

Diurnal Concentrations of 1,3-, 1,6-, 1,8-Dinitropyrenes, 1-Nitropyrene and Benzo[*a*]pyrene in Air in Downtown Kanazawa and the Contribution of Diesel-Engine Vehicles

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Direct-acting mutagenic 1,3-dinitropyrene (1,3-DNP), 1,6-DNP, 1,8-DNP and 1-nitropyrene (1-NP) and indirect-acting mutagenic benzo[*a*]pyrene (BaP) in airborne particulates collected by the side of a busy intersection in downtown Kanazawa were determined by high-performance liquid chromatography (HPLC) with chemiluminescence and fluorescence detection. Time courses of their concentrations were high in the morning (8:00–10:00) and evening (16:00–20:00) and low from the midnight until early morning (0:00–6:00). Levels of each DNP (in the range of fmol/m³) and 1-NP (in the range of sub pmol/m³ or pmol/m³) were, respectively, more than three and about one order of magnitude lower than that of BaP. Large correlation coefficients (0.85–0.91) between their concentrations, traffic volume, and carbon monoxide and nitrogen monoxide concentrations suggested that the main source was vehicles. Utilizing the concentration ratios, $([1,3\text{-DNP}] + [1,6\text{-DNP}] + [1,8\text{-DNP}])/[1\text{-NP}]$, in airborne particulates (0.014), gasoline particulates (0.56) and diesel particulates (0.013), contributions (%) of diesel-engine vehicles to the three DNPs and 1-NP in the air were estimated to be 94.3% and 99.8%, respectively.

Keywords — dinitropyrene; 1-nitropyrene; benzo[*a*]pyrene; airborne particulate; diurnal variation; diesel-engine vehicle

Introduction

There have been many reports concerning carcinogenic and/or indirect-acting mutagenic polycyclic aromatic hydrocarbons (PAHs) such as benzo[*a*]pyrene (BaP) in environmental samples. Several direct-acting mutagenic nitrated polycyclic aromatic hydrocarbons (NPAHs) have also been found. Among the latter, the nitrated pyrenes, 1,3-dinitropyrene (1,3-DNP), 1,6-DNP and 1,8-DNP exhibited the strongest activity in the Ames test without S9 mix.^{1,2)} They exist

on the surface of airborne particulates and their major source in urban air is believed to be automotive vehicles. The number of diesel-powered vehicles in Japan in 1994 was 18% of all vehicles and the ratio continues to increase.³⁾ Moreover, a diesel-engine emits 30–100 times more particulates than a gasoline-engine. These facts indicate the importance of determining the mutagens emitted into the air by vehicles with diesel and gasoline engines in order to take effective countermeasures against air pollution. Their concentrations in air, however, are too low to be determined by

high-performance liquid chromatography (HPLC) with fluorescence detection⁴⁾ or by gas chromatography/mass spectrometry (GC/MS).⁵⁾

HPLC with chemiluminescence detection has been recognized for its high sensitivity and has been applied for the estimation of several amino-polycyclic aromatic hydrocarbons.⁶⁾ We have developed a HPLC method with chemiluminescence detection, for the simultaneous determination of the above three DNPs and 1-nitropyrene (1-NP) after reduction to their corresponding amines.⁷⁻⁹⁾ These compounds were found in as little as a few milligrams of vehicle exhaust particulates¹⁰⁾ and airborne particulates.¹¹⁾ Each DNP level was more than two orders of magnitude lower than that of 1-NP in airborne particulates, and their composition in diesel particulates was much different from that in gasoline particulates.

The purpose of this work was to determine the diurnal concentrations of 1,3-DNP, 1,6-DNP, 1,8-DNP, 1-NP and BaP in urban air collected over an 8 month period, and to estimate the contribution of diesel-engine vehicles to these concentrations.

Experiments

Chemicals—1,3-DNP, 1,6-DNP, 1,8-DNP and 2-fluoro-7-nitrofluorene (FNF) were purchased from Aldrich (Milwaukee, WI, U.S.A.), 1-NP and bis(2,4,6-trichlorophenyl)oxalate (TCPO) were from Tokyo Kasei (Tokyo, Japan), BaP from Wako Pure Chemical Industries and hydrogen peroxide (30% aqueous solution) from Kanto Chemicals (Tokyo, Japan). All other chemicals were of analytical-reagent grade.

