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Effects of Environmental Conditions on Polycyclic Aromatic Hydrocarbons and Mutagenicity of Airborne Particulate Matter

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Much attention has been given to chemical and photochemical reactions of polyaromatic hydrocarbons (PAHs) toward gaseous pollutants such as ozone (O_3) and nitrogen dioxide (NO_2) in the atmospheric environment including sunlight, because they proved to form directly active mutagens (Van Cauwenberghe and Van Vaeck 1983). Pitts et al. (1980) reported the formation of benzo(a)pyrene-4,5-oxide, direct mutagen with Salmonella typhimurium TA98, by ozolysis of benzo(a)-pyrene. Tokiwa et al. (1981) found that 6 PAHs exposed to 10 ppm NO_2 were transformed into nitro-substituted directly active mutagens. Also Gibson et al. (1978) and McCoy et al. (1979) demonstrated such transformations of environmental pollutants by exposure to various radiation sources. In these simulated experiments, PAHs in homogeneous system, not adsorbed onto natural combsution-related particles, had been exposed to high levels and high flow rate of gaseous pollutants or to artificial lights. It is expected that the efficiency of such transformation of PAHs in ambient particles will largely depend on their physical and chemical nature (Tebbens et al. 1971, Korfmacher et al. 1980). The close interaction between aerosol matrix and the adsorbed organic and inorganic materials as sunlight irradiation will induce a different chemical behavior of PAHs from that obtained in the laboratory experiments. For better assessment of toxicological risk of airborne particulate matter during atmospheric transport, chemical and biological information from exposure experiments performed in closely related to real atmospheric conditions should be required.

We have already reported the transformation of pyrene into directly active mutagens following exposure to sunlight in the real atmosphere (Takeda et al. 1984). In the present study, we exposed "natural" airborne particles already collected to the real atmospheric environment including sunlight of Kobe in summer and in winter, and its effect on PAH content and mutagenicity of the particles was evaluated.

MATERIALS AND METHODS

Airborne particulate matter was collected on a glass fiber filter (Toyoroshi GB 100R, 18 x 23 cm) by a high volume air sampler (Kimoto

Electric Co. Ltd., Osaka, Japan) on the roof of our institute from February to April 1983. Approximately 2000 m^3 of air and 200 mg of suspended particulate matter were sampled during a 24-h period. Each of 12 particulate-laden filters was cut into 88 pieces of small quadrilaterals (2 x 2 cm). Twelve quadrilaterals were gathered from 12 different filters and one set of homogeneous particulate-laden filter sample was prepared. The filter sample contains 23 mg of suspended particulate matter and corresponds to 224 m^3 of air. The samples were stored at -20°C under N_2 gas until experiments. Data on ambient NO_2 and O_x levels, and solar radiation intensity were cited from "Annual air pollution report of Kobe, No.26, 1983-1984".

The filter samples were fixed on glass plates by adhesive tape, and exposed to sunlight on bright sunny days in August and September 1983, and in February 1984. In-dark experiment the sample filters were put into a polyethylene vessel (20 x 25 x 30 cm) covered with alminium foil, which was ventilated at a flow rate of 5 l/min. In-light and in-dark experiments were performed simultaneously. After exposure, the samples were stored at -20°C under N_2 gas until the next exposure. The exposed samples were tore off from the glass plate and placed in 100 ml-Erlenmeyer flask. A 50 ml-aliquot of benzene/ethanol (3:1, v/v) was added to the flask and the organic material was extracted by ultrasonic agitation at 15-20°C for 20 min. The solution was filtered and evaporated at 40°C under reduced pressure. The residue was re-dissolved in an appropriate amount of chloroform/methanol (1:1, v/v).

Six PAHs [fluoranthene(Flu), pyrene(Pyr), benzo(a)pyrene(B(a)P), benzo(a)anthracene(B(a)A), coronene(Cor) and perylene(Pery)] were quantitated by high-performance liquid chromatography (HPLC) with fluorescent detection. HPLC was performed on a model ALC/GPC 204 liquid chromatograph (Waters Assoc., Milford, MA, U.S.A.). Detection was by a model RF-530 fluorescence detector (Shimadzu Corporation, Kyoto, Japan). Both excitation and emission wavelength were manually changed during chromatographic runs as shown in Fig.4 and Table 1. The wavelength conditions reported by May and Wise (1984) were used for the determination of PAHs except coronene. The HPLC column was a Radial PAK μBondapak C_{18} cartridge (10 μm, 8 mm x 10 cm) fitted in a Z-module radial compression separation system (Waters). The column was eluted with a linear gradient of 40% to 100% acetonitrile (HPLC grade, Wako Pure Chemicals, Osaka, Japan) in glass-distilled water in 45 min. A flow rate of 1 ml/min was used and the separation was carried out at room temperature.

