Ⅱ．研究成果の刊行に関する一覧表

別紙４

研究成果の刊行に関する一覧表レイアウト

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Miller-Schlze, J. P., Toriba, A., Tang, N., Hayakawa, K., Tamura, K., Dong, L., Simpson, C. D.	Exposures to particulate air pollution and nitro-polycyclic aromatic hydrocarbons amongst taxi drivers in Shenyang, China.	Environmental Science & Technology	**44** (1)	216-221	2010
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Ⅲ. 研究成果の刊行物・別刷

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Exposures to Particulate Air Pollution and Nitro-Polycyclic Aromatic Hydrocarbons among Taxi Drivers in Shenyang, China

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Exposures to particulate matter (PM) of both 10–2.5 μm ($PM_{10-2.5}$) and below 2.5 μm ($PM_{2.5}$) were measured for a cohort of taxi drivers in Shenyang, China, during August 2007. PM samples were collected inside and outside the taxi during the drivers' workshifts, and also inside the drivers' homes when they were off-shift. Ambient PM samples were also collected at a stationary location in Shenyang. Elemental carbon (EC) and organic carbon (OC) were also measured in PM collected on quartz filters inside the taxis as well as at the stationary site. Concentrations of three nitro-polycyclic aromatic hydrocarbons (NPAHs), 1-nitropyrene (1NP), 2-nitropyrene (2NP), and 2-nitrofluoranthene (2NFl), were determined in extracts of the PM samples by using a 2D-HPLC-MS/MS method. The 2NP and 2NFl concentrations did not change substantially with sampling location, but the 1NP concentrations were much higher in samples collected inside and outside the taxis as compared with sampling locations that were more removed from traffic. Concentration ratios of specific NPAHs were used to assess the atmospheric conditions in Shenyang during the sampling period. The relatively high ratios of 2NFl/1NP (~8–50) indicate an important contribution from secondary NPAH formation to ambient NPAH levels, especially for the nontaxi samples. The ratios of 2NFl/2NP (2.5–4.3) indicate that 2NFl is primarily formed via the hydroxyl-initiated reaction.

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Introduction

Particulate matter (PM) of both anthropogenic and natural origin is a well-chronicled potential hazard to human health (*1*–*4*). The EPA has concluded that there are likely causal associations between both coarse ($PM_{10-2.5}$) and fine ($PM_{2.5}$) PM and cardiovascular and respiratory morbidity and mortality (*2*). A variety of the components of anthropogenically generated PM have been associated with these health hazards in epidemiology and toxicology studies, including metals, polycyclic aromatic hydrocarbons (PAHs) and their nitrated analogs, nitro-PAHs (NPAHs), as well as numerous other organic compounds (*5*). Therefore, determining the contribution of specific sources to ambient PM levels is an important step toward more effective management of particulate air pollution and to reduced public health risk associated with PM exposure.

NPAH species are a particularly important constituent of ambient PM, due to their carcinogenicity and mutagenicity (*5*) as well as their utility in source apportionment (*6*). Distinct NPAHs are formed via different routes of production. For example, 1-nitropyrene (1NP) has been proposed as a molecular marker for diesel particulate matter (DPM) since it is by far the most abundant NPAH in DPM while being much less abundant in PM derived from other sources (*7*). Other NPAH isomers, such as 2-nitrofluoranthene (2NFl) and 2-nitropyrene (2NP) have been shown to be formed exclusively via gas-phase atmospheric reactions (*8*, *9*). The concentration ratio of 2NFl to 1NP allows estimation of the contribution to ambient NPAH concentrations of atmospheric reaction formation routes as compared with direct emission from primary PM sources (*5*, *10*). In addition, the ratio of the concentration of 2NFl to 2NP has been used to assess the relative contribution of two distinct production routes for 2NFl (*11*, *12*). For the reasons discussed above, measurements of ambient levels of NPAHs can yield valuable information about the atmospheric reaction conditions, sources of PM emissions, and processes contributing to NPAH formation for a particular geographical region.

In this paper, exposures to the three NPAH species listed above are reported for a cohort of taxi drivers from Shenyang, China. Shenyang is a city of 7.2 million people (as of the 2000 census) in Liaoning Province, the political and economic center of northeast China (*13*). Shenyang and its surrounding areas are home to numerous heavy industries, including smelters, machinery and chemical plants, coke plants, and power plants (*14*). In addition, 8 million tons of coal is burned each year in Shenyang, 20% of which is burned for domestic heating and cooking (*14*). We chose to study taxi drivers in Shenyang because it was anticipated that their exposures to vehicle exhaust (and hence, to 1NP) would be relatively high. However, the data presented herein indicate that exposures to PM derived from secondary sources are also important in this population.

Materials and Methods

Sampling. Twenty-four taxi drivers were recruited for this study, and sample collection took place during August 2007. $PM_{10-2.5}$ and $PM_{2.5}$ were collected inside and outside the subjects' taxis during their workshift (designated as "Inside Car" and "Outside Car") and personal PM samples were collected following the work-shift until the start of the next workshift the following day (samples designated as "Home"). Each driver was monitored over one 24 h period, and either four or five drivers were monitored simultaneously on a particular day. $PM_{10-2.5}$ and $PM_{2.5}$ were also collected at a

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stationary location in Shenyang, the Center for Disease Control building (designated "CDC"). The vehicles driven by the subjects were a mix of diesel vehicles and gasoline-natural gas hybrid vehicles. The samplers for the Inside Car and Outside Car samples were placed on the passenger seat and roof of the drivers' taxis, respectively. All windows were open during the drivers' workshifts. The Home samples were collected by placing samplers in drivers' homes, in their bedrooms or kitchen, for example. The drivers' house windows were expected to be left open during this sampling. The CDC samples were obtained outside the CDC building, but samplers were placed at locations away from streets. The $PM_{10-2.5}$ and $PM_{2.5}$ samples were collected using a Sibata ATPS-20H dual-stage impactor connected to a portable Sibata MP-Σ3 pump (Sibata Sci Tech, Tokyo, Japan). The pumps were operated at a flow rate of 1.5 L/min. Particles greater than 10 μm in diameter were removed on a metal impaction plate coated with grease positioned immediately downstream of the sampler inlet. Particles 10−2.5 μm passed around the impaction plate and were collected on a 10 mm Teflon coated glass fiber filter that was placed on the second impaction stage. Particles less than 2.5 μm were collected on a 20 mm Teflon coated glass fiber filter located in the final stage of the sampler. The $PM_{10-2.5}$ and $PM_{2.5}$ mass concentrations were obtained by weighing the filters before and after the sampling, always after a storage period (24 h) in a temperature- and humidity-controlled room (ambient temperature, 23 ± 0.2 °C, relative humidity, 35 ± 5%), by an ultramicrobalance (sensitivity 0.1 μg, UMX-2, Mettler-Toledo, Inc., Columbus, OH). In addition, $PM_{10-2.5}$ and $PM_{2.5}$ were collected on quartz filters at the Inside Car and CDC locations. These quartz filters were subsequently analyzed for elemental carbon (EC) and organic carbon (OC) by a commercial laboratory using NIOSH method 5040. Sampling was conducted for between 8 and 10 h per filter. The Inside Car and Outside Car filter samples were collected during the day and the Home filter samples were collected during nighttime hours. Separate CDC filter samples were collected both during the day and at night. For the purposes of this sampling, "Day" was classified as the time period from 7−8 a.m. to 5−6 p.m., and "Night" was classified as 6−7 p.m. to 7−8 a.m.

