

Determination of 1,3-, 1,6-, 1,8-Dinitropyrenes and 1-Nitropyrene in Vehicle Exhaust Particulates

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1,3-, 1,6-, 1,8-Dinitropyrenes (DNPs) and 1-nitropyrene (1-NP) in exhaust particulates from idling gasoline and diesel engines were determined using off-line sodium hydrosulfide reduction followed by HPLC with chemiluminescence detection. The mean concentrations of 1,3-, 1,6-, 1,8-DNPs and 1-NP were 0.22, 0.44, 0.35 and 1.8 pmol/mg, respectively, in gasoline particulates ($n=8$) and 0.23, 0.23, 0.21 and 51 pmol/mg, respectively, in diesel particulates ($n=7$). Although the concentrations of DNPs were lower than that of 1-NP in both gasoline and diesel particulates, they cannot be dismissed as negligible because of their extremely high mutagenicity. There was a tendency toward higher DNPs and 1-NP concentrations in particulates, with the lowering of particulate concentration in exhaust gas. The total amount of DNPs and 1-NP emitted from diesel engine vehicles was considered to be remarkably higher than that of gasoline engine vehicles.

Keywords — dinitropyrene; nitropyrene; vehicle exhaust particulate; HPLC; chemiluminescence detection

Introduction

Several mutagenic nitrated polycyclic aromatic hydrocarbons (NPAHs) have been found in environmental samples,^{1,2)} and among them, 1,3-, 1,6- and 1,8-dinitropyrenes (DNPs) have shown extremely high mutagenicities by the Ames test in the absence of S9 mixture.^{3,4)} As a major source of these NPAHs, vehicle exhausts have been considered. Several reports have been published concerning 1-NP concentration in exhaust particulates (330—9300 pmol/mg in extracts from diesel particulates⁵⁾ and 14—700 pmol/mg in extracts from gasoline particulates).⁶⁾ However, neither a direct comparison of diesel and gasoline particulates nor the determination of DNPs in gasoline particulates has been reported. In order to fully understand the risk of human health, a thorough investigation of

their emission is necessary.

Gas chromatography,^{7,8)} gas chromatography/mass spectrometry^{5,9)} and high performance liquid chromatography (HPLC) with fluorescence detection⁶⁾ have been employed in determining NPAHs. Some of these methods have been applied to the determination of 1-NP in diesel engine exhaust particulates. However, these methods are not sensitive enough to determine DNPs because their concentrations are several orders of magnitude lower than that of 1-NP. Although a few reports of DNPs in diesel particulates have been published,¹⁰⁻¹²⁾ gasoline engine vehicles, with their much lower particulate emissions, have so far eluded quantitative analysis.

Recently, HPLC with chemiluminescence detection has attracted much attention because of its high sensitivity and has been applied in the determination of aminopolycy-

clic aromatic hydrocarbons.^{13,14} During the course of our studies on environmental NPAHs, we reported a method for simultaneously determining amino-, nitroso- and nitropyrenes using HPLC with chemiluminescence detection after on-line electrochemical reduction.¹⁵ More recently, an even more sensitive HPLC method using chemiluminescence detection has been developed for determining 1,3-, 1,6-, 1,8-diaminopyrenes (DAPs) and 1-aminopyrene (1-AP).¹⁶ Using this technique we were able to determine trace levels of 1,3-, 1,6-, 1,8-DNPs and 1-NP in environmental samples after a simple clean-up treatment and chemical reduction.¹⁷ All of the four compounds were detected not only in diesel but also in gasoline particulates.¹⁸

In this report, utilizing the same method, we determine the four compounds in exhaust particulates and compare gasoline particulates to diesel particulates.

Experimental

Chemicals — 1,3-, 1,6- and 1,8-DNPs were

obtained from Aldrich (Milwaukee, WI, U.S.A.). 1-NP and pyrene (P) were purchased from Tokyo Kasei (Tokyo, Japan) and benzo[*a*]pyrene (B[*a*]P) was from Wako Pure Chemicals (Osaka, Japan). Bis(2,4,6-trichlorophenyl) oxalate (TCPO) and hydrogen peroxide (30% aqueous solution) were purchased from Tokyo Kasei and Kanto Chemicals (Tokyo, Japan), respectively. Sep-Pak alumina N cartridges were purchased from Millipore (Bedford, MA, U.S.A.). All other chemicals were of analytical grade.