Sampling—A high-volume air sampler (Kimotodenshi, Osaka, Japan) was placed 30 m southwest of a busy intersection in the downtown area of the city (7-10 Showa-machi, Kanazawa, Japan). Airborne particulates were collected on a Pallflex Products (Putnam, CT, U.S.A.) 2500QAT-UP filter (8 × 10 inch) for 24 h at 2 h sampling intervals at a flow rate of 1.5 m³/min. Sampling was performed once a month from July, 1993 to February, 1994. The atmospheric conditions are listed in Table I. The filters were weighed and then stored in a refrigerator (-20°C) until use.

Pretreatment—A piece (2 × 16 cm) of each filter was cut into fine pieces and placed in a flask. After 0.1 ml of FNF solution (4 × 10⁻⁶ M in ethanol) was added as an internal standard, the analytes of interest (1,3-DNP, 1,6-DNP, 1,8-DNP,

TABLE I. Samples and Atmospheric Conditions

Sampling period ^{a)}	Weather	Mean temperature (°C)	Wind	
			Mean speed (m/s)	Mean direction ^{b)}
'93/ 7/28-29	Cloudy→fine (10:00-20:00)	29	1.6	SE
'93/ 8/25-26	Fine	29	1.7	NE
'93/ 9/22-23	Cloudy	20	1.0	ESE
'93/10/27-28	Fine	12	1.9	E
'93/11/24-25	Fine→rainy (10:00-14:00)	6	4.0	SSW
'93/12/22-23	Snowy	2	>8.2	S
'94/ 1/26-27	Fine→rainy (10:00-16:00)	7	3.4	SSE
'94/ 2/23-24	Snowy	3	4.9	WNW

a) Sampling at 2 h intervals began at 10:00 and ended at 10:00 the following day. b) S, south; E, east; W, west; N, north.

1-NP and BaP) were extracted ultrasonically twice with 128 ml of benzene-ethanol (3:1, v/v) for 15 min and then the solution was filtered. The filtrate was cleaned by extraction with 100 ml of 5% (w/v) sodium hydroxide, followed by 100 ml of 20% (v/v) sulfuric acid and then twice with 100 ml of water. After the benzene phase was evaporated to dryness, the residue was dissolved in 1 ml of ethanol and refluxed with 1 ml of 7% (w/v) sodium hydrosulfide for 1 h. After the addition of 4 ml of 0.15 N sodium hydroxide, the corresponding reduced compounds (1,3-diaminopyrene, 1,6-diaminopyrene, 1,8-diaminopyrene, 1-aminopyrene and 2-fluoro-7-aminofluorene) were extracted with BaP into 1.5 ml of benzene. To the benzene phase was added several drops of ascorbic acid-saturated ethanol and the mixture was then evaporated to dryness. The residue was redissolved in 0.2 ml of acetonitrile containing 10 mM ascorbic acid. The reflux apparatus was foil-wrapped to exclude light and the extraction and evaporation were carried out in the dark.

Analysis of 1,3-DNP, 1,6-DNP, 1,8-DNP, 1-NP and BaP — The HPLC system consisted of three pumps, an injector, a switching-valve, a mini column (ODS, octadecyl silica), two analytical columns (ODS), a chemiluminescence detector, a fluorescence detector and an integrator.¹²⁾ Mobile phases were imidazole-perchloric acid buffer (pH 7.6)-acetonitrile (1:1, v/v) for the determination of 1,3-DNP, 1,6-DNP, 1,8-DNP and 1-NP, and water-acetonitrile (1:3, v/v) for BaP. The post-column chemiluminescence reagent solu-

tion was an acetonitrile solution containing TCPO and hydrogen peroxide. Other conditions were the same as reported.¹²⁾

Monitoring of Carbon Monoxide (CO), Nitrogen Monoxide (NO), Suspended Particulate Matter (SPM) and Traffic Volume — Concentrations of CO, NO and SPM in the air at the same sampling site were measured by a Horiba (Kyoto, Japan) APMA-3500 CO analyzer, a DKK (Tokyo, Japan) GPH-74M NO_x analyzer and a DKK DUB-13 SPM analyzer, respectively. Data on traffic volume was provided by the Ishikawa Prefectural Police Headquarters.