Materials. Quartz (2500QAT-UP) and Teflon Fiberfilm (a borosilicate glass fiber coated with fluorocarbon, TX60A20) filters were obtained from Pall Life Sciences, Ann Arbor, MI. The 2NF1, 4-nitropyrene (4NP), 3-nitrofluoranthene (3NFl) and deuterated (d_9) 1NP (1dNP) were obtained from Midwest Research Institute, Kansas City, MO. 1NP used in the calibration solutions and positive control samples was purchased from Sigma-Aldrich, St. Louis, MO, and 2NP was obtained from RT Corp, Laramie, WY. All solvents used in the extraction procedure and 2D-HPLC-MS/MS analysis were of high purity, HPLC grade quality, and were obtained from Sigma-Aldrich or JT Baker, Mallinckrodt Baker, Inc., Phillipsburg, NJ. Ascorbic acid was also obtained from JT Baker. The low-volume Turbovap evaporative concentrator was made by Zymark Corp., Hopkinton, MA. Syringe filters were purchased from PALL Corporation, East Hills, NY. The column used for the separation of the NPAHs (AC1) was obtained from Waters Corporation (Milford, MA), whereas the column used for the separation of the reduced NPAHs (AC2) was from Agilent Technologies, Inc., Santa Clara, CA.

Sample Preparation. The PM samples were extracted and analyzed over a period of 3 months. Approximately 15 PM extracts were analyzed per analysis sequence, along with sets of quality control samples and calibrants. The extraction procedure employed for these filter samples was adapted from an ultrasonic extraction procedure that has been reported previously (*15*). Filters were deposited in silanized 7.5 mL screw top glass test tubes. Filters were spiked with 60 pg internal standard (1dNP) and allowed to age for 30 min. Seven ml extraction solvent (CH_2Cl_2) was added to each test tube and the tubes were then sonicated for 60 min at full power. The CH_2Cl_2 was then decanted into a second silanized test tube and evaporated to dryness in a low volume Turbovap evaporative concentrator. The extracts were reconstituted in 150 μL of 75% ethanol /25% 0.02 M sodium acetate/acetic acid buffer, pH 5.5, sonicated for 10 min at full power, and then vortexed for 10 min in a sample vortexer. The reconstituted extracts were then filtered through PTFE 0.45 μm syringe filters into autosampler vials with silanized vial inserts, crimp capped, and analyzed as described below.

Analytical Method for the Determination of NPAHs. The filter extracts were analyzed with a two-dimensional high performance liquid chromatography tandem mass spectrometry method (2D-HPLC-MS/MS). The method has been adapted from Miller-Schulze et al. (*15*), and is described in detail in the Supporting Information.

The 2D-HPLC-MS/MS method involves an injection of 40 μL of PM extract, and initial separation on the first analytical column (AC-1) followed by online reduction in a column packed with a Pt/Rh catalyst. Passage through the reduction column causes the NPAH species and the deuterated internal standard to be fully reduced to the corresponding amino-PAH derivatives. The two-dimensional method uses a switching valve to toggle between trapping and eluting configurations. In the trapping configuration, the mobile phase carrying the reduced NPAH species is diluted 1:5 with water, causing the NPAH determinants to be retained on the front of the trapping column. In the eluting configuration, a higher strength mobile phase is pumped through the trapping column in the reverse direction. The reduced NPAH determinants and 1dNP are then separated from persistent interfering compounds on the second analytical column (AC2) and detected using an electrospray ionization triple quadrupole mass spectrometer, operated in the multiple reaction monitoring (MRM) mode.

Quantification of Determinants. All three NPAH species were quantified using the same internal standard (1dNP) which was added at a constant level (400 fg/μL) to calibration solutions containing all three NPAHs at concentrations ranging from 2.5 to 5000 fg/μL. This set of calibration solutions was analyzed at the start of each analysis sequence, and selected calibration solutions were reanalyzed periodically throughout each sequence to monitor instrument performance. Calibration curves were constructed by plotting the ratio of the NPAH response to internal standard response versus NPAH concentration. All peaks, including those in the calibrants and for PM sample extracts, were integrated manually within the Agilent Masshunter software. The NPAH concentration in each sample was calculated from the appropriate regression equation, which employed $1/X$ weighting for all determinants.

Quality control (QC) samples were included with each batch of filters to give insight on batch-to-batch variations in assay performance and blank contamination. The relevant results of these QC analyses are summarized here. The average NPAH concentrations calculated to be present in the field blanks were 2NP = 0.32 pg/m^3, 2NFl = 0.55 pg/m^3, and 1NP = 0.20 pg/m^3. Comparison of these blank NPAH concentrations with subsequent data for the NPAH concentrations in Shenyang shows that the blank contamination is negligible. The average NPAH recovery of the extraction and analysis, as measured by dividing the 1dNP internal standard peak area for each sample in a particular analysis batch by the average of the two 1dNP "control" samples analyzed in that batch (vial inserts spiked with an equivalent amount of 1dNP and diluted appropriately with extract matrix) was 92%. Calculated concentrations of NPAH species in spiked filters were within 30% of the expected values: 2NP = 73% ± 8%, 2NFl = 79% ± 8%, 1NP = 84% ± 7%.

TABLE 1. Air Contaminants Measured at Various Sample Locations in Shenyang[a]

location		2NP (pg/m³)	2NFl (pg/m³)	1NP (pg/m³)	EC (μg/m³)	OC (μg/m³)	$PM_{2.5}$ (μg/m³)	$PM_{10-2.5}$ (μg/m³)
inside car (n = 23)	mean ± std dev	192 ± 199	552 ± 477	80.8 ± 26.0	25.7 ± 5.6	53.4 ± 7.1	101 ± 44.5	118 ± 56.1
	median	55.0	195	79.3	25.0	52.8	69.9	110
	range	(15.2–555)	(73.9–1270)	(35.1–150)	(17.2–40.2)	(41.7–67.5)	(51.6–173)	(37.2–219)
outside car (n = 24)	mean ± std dev	232 ± 231	753 ± 614	97.1 ± 31.1			119 ± 62.4	112 ± 50.4
	median	157	690	95.6	NA[b]	NA[b]	92.6	108
	range	(14.9–614)	(89.9–1820)	(38.7–187)			(58.7–322)	(44.4–244)
home (n = 24)	mean ± std dev	210 ± 280	533 ± 485	22.4 ± 11.4			75.2 ± 33.0	51.0 ± 36.7
	median	99.9	368	18.7	NA[b]	NA[b]	73.2	40.3
	range	(14.8–999)	(29.0–1510)	(7.9–49.7)			(25.1–130)	(6.2–124)
CDC (n = 8)	mean ± std dev	369 ± 546	767 ± 723	11.2 ± 6.0	11.0 ± 5.5	28.5 ± 12.1	81.5 ± 54.4	50.6 ± 45.4
	median	110	723	12.0	10.9	32.0	69.7	38.7
	range	(8.4–1550)	(30.5–1720)	(3.3–18.0)	(3.7–17.1)	(12.4–47.1)	(24.6–158)	(7.6–124)

[a] NPAH, EC, and OC concentrations are the total of the determinant concentration in both the $PM_{10-2.5}$ and $PM_{2.5}$ size fractions. Plus/minus values represent 1 standard deviation. [b] EC and OC values for Outside Car and Home locations not available as quartz filters were not deployed at these locations.

