2-Nitrofluoranthene, 1-, 2- and 4-Nitropyrenes and 6-Nitrochrysene in Diesel-Engine Exhaust and Airborne Particulates

Tsuyoshi Murahashi, Ryoichi Kizu, Hitoshi Kakimoto, Akira Toriba, and Kazuichi Hayakawa*

Faculty of Pharmaceutical Sciences, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-0934, Japan

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Diesel-engine exhaust and airborne particulates were collected in downtown and suburban areas and five mutagenic nitroarenes (2-nitrofluoranthene (NF), 1-, 2- and 4-nitropyrenes (NPs) and 6-nitrochrysene (NC)) in benzene-ethanol extracts were determined by high-performance liquid chromatography with chemiluminescence detection. 1- and 4-NPs and 6-NC were found in diesel-engine exhaust particulates, however, 2-NF and 2-NP were not detected. On the other hand, all five nitroarenes were found in airborne particulates. Diurnal concentration patterns of 4-NP and 6-NC in the downtown area were similar to the pattern of 1-NP, but the patterns of 2-NF and 2-NP were different from the pattern of 1-NP. These results strongly suggested that 2-NF and 2-NP are formed in the atmosphere. Of the five nitroarenes, 2-NF had the largest suburban/downtown concentration ratio. 2- and 4-NPs and 6-NC all showed larger ratios than did 1-NP. These results showed that 2-NF and 2-NP were formed in the atmosphere, but that the secondary formation of 4-NP and 6-NC were not known. Taking mutagenic activity and the atmospheric concentration into consideration, the mutagenic contribution of 2-NF was estimated to be comparable with that of 1-NP in the downtown area, and more than that of 1-NP in the suburban area. This result suggested that the mutagenic contribution of the minor nitroarenes formed in urban air might not be negligible.

Key words —— 2-nitrofluoranthene, 2-nitropyrene, 4-nitropyrene, 6-nitrochrysene, diesel exhaust particulate, airborne particulate

INTRODUCTION

Airborne particulates (AP) contain carcinogenic and/or mutagenic nitroarenes.[1)] Among these nitroarenes, 1,3-, 1,6- and 1,8-dinitropyrenes (DNPs) exhibit strongest direct-acting mutagenicity.[2)] We have developed a high-performance liquid chromatographic (HPLC) method with chemiluminescence detection for the simultaneous determination of the three DNPs and 1-nitropyrene (NP)[3)] and analyzed these compounds in diesel exhaust particulates (DEP), gasoline exhaust particulates and AP.[4–7)] Their concentrations in urban air showed both diurnal and seasonal variations and diesel-engine vehicles were considered to be the main contributor.[8,9)]

Besides the above four nitroarenes, other carcinogenic and/or mutagenic nitroarenes such as 2-nitrofluoranthene (NF), 2- and 4-NPs and 6-nitrochrysene (NC) have been found in AP.[10–21)] As possible formation mechanisms for ambient 2-NF and 2-NP, gas phase reactions involving OH or NO_3 radicals have been considered[22–27)] since none of these nitroarenes except 6-NC has been found in DEP.[28–30)] However, the behavior of these nitroarenes in the atmosphere is not known because their ambient concentrations are very low. Although the concentrations of 2-NF and 2-NP in extracts from airborne particulates in several cities in the U.S.A. and Europe have been determined, their concentrations in Japan have not been determined.

We have recently developed an HPLC method for the determination of these nitroarenes in AP.[31)] Here we report the concentrations of 2-NF, 1-, 2- and 4-NPs and 6-NC in DEP and AP collected in downtown and suburban areas of Kanazawa, as determined by this method. We

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*To whom correspondence should be addressed: Department of Hygiene Chemistry, Faculty of Pharmaceutical Sciences, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-0934 Japan. Tel.: +81-76-234-4413; Fax: +81-76-234-4456; E-mail: hayakawa@dbs.p.kanazawa-u.ac.jp

also discuss their atmospheric behavior and relative mutagenicities.

MATERIALS AND METHODS

Chemicals——2-NF and 2- and 4-NPs were kindly provided by Dr. Yoshiharu Hisamatsu of the National Institute of Public Health (Tokyo, Japan) and 6-NC was kindly provided by Dr. Nobuyuki Sera of the Fukuoka Institute of Health and Environmental Science (Fukuoka, Japan). 1-NP and bis(2,4,6-trichlorophenyl) oxalate were purchased from Tokyo Kasei (Tokyo, Japan) and hydrogen peroxide (30% aqueous solution) was from Kanto Chemicals (Tokyo, Japan). All other chemicals were of analytical-reagent grade.