Sampling of Exhaust Particulates

Table I shows characteristics of the vehicles used in this report. Eight vehicles (No. 1–8) were gasoline engine-powered, and the other seven (No. 9–15) were diesel engine-powered. Before sampling exhaust particulates, each engine was allowed to idle for more than 1 h. A Shibata (Tokyo, Japan) L-30 low volume air sampler with a Millipore (Milford, U.S.A.) AP20 glass fiber filter was set at 30 cm from the end of the exhaust pipe of each idling vehicle. Exhaust particulates were collected for 2 h for gasoline and 0.2–2 h for diesel engine vehicles at a flow rate of 0.03 m³/

TABLE I. Gasoline and Diesel Engine Vehicles Used

No.	Vehicle				Engine		Fuel ^{c)}	Particulate (mg/m ³)
	Maker	Type ^{a)}	Year	Mileage (km)	Type ^{b)}	Capacity (cc)		
Gasoline engine-powered ^{d)}								
1	B	S	1983	120 000	RE	1 800	RG	0.39
2	F	V	1984	63 000	RE	550	RG	0.44
3	A	C	1986	73 000	RE	2 000	RG	0.53
4	E	H	1987	41 000	RE, TB	550	RG	0.39
5	C	S	1987	38 000	RE	1 500	RG	0.17
6	D	S	1988	29 000	RE	1 800	RG	0.25
7	A	S	1989	16 000	RE	2 000	HG	0.61
8	C	S	1991	1 000	RE	1 600	RG	0.14
Diesel engine-powered								
9	B	T	1980	90 000	SC	2 200	RL	18.8
10	E	H	1986	72 000	SC, TB	1 000	RL	3.0
11	A	L	1987	6 800	SC	4 000	RL	18.2
12	G	U	1988	14 000	DI	6 700	RL	52.1
13	G	T	1989	7 200	DI	7 400	RL	20.6
14	A	S	1991	10 000	SC, TB	2 000	RL	8.7
15	D	S	1991	20 000	SC	2 000	RL	8.2

a) Vehicle type: S, sedan; V, van; C, coupe; H, hatchback; T, truck; L, land cruiser; U, bus.

b) Engine type: RE, reciprocating; DI, direct injection; SC, swirl chamber; TB, turbo.

c) Fuel: RG, unleaded regular gasoline; HG, unleaded high-octane gasoline; RL, regular light oil.

d) All gasoline engine vehicles had converters packed with ternary catalysts.

min. The atmospheric temperatures during the sampling were 5–15°C. Filters, which were weighed previously, were changed every 20 min to minimize the formation of artifact nitroarene on them. These filters were dried in a desiccator at room temperature for a couple of days in the dark, and then weighed again. Amounts of exhaust particulates collected on the filter were calculated from the difference in the weights before and after the sampling. The filters were stored in a freezer. All filters for each vehicle were cut into small pieces (about 1 cm²) when used.

Pretreatment of Samples — Several pieces of the cut filters (1 ≦ total amounts of particulates ≦ 10 mg) were extracted twice ultrasonically with 50 ml of benzene–ethanol (3+1, v/v) and then filtered using a HLC-DISK 13 (Kanto Chemicals) membrane cartridge (pore size 0.45 μm).

DNPs and 1-NP in the filtrate were prepared by the same method as that reported previously.¹⁷⁾ Namely, the filtrate (100 ml) was washed with 100 ml of 5% sodium hydroxide, 100 ml of 20% sulfuric acid and twice with 100 ml of water, and then evaporated to dryness. This residue was redissolved in 1 ml of ethanol and then reduced with 1 ml of ethanol containing 7% sodium hydrosulfide for 1.5 h. After the addition of 4 ml of 0.15 N sodium hydroxide, the reduced compounds (DAPs and 1-AP) were extracted twice into 15 ml of benzene. The benzene extracts were added with a drop of ascorbic acid-saturated ethanol and evaporated. The remaining residue was redissolved in 0.2–1 ml of ascorbic acid saturated acetonitrile. An aliquot of this solution was injected into the HPLC/chemiluminescence detection system.

For the determination of pyrene (P) and B[*a*]P, the filtrate (100 ml) was evaporated to dryness. The residue was redissolved in 10 ml of *n*-hexane, and then applied to a Sep-pak alumina N cartridge, followed by 50 ml of benzene. The fraction of interest was collected and evaporated to dryness. The residue was redissolved in 0.5 ml of acetonitrile, and an aliquot was injected into a

HPLC/fluorescence detection system.

HPLC System and Conditions — The HPLC system and conditions for the DNPs and 1-NP were as reported previously.¹⁷⁾ The system consisted of a Shimadzu (Kyoto, Japan) LC-6A pump and a Sanuki (Tokyo, Japan) DMX-2200T pump, a Rheodyne (Cotati, CA, U.S.A.) Model 7125 injector (20 μl loop) and a Soma (Tokyo, Japan) S-3400 chemiluminescence detector. The analytical column was a Nacalai Tesque (Kyoto, Japan) Cosmosil 5C₁₈ (4.6 i.d. × 250 mm). The mobile phase was prepared by mixing acetonitrile and 10 mM imidazole–perchloric acid buffer (pH 7.6) (1+1, v/v). The post column chemiluminescence reagent solution was an acetonitrile solution containing 0.02 mM TCPO and 15 mM hydrogen peroxide. The flow-rates of the mobile phase and the post column reagent solution were each 1.0 ml/min.