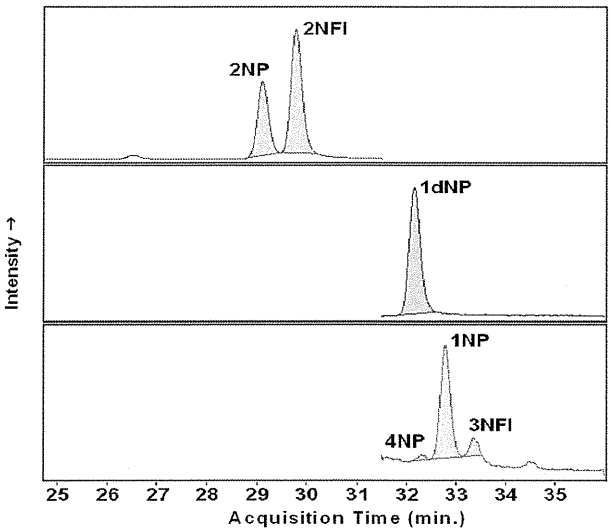

FIGURE 1. 2D-HPLC-MS/MS Chromatograms for a traffic based sampling location. Top pane represents the 1st time segment and the *m/z* transition from *m/z* 218→201. Middle pane represents the *m/z* transition from *m/z* 227→210 in the 2nd time segment. Lower pane represents the *m/z* transition from *m/z* 218→201, also in the 2nd time segment. Peaks annotated in figure, see text for abbreviations.

Results and Discussion

Samples were collected over a 5 day period between August 16 and August 20, 2007. During this period the meteorological conditions were as follows: ambient temperature 22–27 °C; mean visibility 2.6–9.7 miles; average wind speed 1.9–6.9 knots; precipitation 0–1 in./24 h.

The concentrations of the air contaminants measured on the filter samples for each sampling location are given in Table 1. With the exception of the PM metrics, all concentrations given represent the total determinant concentration for each location, i.e., the sum of the concentrations in both the $PM_{10-2.5}$ and $PM_{2.5}$ filters.

Figure 1 shows the multiple reaction monitoring (MRM) chromatograms for the NPAH analysis of a representative $PM_{2.5}$ sample collected at a traffic-based sampling location.

As the chromatogram in Figure 1 shows, all peaks are well resolved. The chromatograms for PM extracts from all sampling locations show similar peaks, albeit with different relative abundances. 1NP, 2NP, and 2NFl were readily detected in all samples at concentrations well above the detection limit of the assay. Two additional compounds are observed in the second (bottom) m/z 218→201 transition. Based on the analysis of additional nitro-PAH standard compounds during method development efforts, we speculated that these peaks were 4-nitropyrene (4NP) and 3-nitrofluoranthene (3NFl). We confirmed this with standard addition experiments, i.e., spiking previously analyzed extracts with 3NFl and 4NP and assessing the increase in response of the unidentified peaks. These experiments confirmed that the additional peaks are 4NP and 3NFl. 4NP has been shown to be a product of the same atmospheric processes that generate 2NFl (*16*), and 3NFl has been shown to be present in the primary emissions of diesel engines, second in concentration only to 1NP (*17*).

NPAH Concentrations. As the NPAH data in Table 1 show, while the average concentrations of 2NP and 2NFl are similar across the different sampling locations, the 1NP concentration is dramatically higher for the traffic-based samples (Inside Car, Outside Car) compared to the sampling locations at least somewhat removed from traffic (Home, CDC). This observation is consistent with the fact that 1NP is present exclusively in primary emissions—predominantly in diesel exhaust. In contrast, 2NP and 2NFl are produced via atmospheric reactions with gas-phase PAH precursors, and, as such, would be expected to vary less between traffic and nontraffic sampling locations, as compared to 1NP.

Table 2 gives some concentrations of 1NP, 2NP, and 2NFl at locations around the world for purposes of comparison with the concentrations of these species found in Shenyang.

As Tables 1 and 2 show, the 1NP concentrations in Shenyang are toward the upper end of the range of values reported previously at other locations around the world. The traffic locations in Shenyang have values of 1NP concentrations in-line with the downtown/high traffic locations in Ho Chi Minh City, Vietnam, and Denmark, while the nontraffic locations in Shenyang are closer to the nontraffic locations in Ho Chi Minh City and Baltimore, MD in the summer.

The 2NP and 2NFl concentrations in Shenyang are substantially greater than those found in the locations listed in Table 2, although the levels of 2NFl in Shenyang are closest to those found in Ho Chi Minh City, as well as Torrance, CA. This comparison suggests that the atmospheric reaction conditions, including the abundance of gas phase precursors, are more favorable for 2NP and 2NFl formation in Shenyang

TABLE 2. Summary of Selected NPAH Concentrations in Literature

sampling location (city)	sampling location (environment type)	sampling date	2NP (pg/m^3)	2NFl pg/m^3	1NP (pg/m^3)	reference number
Ho Chi Minh City, Vietnam	urban	1/05–3/06	n/a	165 ± 65	8.1 ± 3.9	(5)
	urban	1/05–3/06	n/a	190 ± 97	9.1 ± 3.9	
	traffic site	1/05–3/06	n/a	191 ± 64	73 ± 40	
Denmark	urban/high traffic	12/96–6/96,	20 ± 5	91 ± 54	127 ± 44	(12)
	rural/open-land	2/98–2/99	8 ± 4	60 ± 22	30 ± 15	
Torrance, CA	urban	2/86	30	400	30	(18)
Los Angeles (LA) and Riverside (Riv), CA	traffic (LA)	8/02	6.2	62.8	6.5	(19)
	traffic (LA)	1/03	7.8	74.2	21	
	downwind (Riv)	8/02	3.1	154	6.5	
	downwind (Riv)	1/03	4.3	79.2	12.2	
Baltimore, MD Fort Meade, MD	urban (Baltimore)	1/01	6.5	60	27	(20)
	urban (Baltimore)	7/01	2.7	99	8.1	
	rural (Ft. Meade)	1/01	4	49	20	
	rural (Ft. Meade)	7/01	0.76	28	1.4	
Kanazawa, Japan	urban/downtown	10/94	4.5	59	32	(21)
	suburban	10/94	1.6	17	1.7	

during the sampling period than at the locations studied in the works summarized in Table 2.