Sampling and Pretreatment Procedures for DEP——Three diesel-engine vehicles, a land cruiser (4000 cc, swirl chamber type, produced in 1987), a bus (6700 cc, direct injection type, produced in 1988) and a truck (7400 cc, direct injection type, produced in 1989), were used under idling conditions. A Sibata (Tokyo, Japan) L-30 low-volume air sampler was set at 5 cm from the end of the exhaust pipe of each idling vehicle. DEP samples were collected on 55 mm diameter PG60 poly(tetrafluoroethylene)-binding glass fiber filters (Advantec, Tokyo) that were exchanged every 1.5 min for 30 min.

The filters were cut into small pieces and placed in a flask. The nitroarenes were extracted ultrasonically twice with 200 ml of benzene/ethanol (3 : 1, v/v) for 15 min and then the solution was filtered through a No. 6 filter paper (Advantec) and then through a 0.45μm HLCDISK13 membrane filter (Kanto Chemicals). The filtrate was washed with 400 ml of water to remove sulfate. The benzene phase was evaporated to dryness and the residue was dissolved in 2 ml of acetonitrile. An aliquot of the solution was applied to the HPLC system.

Sampling and Pretreatment Procedures for AP——High-volume air samplers (Kimotodenshi, Osaka, Japan) were set at two sites in Kanazawa, Japan (population 450000): site K (1-1 Kata-machi) and site T (1-11 Taiyogaoka) (Fig. 1). Site K was 11 m away from a heavy traffic road in the downtown area. Site T was on the roof of a 3-story building in a suburban area 6 km southeast of site K. AP samples were collected on Pallflex Products (Putnam, CT, U.S.A.) 2500QAT-UP quartz fiber filters (20.3 × 25.4 cm) at a flow rate of 1.5 m^3/min.

