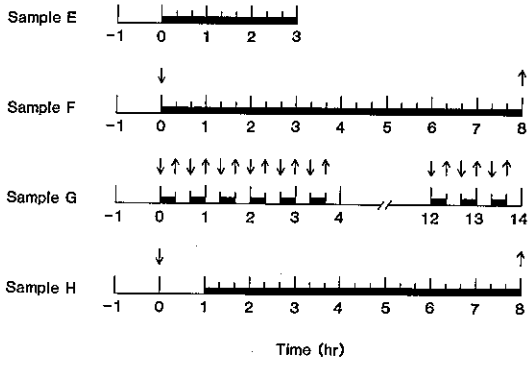

Fig. 2. Sampling methodology of indoor air particulates from a kerosene heater. Sample E was collected in the room without the heater. Sample F was collected continuously for 8 hr from the time of lighting the heater. Sample G was collected 21 times for 20 min at the beginning of burning with intervals of 20 min ventilation. Sample H was collected continuously for 7 hr after 60-min ventilation with burning just after lighting the heater. The filter was changed every 20 min. Then the particulates were extracted with benzene-ethanol (4:1). Symbols: ↓, ignition; ↑, extinction. Thick line, collection of air particulates; thin line, ventilation.

Table I. Mutagenicity of Indoor Air Particulates Exhausted from a Kerosene Heater

Experiment I (Crude extract)

Sample	Particle weight (mg/m^3)	Revertants/plate/m^3			
		TA98		TA100	
		(−)S9	(+)S9	(−)S9	(+)S9
E	ND[a]	1.7	1.2	2.1	1.2
F	0.079	34.9	5.4	24.8	3.7
G	0.20	237	1.4	79.2	12.1
H	0.027	8.9	3.7	7.8	3.7

a) Not determined.

Experiment II (After fractionation, without S9 mix)

Sample	Fraction	Revertants/plate/m^3 (% of TA98)		
		TA98	TA98NR	TA98/1,8-DNP$_6$
E	Crude	1.7	1.2	1.1
F	Crude	34.9 (100)	23.7 (68.0)	7.0 (20.0)
	Neutral	48.6 (100)	34.7 (71.5)	3.6 (7.5)
	Acidic	2.4	0.3	0.2
	Basic	0.3	0.2	0.2
G	Crude	237 (100)	133 (56.7)	17.8 (7.5)
	Neutral	206 (100)	46.4 (22.5)	12.9 (6.3)
	Acidic	1.7	0.2	0.8
	Basic	5.3	0.9	3.8
H	Crude	8.9 (100)	5.7 (64.5)	4.0 (45.2)
	Neutral	5.1 (100)	4.1 (80.6)	1.3 (25.8)
	Acidic	3.1	1.6	1.3
	Basic	3.2	1.6	2.1

sample, is higher for strain TA98 that for TA 100. The indoor air particulates without a heater, sample E (control sample), showed very low mutagenicity, 1.7 His^+ revertants/plate/m^3 of air in strain TA98 in the absence of S9 mix. However, sample G (initial sample), containing emissions from the first 20 min of burning, showed very high mutagenicity, 237 His^+ revertants/plate/m^3. In contrast, sample H (stable sample) showed low mutagenicity, 9 His^+ revertants/plate/m^3. Sample F (continuous sample) showed 35 His^+ revertants/plate/m^3 (Table I-Exp. I). Therefore, the mutagenicity of the stable sample was reduced approximately 27-fold and 4-fold in comparison to the mutagenicity of the initial sample and continuous sample, respectively. These samples were fractionated into diethyl ether-soluble neutral, acidic and basic fractions and the mutagenicity was measured with strains TA98, TA98NR and TA98/1,8-DNP_6 (Table I-Exp. II). Most of the mutagenicity was recovered in the neutral fraction. Furthermore, the mutagenicity of the neutral fraction decreased in the order TA98 > TA98NR > TA98/1,8-DNP_6, which suggested that this fraction contained NPs, especially diNPs.