Another observation regarding the data reported in Table 1 is that the coefficients of variation (CVs) are consistently higher for 2NFl and 2NP (CVs ~100%) compared to 1NP (CVs ~30–50%). A major determinant of the high CVs for 2NFl and 2NP (and also for PM) appears to be substantial day-to-day variation in the concentrations of these contaminants (illustrated for 2NFl in Figure S2 of Supporting Information). In contrast, there is relatively little day-to-day variation in the 1NP concentrations-especially at the traffic locations (Figure S3).

The two-stage impactor utilized in the current study permitted separate analysis of NPAHs in the fine and coarse PM fractions. For all locations approximately 90% of the mass of all NPAH species was found in the $PM_{2.5}$ fraction, and the variation in this distribution between locations was quite low (CV <5%; see Table S1). Over 84% of EC was found in the fine PM fractions and $PM_{2.5}$ constituted 57% of the PM_{10} (range = 47–67%).

Two distinct pathways have been described for the atmospheric formation of 2NFl and 2NP: the hydroxyl radical-initiated pathway, which yields both 2NFl and 2NP, and the NO_3 initiated pathway, which yields primarily 2NFl (*22*). Because the NO_3 radical (and associated NO_x species, NO_2 and N_2O_5) can play an important role in atmospheric chemistry (*11*), particularly at night when these NO_x species exist at higher concentrations, the ratio of 2NFl to 2NP is of interest not only for assessment of NPAH formation, but also because it provides insight into the overall atmospheric chemistry of a specific location or airshed.

Figures 2a and b show the median values for the concentration ratios of 2NFl to 2NP and 2NFl to 1NP, respectively, for each sampling location.

The median values for the 2NFl/2NP concentration ratio range from 2.5 to 4.3 across all four locations. Studies by Arey, Zielinska, and colleagues (*11*) concluded that ratios of 2NFl/2NP close to 10 indicated 2NFl formation mostly via the hydroxyl-initiated pathway, whereas ratios closer to 100 indicated mostly NO_3 initiated 2NFl formation. Therefore, our data indicate that formation of 2NFl and 2NP occurs primarily via the hydroxyl-initiated reaction in this airshed. It is important to note that the concentration of NO_3 radical is episodic, being more abundant at night. Thus, one would expect that the impact of the NO_3 initiated reaction and hence the ratio of 2NFl/2NP would be greater at night. However, examination of data from the CDC location where we collected separate, colocated daytime and nighttime filters, revealed that the 2NFl/2NP ratio was less than 10 in all samples, and on only one occasion was the nighttime 2NFl/2NP ratio significantly greater than the preceding daytime value. This observation supports our conclusion that—at least during the period of the current study—the hydroxyl-initiated reaction is the dominant source of 2NP and 2NFl. 2NFl/2NP ratios significantly below 10 have been observed in numerous areas around the world, with averages of 2.1 in Athens, Greece (*23*), 3.8 in Milan, 6.3 in Rome, 1.6 in Naples, 4.5 in Madrid, and 1.9 in Brazil (*10*), to list a few published values. The relative consistency of the 2NFl/2NP ratio in our study indicates that the formation process and reaction conditions that generate these two compounds are the same at all four locations in Shenyang during the sampling period.

There is also a positive linear correlation between the 2NFl and 2NP concentrations for all four locations, $r^2 = 0.631$

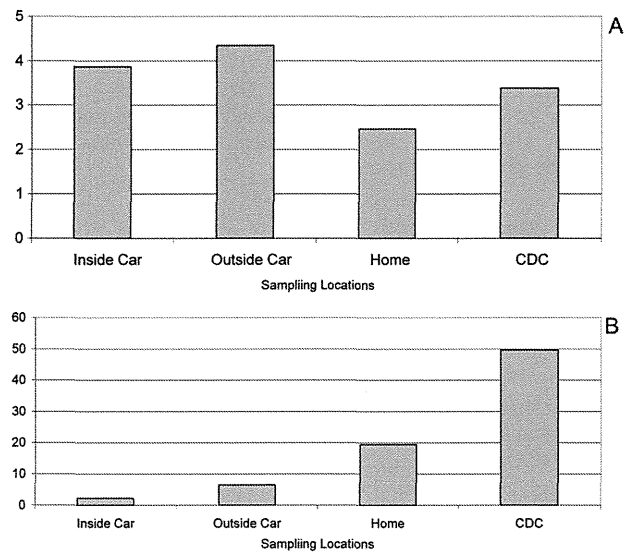

FIGURE 2. (a) Median concentration ratios of 2NFl to 2NP grouped by sampling location. Inside and outside car samples were collected during daytime. Home samples were collected at night and CDC samples were collected both during they day and at night. (b) Median concentration ratios of 2NFl to 1NP grouped by sampling location

($r^2 = 0.709$ if a single potential "outlier" is excluded). While this positive correlation does not necessarily implicate the hydroxyl-initiated pathway specifically for 2NFl and 2NP formation, it does suggest that the same process or combination of processes is prevalent over all sampling locations.

The ratio of the concentrations of 2NFl to 1NP has also been employed to assess the relative influence of primary sources as opposed to atmospheric formation for ambient NPAH levels (*5, 10, 25*). Some studies have implied that a 2NFl/1NP concentration ratio of 5 or greater is indicative of NPAH levels dominated by atmospheric reactions whereas a ratio less than 5 indicated a dominance of primary emissions (*5, 10, 25*). Notably, these benchmark ratios have been based on ambient measurements, as opposed to the chamber studies of Arey and Atkinson described cited earlier. The concentration ratio of 2NFl to 1NP, grouped by sampling location, is illustrated in Figure 2b. As expected given differing formation mechanisms for these two compounds, the sampling locations close to vehicle traffic have lower ratios of 2NFl to 1NP, indicating that ambient NPAH levels are more strongly influenced by vehicle exhaust, specifically diesel exhaust. The median value for the ratio of 2NFl/1NP is below the benchmark ratio of 5 described above for the Inside Car samples (median 2NFl/1NP = 2.3)—indicating a dominance of primary emissions. However, the median 2NFl/1NP ratio is above the benchmark for the Outside Car samples (median 2NFl/1NP = 6.5) as are the mean values for both sample types (Outside Car 9.1 ± 8.7, Inside Car 8.0 ± 7.9). This suggests that even for filter samples collected inside and outside of the taxis, the NPAH concentrations are still significantly influenced by secondary NPAH formation mechanisms in the atmosphere. The sampling locations removed from vehicle traffic have higher ratios of 2NFl to 1NP (median of 2NFl/1NP ratios: CDC 49.7, Home 19.4), indicating that the ambient NPAH concentrations at these locations are dominated by photochemically derived NPAHs. In addition, the ratio of 2NFl to 1NP for the CDC location, which is presumably the most removed from traffic, is greater than the corresponding mean ratio for the home locations.

Associations between 1NP and EC. Elemental Carbon (EC) is currently used as a marker for diesel exhaust (*7*). However, use of EC as a surrogate measure for diesel exhaust exposure may be confounded by combustion sources other than diesel exhaust that emit EC (*26*). In the current study, EC was quantified for the Inside Car and CDC sampling locations contemporaneously with the other PM samples, and associations between EC and 1NP were explored.