The organic matter was re-dissolved in dimethyl sulfoxide and the mutagenicity test with Salmonella typhimurium was performed as described by Ames et al. (1975). A tester strain TA98 was used, because the PAH derivatives formed by various radiations in air had been found to be frame shift mutagens (Gibson et al. 1978). A 9000 x g supernatant (S9) was prepared by the method of Teranishi et al. (1978).

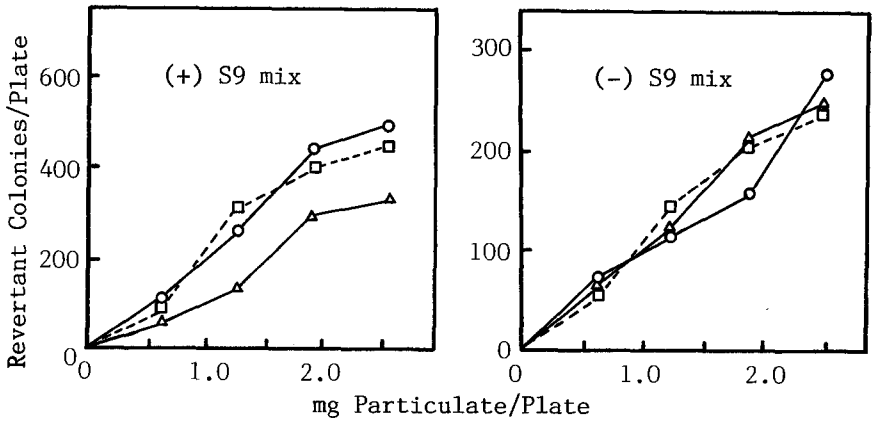

Figure 1. Dose response curves for the mutagenicity of airborne particulate matter exposed to 3 different conditions. Exposure experiments were performed during August 1-4, 1983. Total exposure period was 20 h. Averaged ambient temperature, Ox and NO_2 levels were 33.6°C, 0.035 ppm and 0.023 ppm, respectively.

□----□ : Control (Stored at -20°C under N_2 gas)

o——o : Exposed to ambient air in a ventilated alminium foil covered vessel.

△——△ : Exposed to sunlight and ambient air.

RESULTS AND DISCUSSION

In order to examine whether atmospheric environment including sunlight radiation affects the mutagenicity of airborne particulate matter, benzene/ethanol extracts of the filter samples exposed to sunlight in ambient air and those exposed to ambient air in the dark were tested for mutagenicity using a tester strain TA98 in the presence and absence of metabolic activation system. Both extracts showed mutagenicity with and without S9 mix (Fig. 1). A linear dose-response curves for the mutagenicity of 3 different samples was obtained, and the mutagencity with S9 mix (indirectly active mutagenicity) was about 2 times higher than that without S9 mix (directly active mutagenicity). Sunlight irradiation seemed to reduce the indirectly active mutagenicity, however, it had no effect on the directly active mutagenicity. Both directly and indirectly active mutagenicity of the filter samples seemed not to be affected by pollutant gases such as NO_2 and O_x in the ambient air. These were unexpected results, because several PAHs had been demonstrated to be transformed into strong direct acting mutagens by various radiations and by atmospheric gaseous pollutants (Pitts et al. 1980, Tokiwa et al. 1981, Gibson et al. 1978, Takeda et al. 1984).

To further ascertain the effect of sunlight and ambient pollutant gases on the mutagenicity of the filter samples, the exposure experiments were performed on time-course for a period up to 24 h. Results are shown in Fig. 2 and they were essentially the same as those shown in Fig. 1. The directly active mutagenicity remained unchanged over the exposure periods, while the indirectly active