A piece cut from each filter (20—400 cm^2) was cut

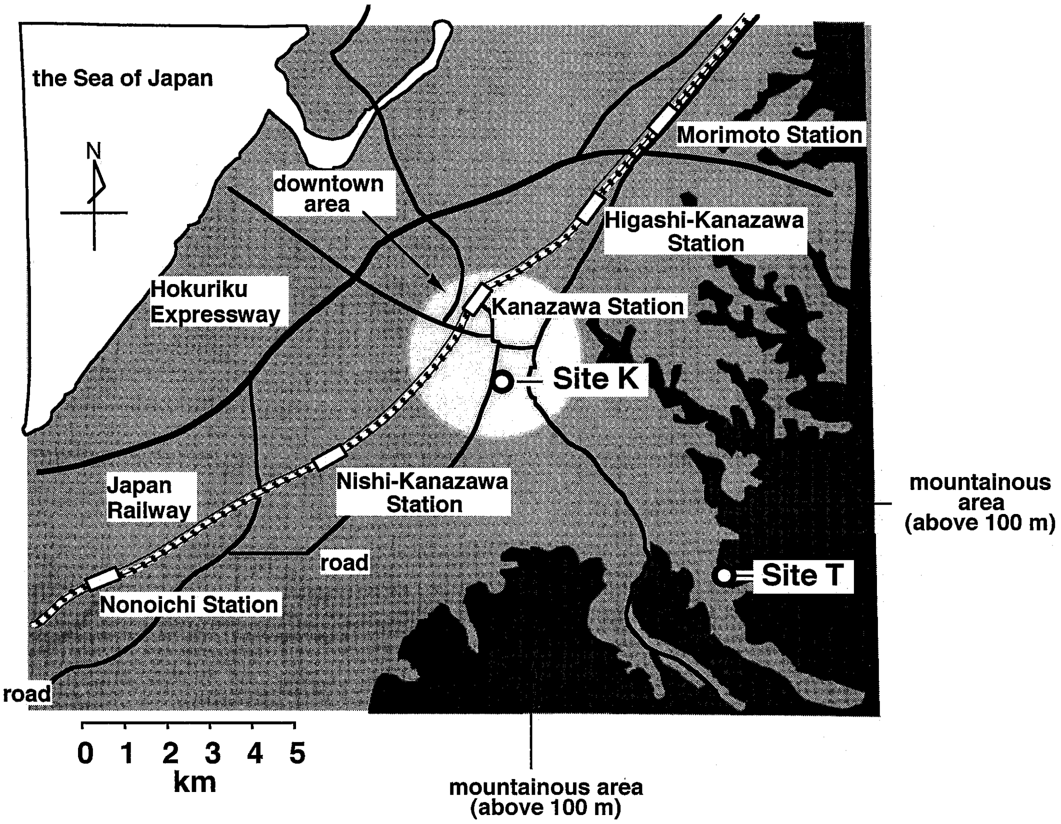

Fig. 1. Sampling Sites in Kanazawa City

into small pieces and placed in a flask. The nitroarenes were extracted ultrasonically twice with 10—200 ml of benzene/ethanol (3 : 1, v/v) for 15 min and then the solution was filtered through a No. 6 filter paper (Advantec) and then through a 0.45μm HLCDISK13 membrane filter. The filtrate was evaporated to dryness and the residue was dissolved in 0.3 ml of acetonitrile. An aliquot of the solution was applied to the HPLC system.

HPLC System——The system consisted of four pumps, an auto sample injector, a switching valve, a chemiluminescence detector, an integrator and a column oven with two separation columns (ODS), a reducer column (zinc/glass beads) and a concentration column (ODS), as previously described in detail.(31)

Mutagenicity Assay——Each nitroarene standard was dissolved in dimethyl sulfoxide. The mutagenicity assay was conducted according to the preincubation technique(32) using *Salmonella typhimurium* TA98 strain(33) without S9 mix. Two plates were used for each dose, and the mutagenic activities (the number of colony reversions/pmol of nitroarene) were calculated from linear regression lines fitted to the increasing portion of the dose-response curves.

RESULTS

Comparison of DEP and AP

Typical chromatograms of 2-NF, 1-, 2- and 4-NPs and 6-NC in extracts from DEP and AP are shown in Fig. 2. The large peak for 1-NP in Fig. 2(A) suggested that 1-NP was a major nitroarene emitted from diesel-engine vehicles and the large peak for 1-NP in Fig. 2(B) suggested that 1-NP was also a major nitroarene in urban air. Not only 4-NP and 6-NC, but also 2-NF and 2-NP were observed as minor nitroarenes in urban air (Fig. 2(B)). However, only 4-NP and 6-NC were observed in DEP (Fig. 2(A)). The concentrations of 2-NF, 1-, 2- and 4-NPs and 6-NC in three DEP samples and AP samples at site K are compared in Table 1.