The correlations between 1NP and EC for the Inside Car location and CDC locations are shown in Figure 3a and b. We observed a high correlation between 1NP and EC concentrations in the PM samples collected at the CDC location. In contrast, the 1NP and EC concentrations at the Inside Car sampling location show no correlation. The lack of correlation between the 1NP and EC concentrations at the Inside Car location is not consistent with the expectation that both EC and 1NP are markers of primary diesel emissions. Both the EC and 1NP concentrations are noticeably higher at the Inside Car location as compared with the CDC location, as would be expected for markers of diesel exhaust. However, in the case of 1NP, the difference is approximately 7-fold, whereas in the case of the EC concentrations, the difference is approximately 2-fold.

The observation of a lack of correlation between the 1NP and EC concentrations at the Inside Car location (Spearman $r = -0.11$, $p = 0.62$) combined with the strong correlation of these concentrations at the CDC site (Spearman $r = 0.905$, $p = 0.002$) warrants some discussion. The ambient air in the city of Shenyang is heavily influenced by both industrial coal burning and residential coal burning, although the residential coal burning may not have been significant during the period

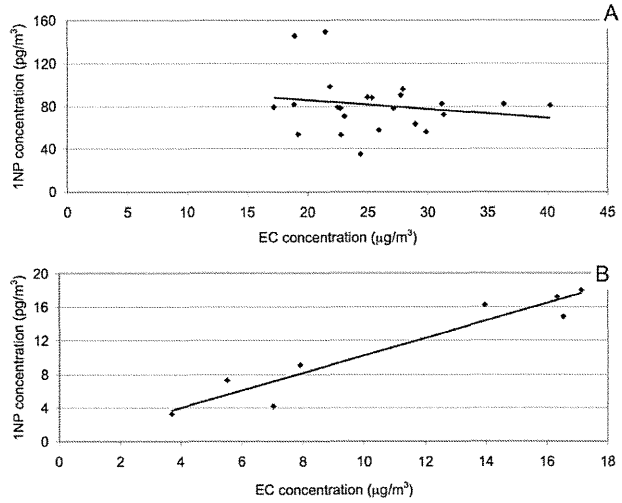

FIGURE 3. Relationship of 1NP to EC for (A) Inside Car and (B) CDC sampling locations.

of this study, as sampling was done in mid-August. Since EC is a significant constituent of coal combustion emissions (*26*), it is likely that coal combustion emissions confounded the expected association between EC and 1NP at the Inside Car sampling location. At the CDC location we found that all the air pollutants we measured were strongly correlated with EC (see Supporting Information, Figure S4a–d). The CDC location is the least influenced by direct traffic emissions, and in the current study it appears to reflect urban Shenyang PM derived from a consistent mixture of sources.

In conclusion, our data showed that for this cohort of taxi drivers, exposures to NPAH, particularly to 2NFl, were high compared to most of the ambient NPAH concentrations that have been previously reported in the literature. 1NP concentrations were ~4–5 fold higher during the drivers' workshift compared to the concentrations measured inside their homes. Therefore, we conclude that the major portion of the drivers' 1NP exposure occurred during their workshift, and, further, we anticipate that 1NP exposures are also high for individuals whose occupations place them in close proximity to traffic. In contrast, the high concentrations we observed for 2NFl exhibited little spatial variation, which indicates that a large fraction of the population of Shenyang is likely to have high exposures to 2NFl. The predominance of 2NFl compared to 1NP indicates a major contribution to ambient NPAH concentrations of secondary aerosol formed via atmospheric reactions with the parent PAHs, as opposed to primary emissions. The significance of atmospheric reactions for ambient NPAH concentrations is important as NPAH species in PM are responsible for at least some of the health risk associated with PM of anthropogenic origin.

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Supporting Information Available

Supporting Information is available for this manuscript. This information is free of charge via the Internet at http://pubs.acs.org.

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Indirect- and direct-acting mutagenicity of diesel, coal and wood burning-derived particulates and contribution of polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons

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Direct-acting mutagenicity

ABSTRACT

Particulates exhausted from two types of diesel engines (DEPs), burning-derived particulates from three types of coal (CBPs) and burning-derived particulates from three types of wood (WBPs) were separated into four fractions by silica-gel column chromatography using *n*-hexane, *n*-hexane–dichloromethane (3:1, v/v), dichloromethane and methanol, as the corresponding eluents. The indirect-acting mutagenicity of each fraction was assayed by the Ames test using the *Salmonella typhimurium* TA100 strain with S9 mix and the direct-acting mutagenicity was assayed using the *S. typhimurium* TA98 strain without S9 mix. The polycyclic aromatic hydrocarbons (PAHs) and nitropolycyclic aromatic hydrocarbons (NPAHs) of each fraction were determined by high-performance liquid chromatography (HPLC). Both direct- and indirect-acting of mutagenicities were the highest in samples of DEPs. The contributions of PAHs in samples of WBPs and NPAHs in DEPs were the largest, respectively.

1. Introduction

Inhalation of high concentrations of suspended particulates causes respiratory, cardiac and lung diseases. The particulates show indirect- and direct-acting mutagenicities, and polycyclic aromatic hydrocarbons (PAHs) are considered to be the main causes of indirect-acting mutagenicity, while nitropolycyclic aromatic hydrocarbons (NPAHs) are considered to be the main causes of the direct-acting mutagenicity [1,2]. Several NPAHs such as, 1,3-, 1,6-, and 1,8-dinitropyrenes (DNPs) and 3-nitrobenzanthrone (NBA) show very strong direct-acting mutagenicities [3,4]. These PAHs and NPAHs are mainly formed through an imperfect combustion process of organic matters. Some NPAHs are also known to be formed through secondary reactions of PAHs and nitrogen oxides in the atmosphere.

Diesel exhaust particulates (DEPs) cause serious urban air pollution in several countries, including Japan [1,3,4]. In China and Russia, coal burning-derived particulates (CBPs) have been considered as the main causes of recent urban air pollution [5–7]. Mutagenicity of DEPs and CBPs has been investigated by some scientists [8–10]. And we previously also showed that DEPs and CBPs are directly mutagenic [11,12]. Wood burning-exhaust particulates (WBPs) are another major source of atmospheric particulate matter [13,14] and PAHs [15]. Kato et al. [16] found that charcoal workers exposed to wood smoke received a systemic exposure to genotoxic compounds by study of urinary mutagenicity. These particulates include several toxic and mutagenic compounds, such as PAHs and NPAHs [17,18], and are one of causes of the above-described diseases in developing countries [19].

Countries in East Asia are undergoing rapid economic development. At the same time, the consumption of energy, such as coal and oil in China, is also increasing rapidly and continuingly. The increase of anthropogenic particulates in the atmosphere has raised health concerns. However, the mutagenicities and the contributions of PAHs and NPAHs to these particulate have not been compared. In this study, extracts from DEPs, CBPs and WBPs were analyzed in order to clarify the compositions of PAHs and NPAHs. On the other hand, indirect- and direct-acting mutagenicities in those extracts were investigated. From these results, the contributions of PAHs to the indirect-acting mutagenicity and NPAHs to

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Table 1
Indirect- and direct-acting mutagenicities (rev. mg^{-1}) of fractions of DEPs, CBPs, and WBPs.