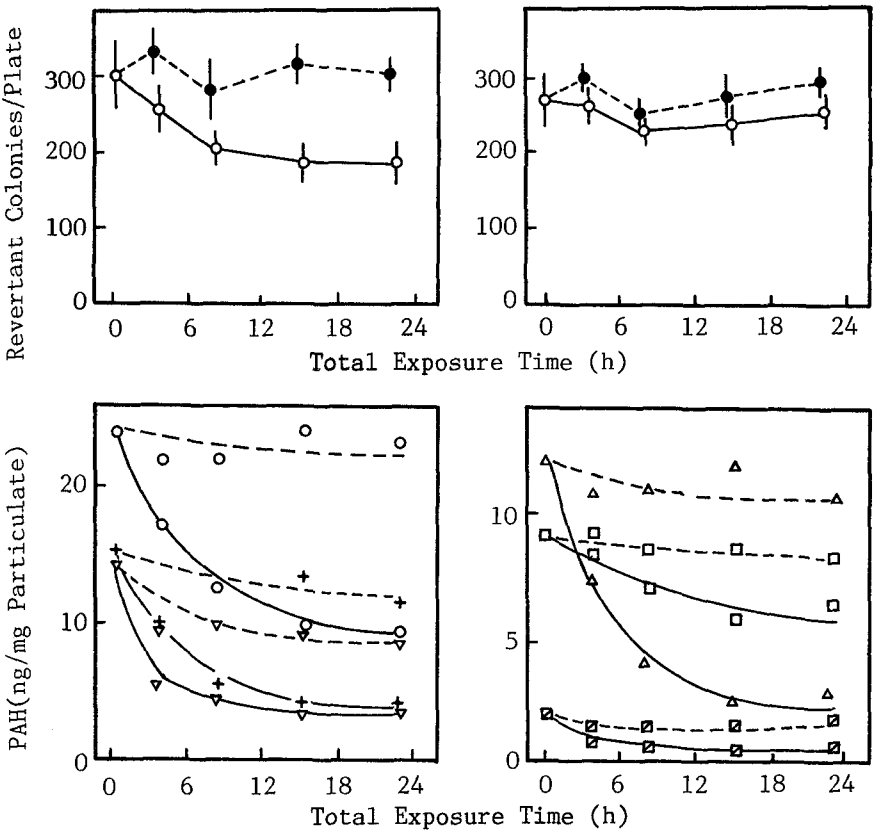

Figure 2. (Upper) Change in the mutagenicity of the filter samples exposed to sunlight (—o—) and to ambient air in the dark (--●--). Exposure experiments were carried out during August 18-22, 1983. Ambient temp. 31.5°C, O_x 0.013 ppm, NO_2 0.010 ppm. The extract equivalent to 1.9 mg of particulate matter was assayed both with and without S9 mix. Values represent the average of 3 determinations in duplicate (6 plates). Vertical bars and asterisks (*) indicate ± S.E. and the significant difference from the control value by Student's t test ($p<0.05$).

Figure 3. (Lower) Change in PAH contents of the filter samples exposed to sunlight (———) and to ambient air in the dark (- - - -). Values represent the average of 3 determinations.

o : B(a)P	+ : Flu	▽ : Pyr
△ : B(a)A	□ : Cor	▨ : Pery

mutagenicity of the samples exposed to sunlight for 8 h was reduced to about two third of the control value ($p<0.05$), and thereafter it was nearly constant up to 24 h. As the significantly lowered indirect mutagenic activities of the sunlight-exposed samples seemed to be due to losses of mutagenic compounds such as B(a)P which requires metabolic conversion to active products, the amount of PAHs associated with the particles was determined by HPLC with fluorescent detection. Typical chromatograms are shown in Fig. 4. The

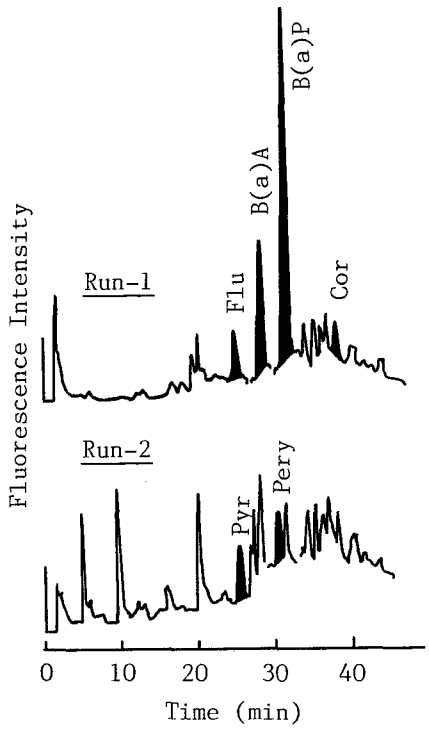

Table 1. Fluorescence conditions for the HPLC determinations of PAHs in particulate matter

PAH	Wavelength (nm) Excitation	Emission
Run-1		
Flu	285	450
B(a)A	285	385
B(a)P	295	405
Cor	305	445
Run-2		
Pyr	330	385
Pery	400	445

Figure 4. Reversed-phase LC analysis of PAHs extracted from airborne particulate matter exposed to sunlight in ambient air.

results show that sunlight considerably accelerates the losses of PAHs. Susceptibility of them toward sunlight under the conditions was the following order: Pyr, B(a)A > Flu, B(a)P > Pery, Cor. Approximate half-lives of B(a)P, B(a)A, Pyr and Flu were 8, 3, 2.5 and 6 h, respectively. These analytical data of PAHs suggest that the observed decrease in the indirect mutagenic activity can be explained in terms of the decreased amount of indirectly active mutagenic compounds in the particulates such as PAHs. Moreover losses of co-mutagenic substances such as fluoranthene (Tokiwa et al. 1977, Ashby and Style 1978) might accelerate the reduction of the indirectly active mutagenicity.