Both P and B[*a*]P were analyzed using the HPLC system described in the report¹⁹⁾ with several modifications. Two analytical columns were combined in series (Vydac 201TP54 C₁₈, 4.6 i.d. × 250 mm, Separations Group, Hesperia, CA, U.S.A.). The monitor was a Jasco (Tokyo, Japan) 820-FP intelligent spectrofluorometer (Ex 300 nm, Em 385 nm for P and Ex 384 nm, Em 430 nm for B[*a*]P). The mobile phase was an acetonitrile–water solution (17+3, v/v). The flow rate was set at 1.0 ml/min.

Results and Discussion

Figure 1 shows chromatograms of the benzene–ethanol extracts from (A) gasoline and (B) diesel particulates after off-line reduction. When the sampler was operated without idling the engines, the benzene–ethanol extracts did not show any peak. The fact that 1,3-, 1,6-, 1,8-DAPs and 1-AP were detected on both chromatograms suggests the existence of their corresponding nitrated forms (1,3-, 1,6-, 1,8-DNPs and 1-NP) in both samples.

Utilizing our HPLC method, all four compounds could be determined in not only the

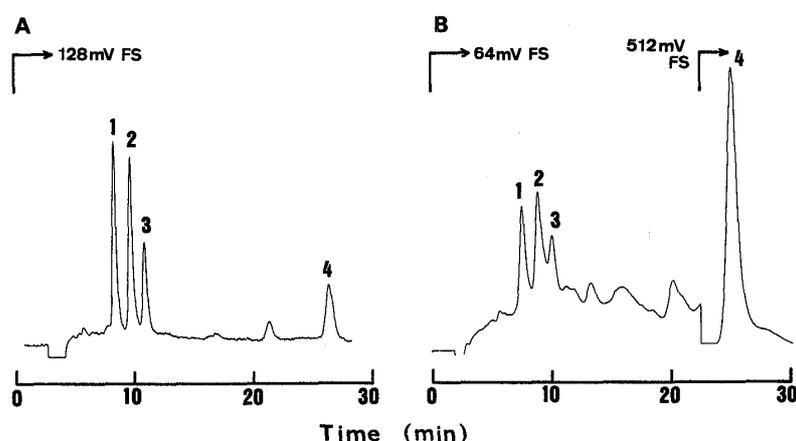


Fig. 1. Chromatograms of Benzene—Ethanol Extracts from (A) Gasoline (No. 7) and (B) Diesel (No. 10) Particulates after Off-Line Reduction

Peaks: 1=1,6-DAP; 2=1,8-DAP; 3=1,3-DAP; 4=1-AP.

TABLE II. Concentrations^{a)} of DNPs, 1-NP, B[*a*]P and P in Particulates

No. ^{b)}	1, 3-DNP	1, 6-DNP	1, 8-DNP	1-NP	B[<i>a</i>]P	P
1	0.029	0.093	0.079	0.70		
2	0.041	0.20	0.13	1.2	16	16 000
3	0.17	0.39	0.42	2.5		
4	0.079	0.16	0.19	1.9		
5	0.33	0.43	0.38	2.2	46	30 000
6	0.40	0.54	0.54	1.9		
7	0.30	0.43	0.45	0.73	1.1	5 700
8	0.44	1.3	0.60	3.4		
9	0.077	0.046	0.063	3.0		
10	0.28	0.39	0.44	170		
11	0.13	0.22	0.22	32		
12	0.14	0.092	0.086	4.5	7.1	5 400
13	0.54	0.51	0.38	32	3.1	1 200
14	0.13	0.079	0.067	44		
15	0.29	0.24	0.20	72	9.8	660

^{a)} Data are presented as the mean values of three determinations. Unit, pmol/mg.

^{b)} Numbers correspond to Table I.

diesel particulates but also the gasoline particulates at low sub pmol/mg levels (Table II). The mean \pm SD concentrations of 1,3-, 1,6-, 1,8-DNPs and 1-NP were 0.22 ± 0.15 , 0.44 ± 0.36 , 0.35 ± 0.18 and 1.8 ± 0.85 pmol/mg, respectively, in gasoline particulates and 0.23 ± 0.15 , 0.23 ± 0.16 , 0.21 ± 0.14 and 51 ± 53 pmol/mg, respectively, in diesel particulates.