Fractions	DEP		CBP			WBP		
	Isuzu (3059 cc)	Yamaha (1007 cc)	China solid	China crushed	Hokkaido solid	Oak	Cercidiphyllum	Cherry blossom
Indirect								
Order	11219	2058	1067	538	3556	622	169	237
Fr. 1	12	0	1230	15	656	131	62	536
Fr. 2	3177	862	546	262	1516	223	133	1291
Fr. 3	3955	299	309	67	1139	122	100	741
Fr. 4	763	74	274	73	213	48	73	236
$\sum$1234	12906	1234	2358	417	3529	575	367	2814
Direct								
Order	13520	789	330	39	629	141	34	323
Fr. 1	0	0	32	0	0	0	0	0
Fr. 2	705	269	54	20	366	49	16	83
Fr. 3	10009	546	491	83	683	73	69	304
Fr. 4	3918	94	39	26	313	41	27	153
$\sum$1234	14630	909	667	129	1369	164	112	540

(1) *n*-Hexane; (2) *n*-hexane DCM; (3) DCM; (4) *n*-hexane; $\sum$1234, some of the four fractions.

direct-acting mutagenicity in DEPs, CBPs and WBPs were calculated and compared.

2. Experimental

2.1. Chemicals

EPA 610 PAHs mix including naphthalene (Nap), acenaphthene (Ace), fluorene (Fle), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benz[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), dibenz[*a,h*]anthracene (DBA), benzo[*ghi*]perylene (BghiPe), indeno[1,2,3-*cd*]pyrene (IDP) were purchased from Supelco Park (Bellefonte, PA, USA). Pyrene-d_{10} (Pyr-d_{10}) and benzo[*a*]pyrene-d_{12} (BaP-d_{12}), internal standards for PAHs, were purchased from Wako Pure Chemicals (Osaka, Japan).

1,3-, 1,6-, 1,8-Dinitropyrenes (DNPs), 1-nitropyrene (1-NP), 1-nitrofluoranthene (1-NFR), 3-nitrofluoranthene (3-NFR), 4-nitrophenanthrene (4-NPh), 9-nitrophenanthrene (9-NPh), 2-nitropyrene (2-NP), 4-nitropyrene (4-NP), 2-nitrofluoranthene (2-NFR), 6-nitrochrysene (6-NC), 3-nitrobenz[*a*]anthracene (3-NBaA), 5-nitroacenaphthene (5-NAc), 2-nitrofluorene (2-NF), 7-nitrobenz[*a*]anthracene (7-NBaA), 10-nitrobenz[*a*]anthracene (10-NBaA), 1-nitroperylene (1-NPer), 3-nitroperylene (3-NPer), 6-nitrobenzo[*a*]pyrene (6-NBaP), 2-nitroanthracene (2-NA), 9-nitroanthracene (9-NA), 2-nitrotriphenylene (2-NTP) and 2-fluoro-7-nitrofluorene (FNF), internal standard for NPAHs, were purchased from Chiron AS (Trondheim, Norway). All other chemicals used were of analytical reagent grade.

2.2. Sampling and pretreatment of WBP, DEP and CBP

Two different DEP samples investigated in this study were kindly given by Dr. H. Takano of NIES. They were respectively collected from the dilution tunnel of Isuzu 4JG2-type engine (3059 cc) and Yamaha NF-19 engine (1007 cc) by a high-volume air sampler (Kimoto 123-XL) with a quartz-fiber filter (8 in. × 10 in., 2500QAT-UP, Pallflex Products, Putnam, CT, USA). The sampling temperature was 50 ± 5 °C. Each sample was extracted twice with benzene–ethanol (3:1, v/v) ultrasonically. And the extract was evaporated to remove solvent. The procedures of extraction and evaporation were the same as those in our previous reports [11,12].

Chinese Fushun solid coal and crushed coal, and Japanese Hokkaido solid coal were burned by a domestic coal stove respectively for sampling of CBPs. Wood of cercidiphyllum, cherry blossom and oak were also burned by the same stove as described above for sampling of WBPs. A high-volume air sampler (Kimoto 123-XL) with a quartz-fiber filter (8 in. × 10 in., 2500QAT-UP, Pallflex Products, Putnam, CT, USA) was set about 50 cm after the end of the 4 m flue of the stove. The sampling temperature was 50 ± 5 °C. Each sample of these three CBPs and three WBPs was extracted twice with benzene–ethanol (3:1, v/v) ultrasonically. And the extract was evaporated to remove solvent under the same conditions as described above.

2.3. Silica-gel column chromatography

Each crude extract solution was evaporated to dryness and the residue was redissolved in 10 ml of *n*-hexane. The solution was applied to a silica-gel column (Wakogel Q200, 1.5 cm i.d. × 21.3 cm bed). The column was then eluted successively with *n*-hexane (200 ml), *n*-hexane–dichloromethane (DCM) (3:1, v/v) (200 ml), DCM (200 ml) and methanol (450 ml). The corresponding fractions (fractions 1–4) were collected according to our previous paper [11]. Each fraction was divided into two equal volume solutions and both solutions were evaporated to dryness. The one dried sample was added with the internal standard solution of deuterated PAHs and FNF, and dissolved in acetonitrile for the determination of PAHs and NPAHs. The other one was dissolved in dimethylsulfoxide (DMSO) for the mutagenicity assay.

2.4. Determination of PAHs and NPAHs

The 15 PAH species having two to six rings described in Section 2 were determined by using HPLC with fluorescence detection. A reversed-phase column (Inertsil ODS-P, 4.6 mm i.d. × 250 mm, GL Sciences Inc., Tokyo, Japan) was used with an acetonitrile/water gradient. The flow rate was 1 ml min^{-1}. The time program of the fluorescence detector was set to detect at the optimum excitation and emission wavelengths for each PAH. The other conditions were the same as those in our previous report [20].

The 21 NPAH species having three to six rings described in Section 2 were determined by using HPLC with chemiluminescence detection. The HPLC system consisted of two reversed-phase columns (Cosmosil 5C18-MS, 4.6 i.d. × (250 + 150) mm, Nacalai Tesque, Tokyo, Japan) connected in series a chemiluminescence detector. The mobile phase was 10 mM imidazole buffer (pH 7.6)–acetonitrile (1:1, v/v), and the chemiluminescence reagent solution was an acetonitrile solution containing 0.02 mM bis(2,4,6-trichlorophenly)oxalate and 15 mM hydrogen peroxide. The flow rate was 1 ml min^{-1} for each solution. The other conditions were the same as those in our previous report [11,12].

2.5. Mutagenicity assay

Indirect- and direct-acting mutagenicities were assayed by the Ames test using *Salmonella typhimurium* TA100 strain with S9 mix and *S. typhimurium* TA98 strain without S9 mix, respectively [21,22]. At least two plates were used for each dose, and the mean values of the revertants (pmol^{-1} plates^{-1}) were calculated from linear regression lines fitted to the increasing portion of the dose–response curve. And the mutagenicities presented in this study were quantified by revertants per milligram particles. The other conditions were the same as in our previous study [11,12].