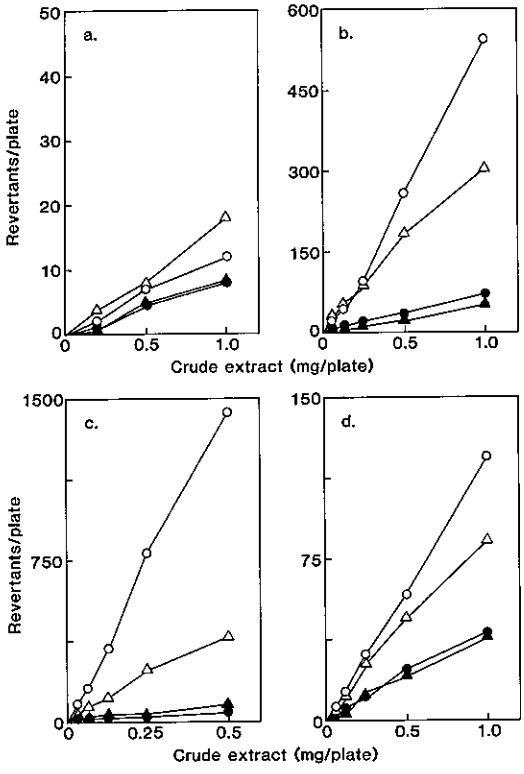

Fig. 3. Dose-response curves of mutagenicity of crude extracts of indoor air particulates. Symbols: ○, TA98 (−)S9; ●, TA98 (+)S9; △, TA100 (−)S9; ▲, TA100 (+)S9. a, control sample; b, continuous sample; c, initial sample; d, stable sample.

Determination of 1-NP and 1,6-diNP Contents We determined the concentrations of 1-NP and 1,6-diNP in the crude extract (Table II). The amounts of 1-NP and 1,6-diNP in sample G (initial sample) were 1.62 and 0.149 ng/m^3, respectively, and accounted for 1.2 and 17.6%, respectively, of the mutagenicity in strain TA98 in the absence of S9 mix. However, in sample H (stable sample), the amounts of 1-NP and 1,6-diNP were smaller than those in the initial sample; 0.044 and 0.001 ng/m^3, accounting for 0.8 and 4.4% of the mutagenicity, respectively. Sample F (continuous sample) contained 0.147 and 0.025 ng/m^3 of 1-NP and 1,6-diNP, respectively, accounting for 0.7 and 20.1%, respectively, of the mutagenicity of particles ex-

Table II. Mutagenicity and Nitropyrene Concentration of Indoor Particulates Exhausted from a Kerosene Heater

Sample	Revertants/plate/m^3 of air			ng/m^3		% Mutagenicity[a]	
	TA98	TA98NR	TA98/1,8-DNP_6	1-NP	1,6-diNP	1-NP	1,6-diNP
E	1.7	1.2	1.1	ND[b]	ND	ND	ND
F	34.9	23.7	7.0	0.147	0.025	0.7	20.1
G	237	133	17.8	1.62	0.149	1.2	17.6
H	8.9	5.7	4.0	0.044	0.001	0.8	4.4

a) 1-Nitropyrene and 1,6-dinitropyrene induced 1.69 and 280 His^+ revertants/plate/ng, respectively.
b) Not determined.