Results and Discussion

Diurnal Concentrations of 1,3-DNP, 1,6-DNP, 1,8-DNP, 1-NP and BaP

In earlier reports, the concentration of BaP in airborne particulates was determined to be a mutagen.^{13,14)} For comparison, the level of BaP in airborne particulates to those of 1,3-DNP, 1,6-DNP, 1,8-DNP and 1-NP was determined in the present report. Mean concentrations of the five compounds in collections every 2 h for 24 h over 8 months are listed in Table II. Generally, the levels of each DNP (in the range of fmol/m³) and 1-NP (in the range of sub pmol/m³ or pmol/m³) were, respectively, more than three and about one order of magnitude lower than that of BaP. However, there were large sample differences and the seasonal variation of the concentrations being higher in winter than in summer¹¹⁾

TABLE II. Mean Concentrations^{a)} of 1, 3-DNP, 1, 6-DNP, 1, 8-DNP, 1-NP and BaP in Air in Downtown Kanazawa

Sample ^{b)}	1, 3-DNP	1, 6-DNP	1, 8-DNP	1-NP	BaP
'93/ 7/28-29	4.6	4.2	6.5	1220	9800
'93/ 8/25-26	2.9	2.5	4.1	780	4500
'93/ 9/22-23	2.7	1.9	2.9	700	8000
'93/10/27-28	4.1	3.3	4.3	960	12800
'93/11/24-25	8.6	6.9	7.7	1450	11800
'93/12/22-23	1.4	1.3	1.5	410	6100
'94/ 1/26-27	8.5	6.3	7.9	1420	20100
'94/ 2/23-24	1.9	1.9	2.5	440	6400
Mean ± S. D.	4.3 ± 2.8	3.5 ± 2.1	4.7 ± 2.4	920 ± 410	9900 ± 5000

a) Unit, fmol/m³. b) Sampling period, see Table I.

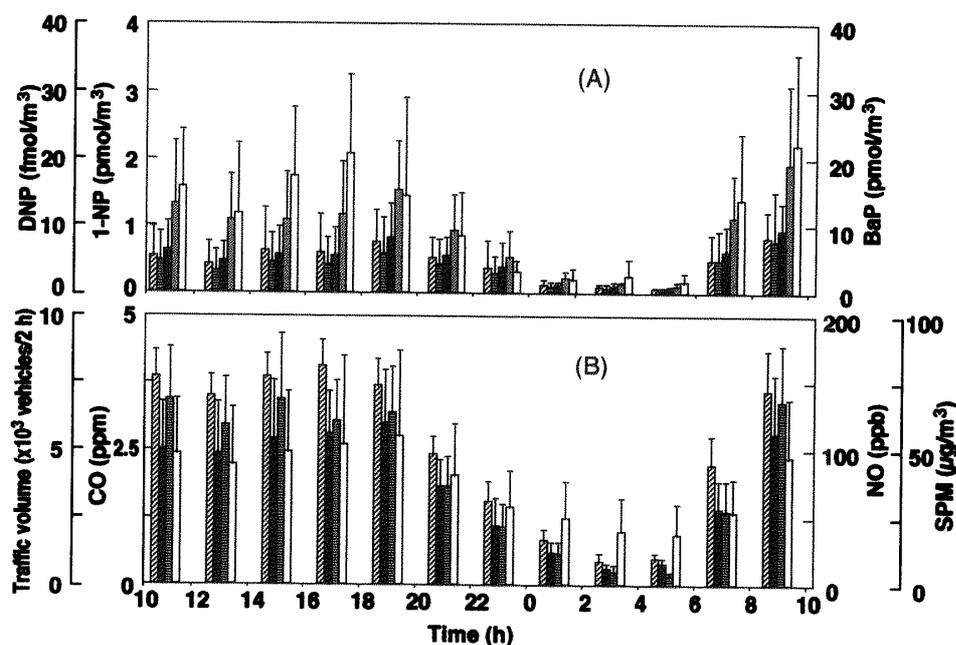


Fig. 1. Diurnal Concentrations of 1,3-DNP, 1,6-DNP, 1,8-DNP, 1-NP, BaP, CO, NO, SPM and Traffic Volume in Downtown Kanazawa

Conditions of air sampling and determination method were described in the text. Symbols: (A) ▨, 1,3-DNP; ▩, 1,6-DNP; ▣, 1,8-DNP; ▤, 1-NP, □, BaP; (B) ▨, traffic volume; ▩, CO; ▣, NO; □, SPM. Each box and bar represent the mean + S.D. ($n=8$).

was not observed by only samplings taken for one 24 h period a month in Table II. Table I suggests that the concentrations might be decreased by sunlight in summer (August 25–26, 1993) and snow or wind in winter (December 22–23, 1993 and February 23–24, 1994). Effects of these atmospheric factors will be reported elsewhere.