3. Results and discussion

3.1. Mutagenicities of extracts from DEPs, CBPs and WBPs

Indirect- and direct-acting mutagenicities of DEPs, CBPs and WBPs calculated by using the mutagenicities of each extract were shown in Table 1. The indirect-mutagenicities of all the samples represent higher value in fraction 2 (*n*-hexane/DCM) than fraction 3 (DCM) and opposite results can be found for direct-mutagenicities. Regarding the indirect-acting mutagenicities of crude extraction of each kind of particulate, Isuzu (3059 cc) (11,219 rev. mg^{-1}), Hokkaido solid (3556 rev. mg^{-1}) and Oak (622 rev. mg^{-1}) show the highest value, respectively. And the highest indirect-acting mutagenicities of crude extraction of each kind of particulate are Isuzu (3059 cc) (13,520 rev. mg^{-1}), Hokkaido solid (629 rev. mg^{-1}) and Cherry blossom (323 rev. mg^{-1}), respectively.

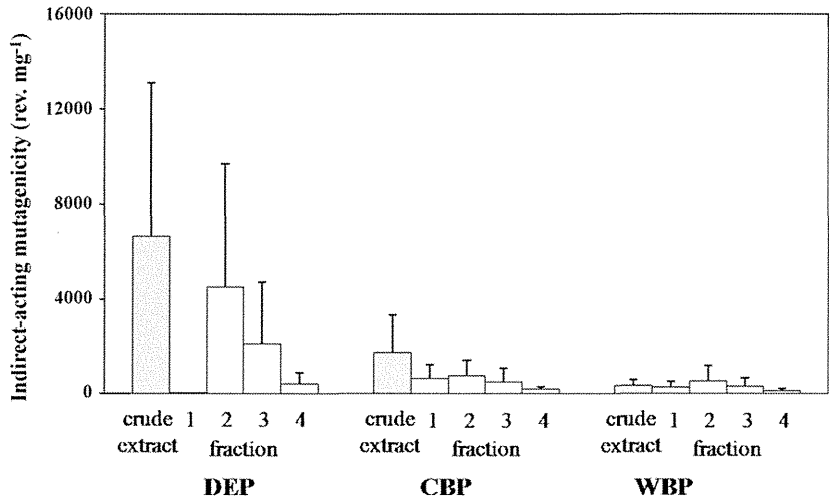

Fig. 1. Indirect-acting mutagenicities of DEP, CBP, and WBP extracts after silica-gel column chromatography in *S. typhimurium* TA100 strain with S9 mix (DEP: $n = 2$; CBP and WBP: $n = 3$).

The average values of indirect- and direct-acting mutagenicities are summarized in Figs. 1 and 2, respectively, in order to investigate the comparison between DEP, CBP and WBP. As shown in Fig. 1, DEP showed the highest indirect-acting mutagenicity (6639 ± 6478 rev. mg^{-1}) being about 4 times higher than the value of CBP (1720 ± 1612 rev. mg^{-1}) and 20 times higher than the value of WBP (343 ± 245 rev. mg^{-1}). When the indirect-acting mutagenicities were calculated by the total of the four separated fractions, the values increased to 7070 ± 8285 rev. mg^{-1} for DEP, 2101 ± 1931 rev. mg^{-1} for CBP and 1252 ± 1362 rev. mg^{-1} for WBP. We previously reported that PAH inhibited the direct-acting mutagenicity of NPAH [11]. Therefore, like the inhibitors of direct-acting mutagenicity, the increase of the indirect-acting mutagenicities after the separation by silica-gel column chromatography is probably because the inhibitors of indirect-acting mutagenicities are also present. It is important to identify these inhibitors and to clarify their inhibitory activities.

Regarding direct-acting mutagenicities, fraction 3 showed the highest activity for each kind of particulate (Fig. 2). Among the crude extracts, the order of mutagenicity is DEP (7155 ± 9002 rev. mg^{-1}) > CBP (333 ± 295 rev. mg^{-1}) > WBP (166 ± 146 rev. mg^{-1}). The total direct-acting mutagenicity of the four separated fractions were DEP (7769 ± 9702 rev. mg^{-1}) > CBP (727 ± 675 rev. mg^{-1}) > WBP (272 ± 237 rev. mg^{-1}). This result might be due to the higher concentrations of the NPAHs in DEPs than in CBPs and WBPs. In all types of particulates, except for DEP, the total mutagenicity of the four fractions was higher than the mutagenicity of the crude extracts. The comparison of Figs. 2 and 3 suggested that this might be because major inhibitors such as PAHs were mainly separated from the NPAH fractions by silica-gel column chromatography as reported previously [11].

3.2. PAHs and NPAHs in DEPs, CBPs and WBPs

Concentration of PAHs in DEPs, CBPs and WBPs, respectively, are compared in Table 2. The total concentration of PAHs was the highest in WBP ($52{,}323 \pm 48{,}935$ pmol mg^{-1}), being about twice as high as the level of CBP ($22{,}830 \pm 21{,}300$ pmol mg^{-1}), although concentrations of several volatile PAHs such as Ace, Phe and Ant were higher in CBP. On the contrary, the total PAH concentration in DEP (586 ± 568 pmol mg^{-1}) was the lowest in these three kinds of particulates. Concentrations of NPAHs in DEPs, CBPs and WBPs are shown in Table 3. Total NPAHs in WBP (5.2 ± 8.4 pmol mg^{-1}) and CBP (4.7 ± 5.8 pmol mg^{-1}) was at similar level. On the contrary, the total concentration of NPAHs was the highest in DEP (191 ± 99 pmol mg^{-1}). Especially the concentra-

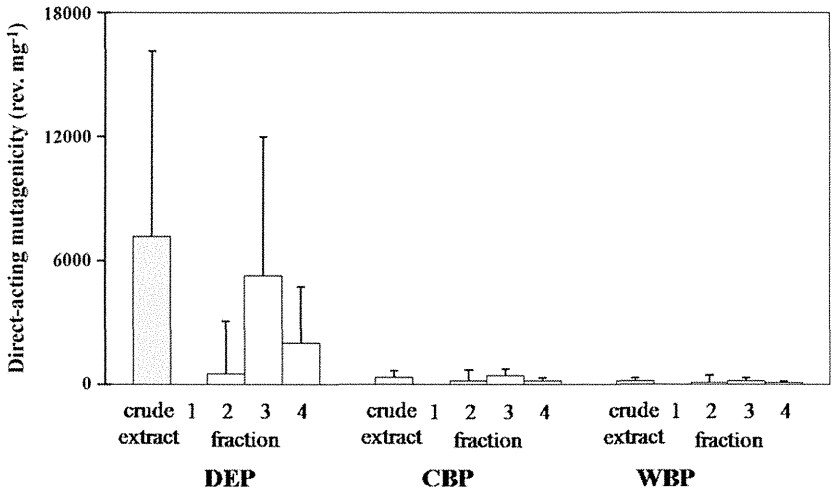

Fig. 2. Direct-acting mutagenicity of DEP, CBP, WBP extracts after silica-gel column chromatography in *S. typhimurium* TA98 strain without S9 mix (DEP: $n = 2$; CBP and WBP: $n = 3$).