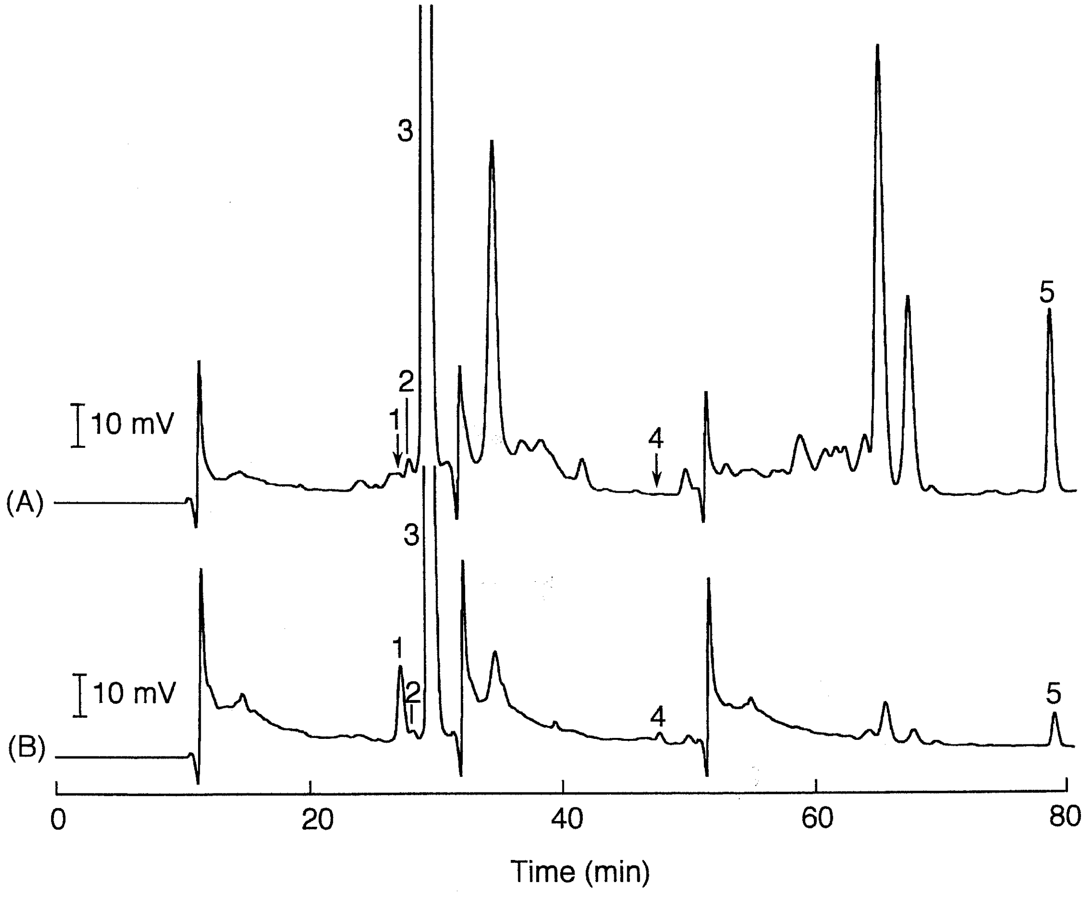

Fig. 2. Chromatograms of Extracts from DEP and AP

(A), 0.13 mg of DEP ; (B), 0.95 mg of AP. Peaks ; 1=2-NF, 2=4-NP, 3=1-NP, 4=2-NP, 5=6-NC. Arrows 1 and 4 on the chromatogram (A) indicate the elution times of 2-NF and 2-NP, respectively.

Table 1. Concentrations (pmol/mg) of 2-NF, 1-, 2- and 4-NPs and 6-NC in DEP and AP

Sample	2-NF	1-NP	2-NP	4-NP	6-NC
DEP					
Land cruiser	nd[a]	26	nd[b]	0.46	4.9
Bus	nd[a]	6.8	nd[b]	0.023	0.82
Truck	nd[a]	22	nd[b]	1.9	0.93
Mean±S.D.		18±10		0.79±0.98	2.2±2.3
AP (site K)					
Mean±S.D.	2.1±1.6[c]	3.8±2.3[c]	0.30±0.16[c]	0.053±0.027[c]	0.68±0.50[c]

a) Not detected (detection limit, 0.36 pmol/mg). *b)* Not detected (detection limit, 0.023 pmol/mg). *c)* n=12.

Table 2. Concentrations (fmol/m^3) of 2-NF, 1-, 2- and 4-NPs and 6-NC at Sites K and T in Kanazawa

Site	2-NF	1-NP	2-NP	4-NP	6-NC
Site K (n=12)[a]					
Min–Max	23–240	70–190	2.5–20	0.67–2.7	5.6–45
Mean±S.D.	90±79	130±40	12±6	1.9±0.6	24±13
Site T (n=12)[a]					
Min–Max	13–260	6.8–44	0.50–9.4	0.19–1.3	4.9–14
Mean±S.D.	53±68	15±8	3.4±2.5	0.57±0.31	7.6±3.3
T/K[b]	0.59	0.11	0.28	0.30	0.32

a) Twenty-four h AP samples were collected for three days in each of the four seasons in 1994 and 1995. *b)* Mean concentration at site T relative to mean concentration at site K.

Comparison of AP at Downtown and Suburban Sites

Airborne particulates were collected simultaneously at two different sites in Kanazawa, site K in a downtown area and site T in a suburban area. The sampling was performed on three consecutive days in each season for one year (from summer, 1994 to spring, 1995). The nitroarene concentrations at different times of the year at the two sites are compared in Table 2. The mean concentrations of all five nitroarenes at site K were higher than those at site T. However, the concentration orders of 1-NP and 2-NF were different at the two sites: 1-NP had the highest concentration at site K (130 fmol/m^3) while 2-NF had the highest concentration at site T (53 fmol/m^3). Although the concentrations of the other compounds were in the same decreasing order, 6-NC>2-NP>4-NP, at both sites, the ratios of the concentrations of the nitroarenes at the suburban site to their concentrations at the downtown site (T/K) varied in the range from 0.11 (1-NP) to 0.59 (2-NF).