It is obvious that, for all samples in Table II, the concentration of any of the three DNPs was lower than that of the corresponding 1-NP, although individual differences were

observed. To compare gasoline particulates with diesel particulates, the mean values and standard deviations of the four compounds are graphed in Fig. 2(A). The three DNPs in the two particulates were at similar levels without any significant difference. However, the concentration of 1-NP in diesel particulates was much higher than that in gasoline particulates.

As described in the Introduction, the mutagenicity of the DNPs is much stronger than that of 1-NP. For example, the Ames

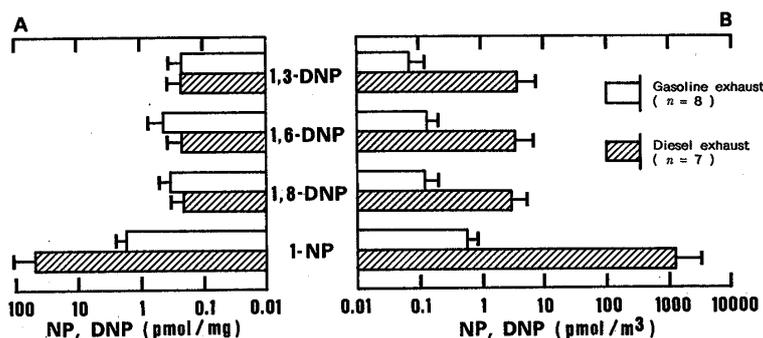


Fig. 2. Concentrations of 1,3-, 1,6-, 1,8-DNPs and 1-NP in (A) Particulates and (B) Exhaust Gas Emitted from Gasoline and Diesel Vehicles

Data are expressed as the mean \pm S.D.

test activity of each DNP isomer is reported to be 400–2000 times as strong as that of 1-NP using YG1024.⁴⁾ Thus we made comparisons of their concentrations with regard to their relative contribution to the mutagenicity of the particulates. The ratios of the mean concentrations of the total of the three DNPs to that of 1-NP in gasoline and diesel particulates were 1/1.8 and 1/76, respectively. These values are greater than the sum of the reciprocal of the above mutagenicity ratios. Although 1-NP is a strong mutagenic compound in diesel particulates, its activity is less than 15% of the total, with the remaining activity being observed in more polar fractions.³⁾ Our findings suggest that DNPs contribute greatly to the mutagenic activity of vehicle exhaust particulates.

In order to compare the formation efficiencies of the four compounds in gasoline and diesel engines, DNPs/1-NP and 1-NP/P ratios calculated from Table II are listed in Table III. The DNPs/1-NP ratio for diesel particulates was about one order of magnitude smaller than that of gasoline particulates, although the 1-NP/P ratio was about one order of magnitude greater for diesel engines than for gasoline engines. As the main formation pathway of 1-NP and DNPs in engines and exhaust pipes, Eqs. 1 and 2 are applicable:

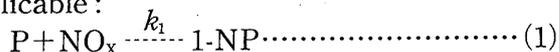


TABLE III. Concentration Ratios of P, 1-NP and DNPs in Gasoline and Diesel Particulates

Engine type	1-NP/P	DNPs ^{a)} /1-NP
Gasoline engine ($n=3^b$)	2.7×10^{-4}	5.6×10^{-1}
Diesel engine ($n=3^c$)	3.5×10^{-3}	6.1×10^{-2}

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

b) No. 2, 5 and 7 in Table II.

c) No. 12, 13 and 15 in Table II.



It is known that the concentration of NO_x in diesel exhausts is much higher than that in gasoline exhausts. Equation 1 suggests that the higher NO_x concentration might be the main reason for the greater 1-NP/P ratio for diesel engines. However, this difference in the NO_x level cannot explain the greater DNPs/1-NP ratio for gasoline engines. The formation of DNPs from 1-NP defined by Eq. 2 requires a high temperature.²⁰⁾ The higher ignition temperature of gasoline than that of light oil may be one reason for the difference.

Diesel engines are known to emit many more particulates than gasoline engines. The calculated concentrations of particulates in exhaust gas (mg/m^3) from the vehicles are listed in Table I. Taking these values into consideration, the concentration of each compound in exhaust gas (pmol/m^3) was calcu-

lated by a formula, [DNP or 1-NP concentration in particulate, pmol/mg] \times [particulate concentration in exhaust gas, mg/m³], and the result is graphed in Fig. 2(B). This graph shows that the concentrations of all four compounds in exhaust gas are much greater from diesel than gasoline vehicles. Taking Japan as an example, the number of vehicles at the end of 1991 was over forty million and 25% of them were diesel engine-powered.²¹⁾ Our results suggest that the contribution of diesel engine vehicles to the total amounts of

1-NP and DNPs emitted from all vehicles might be much greater than that of gasoline engine vehicles, although driving conditions are different. If a sampling of particulates was possible under driving conditions, this point could be clarified.

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