In spite of these sampling differences, characteristic diurnal variations were revealed after statistical calculations using the data of all 8 months. Figure 1(A) shows that concentrations of the five compounds were high in the morning (8:00–10:00) and evening (16:00–20:00) and low from midnight to early morning (0:00–6:00). Diurnal variations of traffic volume and concentrations of CO, NO and SPM at the same sampling point are shown in Fig. 1(B). There were strong correlations between traffic volume and DNPs (1,3-DNP + 1,6-DNP + 1,8-DNP) ($r=0.85$) as well as between traffic volume and 1-NP ($r=0.89$) (Table III). Large correlation coefficients were also observed

TABLE III. Correlation Coefficients of Analytical Results in Fig. 1

Compound	Traffic volume	CO	NO	SPM
DNPs	0.85	0.89	0.85	0.83
1-NP	0.89	0.91	0.89	0.84

DNPs=1,3-DNP+1,6-DNP+1,8-DNP.

for CO (0.89–0.91) and NO (0.85–0.89), which were primarily from vehicle emissions,¹⁵⁾ while the correlation coefficients for SPM were the smallest (0.83–0.84). The reason for this was that SPM contained particulates not only from vehicles but from other sources whose fraction might increase when traffic volume was low. These results indicated that the major contributor of 1,3-DNP, 1,6-DNP, 1,8-DNP and 1-NP in urban air was the automotive vehicle.

Contribution of Diesel-Engine Vehicles to 1,3-DNP, 1,6-DNP, 1,8-DNP and 1-NP in Air

Diesel particulates must be distinguished from gasoline particulates to study air pollution in detail. The relationship between the

TABLE IV. Concentrations of 1,3-DNP, 1,6-DNP, 1,8-DNP and 1-NP in Diesel, Gasoline Exhaust and Airborne Particulates

Particulates	Concentration				[DNPs]/[1-NP]
	[DNPs] ^{a)}		[1-NP]		
Diesel (<i>n</i> =8)	0.66±0.47	pmol/mg ^{b)}	51±53	pmol/mg ^{b)}	0.013
Gasoline (<i>n</i> =7)	1.02±0.70	pmol/mg ^{b)}	1.82±0.85	pmol/mg ^{b)}	0.56
Air					
Total (<i>n</i> =96)	12.6±12.0	fmol/m ³	920±840	fmol/m ³	0.014
10:00–20:00 (<i>n</i> =40)	15.9±12.7	fmol/m ³	1230±760	fmol/m ³	0.013
0:00–6:00 (<i>n</i> =24)	2.38±1.45	fmol/m ³	147±79	fmol/m ³	0.016

a) [DNPs]=[1,3-DNP]+[1,6-DNP]+[1,8-DNP]. b) Ref. 10.

methylthiophene/methylphenanthrene ratio in heavy traffic street dust and in diesel particulates was reported.¹⁶⁾ We evaluated the BaP and pyrene concentrations in diesel and gasoline particulates but found no great difference. We previously reported the concentrations of the three DNPs and 1-NP in particulates emitted from vehicles¹⁰⁾; the concentration ratio ([DNPs]/[1-NP]) in diesel particulates (0.013) was much smaller than that in gasoline particulates (0.56). In the present report, the ratio in air calculated from mean concentrations of these four substances over the 8 months (Table II) was 0.014, which was not close to that in gasoline but was to that in diesel particulates (Table IV).

This result suggested the value of estimating the contribution of diesel-engine vehicles since they were the only source near the sampling point. Concentrations of the three DNPs (D_A) and 1-NP (N_A) in air are given by Eqs. 1 and 2, respectively, where D_D , N_D and P_D were concentrations in diesel particulates (mol/mg) and diesel particulates in air (mg/m³), respectively. Concentrations in gasoline particulates (mol/mg) and gasoline particulates in air (mg/m³) were D_G , N_G and P_G , respectively.

$$D_A = D_D \cdot P_D + D_G \cdot P_G \quad (1)$$

$$N_A = N_D \cdot P_D + N_G \cdot P_G \quad (2)$$

Percentages of contributions of diesel-engine

vehicles are defined by Eqs. 3 and 4, respectively.