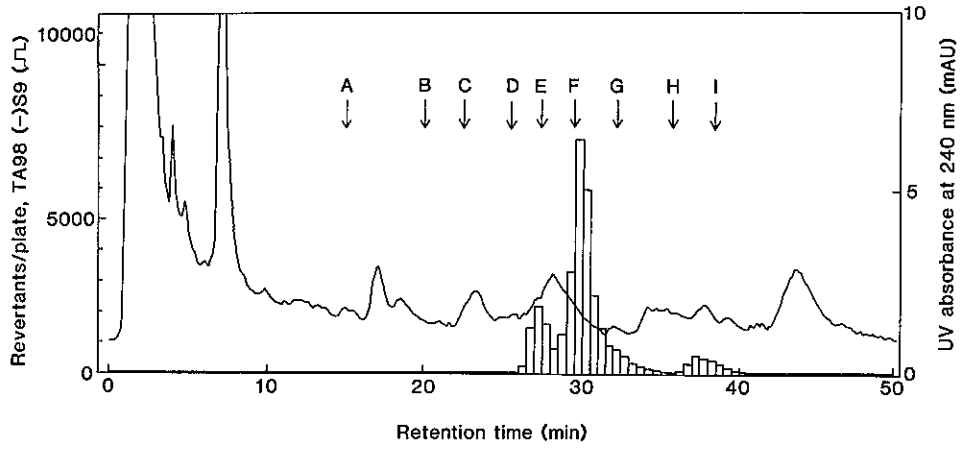

Fig. 4. HPLC pattern of the mutagenicity of benzene-ethanol extract of particulate emissions from a kerosene heater at the beginning of burning (initial sample). The sample (46,100 revertants/plate) was applied to a Chemcosorb 5-ODS-H column (4.6×250 mm) and eluted with 60% methanol at 2 ml/min. Fractions of 1 ml each were collected for measurement of mutagenicity in *Salmonella typhimurium* strain TA98 in the absence of S9 mix. The solvent of each fraction was evaporated off and the residue was dissolved in 0.1 ml of DMSO and assayed for mutagenicity. Arrows indicate the retention times of standard chemicals: A, 1-nitro-6/8-hydroxypyrene; B, 1-nitro-6/8-acetoxypyrene; C, 1-nitro-3-hydroxypyrene; D, 1-nitro-3-acetoxypyrene; E, 1,6-diNP; F, 1,8-diNP; G, 1-NP; H, 3-nitrofluoranthene; I, 1,3-diNP.

hausted from the kerosene heater for 8 hr. These results suggest that the particulates from the kerosene heater at the beginning of burning contained mutagenic NPs, especially diNPs.

In order to determine if other mutagenic compounds were present, the crude extract of the initial sample was separated by HPLC and 30 sec fractions were collected for mutagenicity assay with strain TA98 in the absence of S9 mix. Figure 4 shows the UV absorbance and Ames histogram of the crude extract of the initial sample. The total recovery of mutagenicity from the HPLC was 80.2% for strain TA98 in the absence of S9 mix. When analyzed by HPLC, three mutagenic peaks (E, F and I) were observed and these peaks corresponded to 1,6-diNP (13.7 and 17.1% of the HPLC-applied mutagenicity and of the HPLC-recovered mutagenicity, respectively), 1,8-diNP (37.1 and 46.3%) and 1,3-diNP (5.9 and 7.4), respectively. The peak corresponding to 1,8-diNP (peak F) had the highest mutagenic activity. Fractions 64 and 65, corresponding to 1-NP, had 3.9 and 4.9% of the HPLC-applied mutagenicity and of the HPLC-recovered mutagenicity, respectively. Other NP derivatives, nitrohydroxypyrenes, nitroacetoxypyrenes and nitrofluoranthene (NF), were not detected on the HPLC-Ames histogram. The total concentration of 1-NP and diNP isomers in the particulate extract accounted for 60.6% of HPLC-applied mutagenicity and 75.7% of the HPLC-recovered mutagenicity.

Discussion

The increase in energy cost as a result of the energy crisis has caused a substantial change in fuel use patterns. This has led to an increase in pollutant levels in indoor air, especially in places where the air exchange rates have been reduced, or heaters that are not vented to the outside (e.g., kerosene heaters) are used. Even if the concentration of indoor air pollutants is low, it may make a substantial contribution to the time-weighted exposure.

Several investigators have reported the presence of pollutants such as NO_2, SO_2, CO, and particulate matter in the emissions from

kerosene heaters.[36, 37] In addition, Kaden *et al.*[19] reported that kerosene soot, obtained by burning kerosene fuel, contained various PAHs (e.g., acenaphthylene, pyrene, fluoranthene, naphthalene, benzo[*a*]pyrene, etc.). However, the mutagenicity of the soot extract was 10 to 20 times higher than could be accounted for by the amount of benzo[*a*]-pyrene present. Recently, Tokiwa *et al.*[38] demonstrated that NPs were generated by kerosene heaters. Our results confirm and extend their study by indicating that the particulates from the beginning of the burning period were more mutagenic and contained higher concentrations of NPs than particulates from the heater after it was burning stably. We also measured the pollutant levels that resulted from the residential use of a kerosene heater. The high mutagenicity of the initial sample may be due to the difference of combustion efficiency between the initial phase and the stable phase of combustion or due to evolution of widely-adsorbed mutagenic compounds. Many kinds of mutagenic PAHs can be exhausted at the beginning of burning of organic fuel, because the initial phase is at a low temperature, causing incomplete combustion. In contrast, since the stable phase is at a high temperature, and fuel and wick-adsorbed compounds are completely burned out, production of mutagenic activity can be minimized in the stable phase.