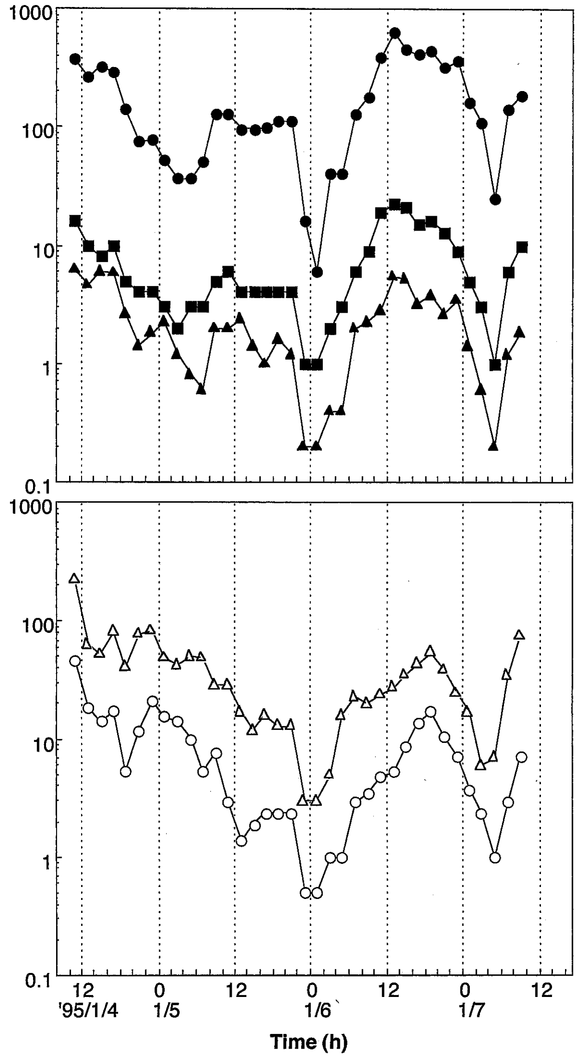

Fig. 3. Diurnal Concentrations (fmol/m^3) of 2-NF, 1-, 2- and 4-NPs and 6-NC in Winter at Site K

Diurnal and Seasonal Concentrations

Figure 3 shows the time courses of the five nitroarene concentrations for three days in winter (January 4—7, 1995) at site K. The highest concentrations of 4-NP and 6-NC were observed around 10 : 00—14 : 00 every day. These variations were similar to that of 1-NP, although the concentration levels were different. We previously reported that the increase of 1-NP concentration in the atmosphere was caused mainly by direct emission from diesel-engine vehicles.[9] However, the patterns for 2-NF and 2-NP were different from the pattern for 1-NP. For example, on January 6, the highest concentrations of 2-NF and 2-NP were observed around 18 : 00—20 : 00, which was about 6 h later than the time of highest concentration of 1-NP.

Figure 4 shows the seasonal concentrations

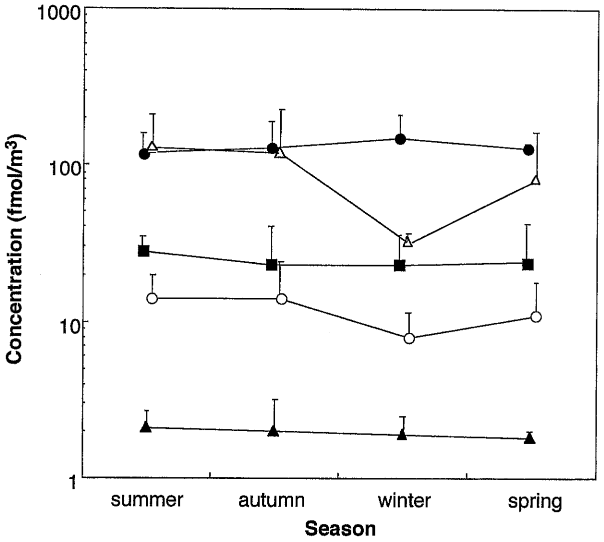

Fig. 4. Seasonal Concentrations (fmol/m^3) of 2-NF, 1-, 2- and 4-NPs and 6-NC at Site K

Each plot and bar indicate mean and S.D., respectively of three days.

of the five nitroarenes at site K. No clear seasonal differences were observed in the cases of 1- and 4-NPs and 6-NC, although a slight increase in 1-NP concentration was observed in winter. However, the concentrations of 2-NF and 2-NP were significantly lower in winter than in the other seasons.