$$A = 100 \cdot D_D \cdot P_D / D_A \quad (\%) \quad (3)$$

$$B = 100 \cdot N_D \cdot P_D / N_A \quad (\%) \quad (4)$$

Values of D_D , N_D , D_G , N_G , D_A and N_A are all available from Table IV. By substituting these values for Eqs. 1 and 2, P_D and P_G were obtained.

$$P_D = 18.0 \quad (\mu\text{g}/\text{m}^3)$$

$$P_G = 0.704 \quad (\mu\text{g}/\text{m}^3)$$

Substitutions of these values into Eqs. 3 and 4 gave A and B (%) as 94.3% and 99.8%, respectively. As listed in Table IV, the [DNP]/[1-NP] ratio from 0:00 to 6:00 (0.016) was slightly larger than that from 10:00 to 20:00 (0.013), the reason being that diesel-engine vehicles such as busses were not used at midnight and early morning hours. This meant that contributions of diesel-engine vehicles to the three DNPs and 1-NP (66.3%, 82.3%) from 0:00 to 6:00 were less than those from 10:00 to 20:00. Thus, the contribution of these vehicles to all four substances was extremely large.

Conclusion

Diurnal concentrations of 1,3-DNP, 1,6-DNP, 1,8-DNP, 1-NP and BaP in air in downtown Kanazawa were measured by HPLC with chemiluminescence and fluorescence detection. Time courses of their concentra-

tions were high in the morning (8:00–10:00) and evening (16:00–20:00) and low at midnight and early morning (0:00–6:00). Levels of each DNP and 1-NP were, respectively, more than three and about one order of magnitude lower than that of BaP. Large correlation coefficients between these compounds and traffic volume, CO and NO suggested that automotive vehicles were the major source. Utilizing concentrations of the three DNPs and 1-NP in diesel, gasoline, and airborne particulates, the contributions made

by diesel-engine vehicles in the air were calculated to be 94.3% and 99.8%, respectively, identifying these vehicles as the major source of DNP and 1-NP.

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References

- 1) H.S. Rosenkranz, E.C. McCoy, D.S. Sanders, M. Butler, D.K. Kiriiazides and R. Mermelstein, *Science*, **209**, 1039 (1980).
- 2) G. Klopman, D.A. Tonucci, M. Holloway and H.S. Rosenkranz, *Mutat. Res.*, **126**, 139 (1984).
- 3) The Ministry of Transportation, "The Monthly Report of the Number of Automobiles, Sep. 1994," Tokyo, 1994.
- 4) K. Tanabe, H. Matsushita, C.T. Kuo and S. Imamiya, *J. Jpn. Soc. Air Pollut.*, **21**, 535 (1986).
- 5) D. Schuetle, T.L. Riler, T.J. Prater, T.M. Harvey and D.F. Funt, *Anal. Chem.*, **54**, 265 (1982).
- 6) K.W. Sigvardson, J.M. Kennish and J.W. Birks, *Anal. Chem.*, **56**, 1096 (1986).
- 7) N. Imaizumi, K. Hayakawa, Y. Suzuki and M. Miyazaki, *Biomed. Chromatogr.*, **4**, 108 (1990).
- 8) K. Hayakawa, R. Kitamura, M. Butoh, N. Imaizumi and M. Miyazaki, *Anal. Sci.*, **7**, 573 (1991).
- 9) K. Hayakawa, M. Butoh and M. Miyazaki, *Anal. Chim. Acta*, **266**, 251 (1992).
- 10) K. Hayakawa, M. Butoh, Y. Hirabayashi and M. Miyazaki, *Jpn. J. Toxicol. Environ. Health*, **40**, 20 (1994).
- 11) K. Hayakawa, T. Murahashi, M. Butoh and M. Miyazaki, *Environ. Sci. Technol.*, **29**, 928 (1995).
- 12) T. Murahashi, K. Hayakawa, Y. Iwamoto, M. Miyazaki, *Bunseki Kagaku*, **43**, 1017 (1994).
- 13) R.E. Waller, *Brit. J. Cancer*, **6**, 8 (1952).
- 14) A. Suzuki, *J. Nor. Occ. Health*, **18**, 49 (1958).
- 15) Ishikawa Prefecture, "Annual Report of Air Pollution Survey in Ishikawa Prefecture," Kanazawa, 1993, pp.11–44.
- 16) H. Takada, T. Onda and N. Ogura, *Environ. Sci. Technol.*, **24**, 1179 (1990).