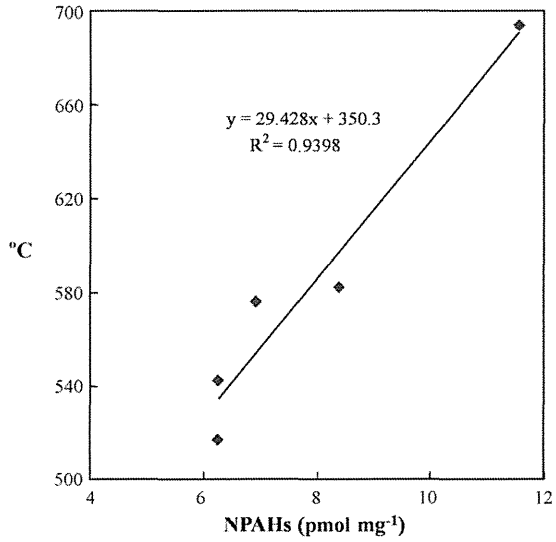

Fig. 3. Correlation between NPAHs from CBPs and the burning temperature.

Table 2
Contributions of PAHs in DEP, CBP and WBP (pmol mg^{-1}).

	DEP	CBP	WBP
Nap	N.D.	N.D.	N.D.
Ace	5.0 ± 10.6	410 ± 720	43 ± 75
Fle	N.D.	910 ± 1400	2300 ± 3900
Phe	129 ± 172	4500 ± 3900	2600 ± 1600
Ant	N.D.	2300 ± 2200	580 ± 280
Flu	180 ± 85	4200 ± 4000	6600 ± 3700
Pyr	207 ± 216	4200 ± 3500	7500 ± 3800
BaA	12 ± 11	1800 ± 950	5300 ± 4400
Chr	12 ± 7.8	1700 ± 1400	7500 ± 7200
BbF	23 ± 29	830 ± 1100	4000 ± 5300
BkF	4.7 ± 9.2	400 ± 280	2400 ± 2900
BaP	0.6 ± 1.3	950 ± 800	6100 ± 7300
DBA	N.D.	N.D.	100 ± 180
BghiPe	8.7 ± 18	500 ± 860	3900 ± 3900
IDP	5.0 ± 7.1	130 ± 190	3400 ± 4400
Total	586 ± 568	22830 ± 21300	52323 ± 48935

DEP, CBP and WBP: all data represent mean concentrations ±S.D. (DEP: $n = 2$; CBP and WBP: $n = 3$). N.D., not detected.

Table 3
Contributions of NPAHs in DEP, CBP and WBP (pmol mg^{-1}).

	DEP	CBP	WBP
2-NF	63 ± 52	N.D.	0.7 ± 1.1
4-Nph	N.D.	0.7 ± 1.2	N.D.
9-Nph	N.D	0.6 ± 0.9	0.5 ± 0.8
5-NAc	N.D.	N.D.	N.D.
2-NA	0.0 ± 0.0	0.8 ± 1.3	0.8 ± 1.3
9-NA	N.D.	0.2 ± 0.4	1.6 ± 2.7
1-NP	102 ± 11	1.1 ± 0.7	1.1 ± 1.8
2-NP	N.D.	N.D.	N.D.
4-NP	0.1 ± 0.2	0.6 ± 0.4	0.0 ± 0.1
3-NFE	0.2 ± 0.3	0.1 ± 0.2	0.0 ± 0.0
2-NTP	N.D.	N.D.	N.D.
7-NBaV	4.9 ± 6.9	0.06 ± 0.08	0.1 ± 0.2
6-NC	5.5 ± 7.8	0.4 ± 0.7	0.2 ± 0.3
3-NBA	14 ± 19	N.D.	N.D.
10-NBA	N.D.	N.D.	N.D.
1,3-DNP	0.9 ± 1.3	0.01 ± 0.01	0.2 ± 0.02
1,6-DNP	0.2 ± 0.2	0.003 ± 0.003	0.004 ± 0.007
1,8-DNP	0.4 ± 0.3	0.06 ± 0.01	0.04 ± 0.1
6-NBaP	0.02 ± 0.02	0.03 ± 0.003	0.02 ± 0.02
1-Nper	N.D.	N.D.	0.01 ± 0.01
3-NPer	N.D.	0.01 ± 0.02	0.01 ± 0.01
Total	191 ± 99	4.7 ± 5.8	5.2 ± 8.4

DEP, CBP and WBP: all data represent mean concentrations ±S.D. (DEP: $n = 2$; CBP and WBP: $n = 3$). N.D., not detected.

Table 4
Indirect-acting mutagenicities of PAHs in *S. typhimurium* TA100 strain with S9 mix.

PAH	Mutagenicity (rev. nmol^{-1})
Nap	<1
Ace	<1
Fle	<1
Phe	<1
Ant	<1
Flu	10
Pyr	<1
BaA	10
Chr	<1
BbF	34
BkF	15
BaP	64
DBA	20
BghiPe	1
IDP	3

tion of 1-NP in DEP was about 100 times higher than that in both CBP and WBP. Moreover, the contribution of 1-NP to total NPAHs of DEP ([1 – NP]/[$\sum$NPAH] = 0.53) is also higher than that of CBP (0.23) and WBP (0.21) (Table 3). As it was mentioned in last section, the higher concentration of NPAHs resulted in much higher direct-acting mutagenicity in DEPs than the other two. And it is worthwhile to note that, although NPAHs show a highest level in DEP among those three kinds of particulates, the PAHs concentration is the lowest in DEP. NPAHs that are produced from combustion reactions are generally formed via electrophilic nitration reactions in the presence of NO_2 [23] and these kinds of reactions are accelerates by the burning temperature. We previously reported that the [NPAH]/[PAH] concentration ratio was in the order DEP > CEP > WBP [24]. In this study, we found that the NPAH concentration in CBPs (Chinese solid) was strongly and positively correlated with the combustion temperature ($r = 0.97$, $n = 5$, $p < 0.01$) (Fig. 3).

3.3. Contributions of PAHs and NPAHs to indirect- and direct-acting mutagenicities respectively, of DEPs, CBPs and WBPs

To examine the contributions of PAHs to the indirect-acting mutagenicities of DEPs, CBPs and WBPs, the indirect-acting mutagenicities of the fifteen PAHs were assayed independently.

Table 5
Direct-acting mutagenicities of NPAHs in *S. typhimurium* TA98 strain without S9 mix.

	Mutagenicity (rev. pmol^{-1})
2-NF	<0.1
4-Nph	<0.1
9-Nph	0.1
5-NAc	<0.1
2-NA	2
9-NA	<0.1
1-NP	0.3
2-NP	3
4-NP	37
3-NFR	7
2-NTP	26
7-NBaV	<0.1
6-NC	<0.1
3-NBA	53
10-NBA	14
1,3-DNP	48
1,6-DNP	7
1,8-DNP	116
6-NBaP	<0.1
1-NPer	<0.1
3-NPer	<0.1