DISCUSSION

At site K, the airborne sampler was set on the sidewalk of a T-junction of two heavy traffic roads. The average traffic volumes in daytime (6 : 00—18 : 00) and nighttime (18 : 00—6 : 00) were 2040 and 1250 vehicles/h, respectively, and the main contributors of 1-NP were not gasoline-engine vehicles but diesel-engine vehicles.[9] Moreover, there was no power plant or large factory in the vicinity of site K. Considering these facts, Fig. 1 and Table 1 suggested that both 2-NF and 2-NP were formed in the atmosphere after emission from diesel-engine vehicles.

A comparison of 1-NP concentration in DEP and AP (Table 1) and a comparison of AP at sites K and T (Table 2) indicate that 1-NP was diluted with other particles such as soil in the atmosphere. As other factors affect the nitroarene concentrations, it is possible that nitroarenes are formed or degraded in the atmosphere. In order to study these factors, the concentrations of nitroarenes at the suburban site (site T) were compared with those at the downtown site (site K). The concentrations of 4-NP and 6-NC at site T relative to those at site K (T/K) were larger than that of 1-NP, as shown in Table 2. Site T is 6 km southeast of downtown Kanazawa. The most frequent wind direction of the year at site T was northwest, suggesting that site T was usually downwind of downtown. At a wind speed of 1 m/sec, it takes 1 h and 40 min to move from downtown to site T. If little or no degradation of 1-NP occurred in the atmosphere, the larger concentration ratios of 2-NF and 2-NP might be due to the formation of these compounds during their transport.

Smog chamber experiments have shown that 2-NF and 2-NP are formed by a two-step reaction mechanism. For 2-NF, an OH radical first attacks position 3 of fluoranthene and then a NO_2 is added to position 2. Similarily for 2-NP, an OH radical first attacks position 1 of pyrene and then a NO_2 is added to position 2. It has been also reported that OH radicals can be formed by a photochemical reaction.[34] Therefore, the formation of OH radicals in the atmosphere is slow in winter in Kanazawa, because of snowy weather with low temperatures. These meteorological conditions might be the main reason why the concentrations of both 2-NF and 2-NP were lower in winter than in the other seasons (Fig. 4).

4-NP was detected not only in AP, but also in DEP in our study. However, there are no reports of the detection of 4-NP in DEP. The formation of 4-NP can only occur by a two-step reaction mechanism in which an OH radical and/or a NO_3 radical first attacks position 1 of pyrene, and then a NO_2 is added to position 4. This then becomes 4-NP by elimination of water or HNO_3. Studies carried out in smog chambers have shown that 4-NP can be generated by gas phase reactions, but in very low yield, when compared with 2-NP.[35,36] The high sensitivity of the HPLC method with chemiluminescence detection could easily detect 4-NP at trace levels in DEP. Another possible explanation for the detection of 4-NP in DEP in our study is that it was generated in the tail pipe or formed just after emission from the tail pipe. 6-NC was formed from chrysene and nitric acid.[37] The detection of 6-NC in DEP in our study supports the possibility that 6-NC was emitted directly from diesel-engine vehicles. Consequently, these results clarified only the formation of 2-NF and 2-NP in the atmosphere, and not

Table 3. Mutagenic Activities in *Salmonella typhimurium* Strain TA98 and Mutagenic Contributions of Nitroarenes

Compound	Mutagenic activity, rev./pmol	Mutagenic contribution, 10^3rev./m^3 [a]	
		Site K	Site T
2-NF	0.57	51	30
1-NP	0.48	62	7.2
2-NP	2.2	26	7.5
4-NP	3.7	7.0	2.1
6-NC	0.25	6.0	1.9

a) Mutagenic contribution was calculated by multiplying the mutagenic activity (rev./pmol) by the mean concentration (pmol/m^3) based on the yearly averages shown in Table 2.

the formation of 4-NP and 6-NC.

The mutagenic activities (rev./pmol) of the nitroarenes in the *S. typhimurium* TA98 strain were in the order 4-NP>2-NP>2-NF>1-NP>6-NC (Table 3). These results suggest that the longer the duration time of DEP in the atmosphere, the larger the amounts of strong mutagenic nitroarenes such as 2-NF and 2-NP. Therefore, the mutagenic contributions of the five nitroarenes at the two sites were obtained by multiplying their mutagenic activity (rev./pmol) by their concentration (pmol/m^3). The results are shown in Table 3. The value for 2-NF was the largest at site T and close to that of 1-NP at site K. This result suggested that the mutagenic contribution of 2-NF became larger in suburban areas or when the time the particulates were in the atmosphere was longer. It also suggested that the mutagenic contribution of nitroarenes such as 2-NF, formed in the atmosphere may not be negligible.

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