The mutagenic activity of each particulate extract was determined in the *Salmonella typhimurium* bioassay using strains TA98, TA 98NR and TA98/1,8-DNP_6. Since strains TA 98NR and TA98/1,8-DNP_6 are defective in specific activating enzymes (i.e., nitroreductase and transacetylase, respectively), they show low mutagenicity in the presence of 1-NP and diNPs, respectively.[39] Both strains exhibited reduced mutagenic responses, and this was especially pronounced with strain TA 98/1,8-DNP_6 (Fig. 1 and Table I-Exp. II). This suggested that diNPs are responsible for most of the mutagenicity observed. In addition, the elution profile of the HPLC revealed that the mutagenicity of the initial sample was distributed in the fractions approximately corresponding to diNPs (Fig. 4). Furthermore, chemical analysis of the kerosene-heater particulate extract showed considerable amounts of 1-NP and 1,6-diNP (Table II). Although 1,6-diNP was formed in a much lower concentration than 1-NP, the amounts of 1,6-diNP and other diNPs accounted for a major fraction of the mutagenic activity because of the high specific mutagenicity of diNPs. Salmeen *et al.*[9] suggested that NF and diNPs are the principle mutagens in diesel emission particulates. We also suggested that nitrohydroxypyrenes, nitroacetoxypyrenes and diNPs are the main mutagens in diesel particulates.[10] However, NF, nitrohydroxypyrenes and nitroacetoxypyrenes were not detected in the kerosene particulates as principle mutagens (Fig. 4).

PAHs are produced by incomplete combustion of fuel. Since nitro-PAH derivatives are formed easily by nitration of PAHs with NO_2 under acidic conditions.[30, 40] NPs in indoor air pollutants may be artifacts produced during filter sampling of the particulates. Schuetzle[41] reported that nitro-PAH formation during filter sampling of diesel particulates collected on Teflon filters from diluted exhaust containing less than 3 ppm of NO_2 can be minimized by sampling for less than 23 min at temperatures less than 43°. Under these conditions, Schuetzle[41] estimated that the maximum conversion of pyrene to NPs would be 10% of the total NPs. The particulates exhausted from the kerosene heater in our experiments were collected under these conditions. In addition, the NO_2 concentration in the experiment was very low, at most 0.1 ppm (Fig. 1). Therefore, it is improbable that formation of all of the NPs is artificial.

Since the main route of human exposure to kerosene heater emissions is by inhalation, reports that the administration of diNPs by the respiratory route or directly into the lung induced lung cancer are matters of primary concern.[42, 43] Sun *et al.*[44] reported that in rats exposed to radioactive 1-NP, which was coated on gallium oxide particles, the majority of the radioactivity appeared in the feces, presumably due to mucociliary clearance followed by ingestion. Exposure to NO_2 has been associated with toxicological effects including pulmonary edema, bronchoconstriction and an increase of infection rates.[13–17] Furthermore, exposure to NO_2 has an adverse effect on host defense systems such as mucociliary clearance.[45, 46] A decrease of mucociliary clearance would be expected to increase

the residence time of particulates in the lung. When such particulates become accumulated in the lung, they may be harmful.

The concentration of NO_2 and mutagenicity were decreased (about 70%) by ventilation (Fig. 1). This decrease indicates that pollutants can accumulate in a house as a result of using heaters that are not vented to the outside. Since the concentration of NPs during the first 20 min after lighting the kerosene heater was very high, rooms should be ventilated by opening the window and using a ventilator and/or by air-cleaning with air filters just after lighting the kerosene heater. If the ventilation is effective, there should be relatively little effect of indoor combustion, even when kerosene heaters are used.

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