In further series of experiments, a contribution of seasonal factors to the change of both PAH content and the mutagenicity was evaluated. To standardize the exposure conditions, the exposure period for 4 h a day (10:30-14:30) was set both in summer and in winter. Results are shown in Fig. 5 and Table 2. Only the indirectly active mutagenicity of the filter samples upon exposure to sunlight during summer decreased significantly ($p<0.05$), but the others essentially unchanged over the experimental periods in both the seasons. Although pyrene was likely to be somewhat susceptible to dark reactions in summer, other PAHs determined here were rather resistant toward ambient air in the dark in both the two seasons (Fig. 3 and

Table 2. Comparison of the particulate PAH content between summer and winter after exposure to ambient air in sunlight or in the dark

PAH (ng/mg particulate)	Control (non-exposed)	Summer				Winter			
		Sunlight		Dark		Sunlight		Dark	
		8	24	8	24	8	24	8	24(h)
Pyr	14.4	5.2	5.2	8.0	8.0	9.8	6.3	12.6	14.9
B(a)A	12.0	3.5	3.5	8.9	9.8	7.9	5.8	10.7	11.1
B(a)P	24.2	13.2	8.7	20.1	19.2	18.7	15.6	21.7	23.4
Cor	9.8	11.0	11.6	8.7	11.0	9.6	7.0	11.0	11.0

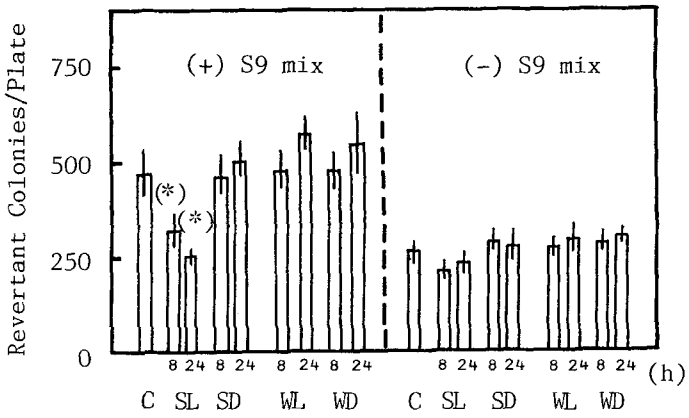

Figure 5. Seasonal change in the mutagenicity of the filter samples exposed to sunlight (L) and to ambient air in the dark (D) both in summer (S) and in winter (W). (C) indicates the control value. Exposure periods and atmospheric conditions: Summer; August 30-September 6, 1983. Temp. 34.7°C, O_x 0.015 ppm, NO_2 0.020 ppm, Solar radiation intensity 25 KJ/m²/h. Winter; February 14-20, 1984. Temp. 9.4°C, O_x 0.022 ppm, NO_2 0.017 ppm, Solar radiation intensity 9 KJ/m²/h. Values represent the average of 3 determinations in duplicate(6 plates). Vertical bars and asterisks (*) indicate ± S.E. and the significant difference by Student's t test ($p<0.05$).

Table 2). Upon exposure to sunlight for 8 h, losses of more than 60% were observed for pyrene and benzo(a)anthracene in summer, but about two third of those of the initial amount was still present in the winter samples. Coronene appeared much more resistant to both light and dark reactions in summer and in winter.

Losses of PAHs from the particles could be considered to be due to either vaporization, photochemical oxidation with activated oxygen or chemical reactions with gaseous pollutants. The contribution of vaporization to the losses of 4-membered rings such as pyrene in summer (Yamasaki et al. 1978) is thought to be very small, judging

from our previous results that 96% of initial weight of pyrene adsorbed on a filter paper was recovered upon exposure to sunlight in summer (Takeda et al. 1984). Chemical reaction of PAHs with atmospheric pollutants gases in the absence of sunlight might partially contribute to their losses in summer, but not in winter. With respect to NO_2 and O_x levels, those prevailing in Kobe were much lower than those used in other several simulated experiments. Grosjean et al. (1983) also showed no evidence for chemical removal of B(a)P, perylene and 1-nitropyrene, irrespective of filter paper, carrier particle and polluted gases (0.1 ppm of NO_2, O_3 or SO_2). According to Tebbens et al. (1971), the disappearance of PAHs from soot increases with increasing intensity of light irradiation. The slower decreasing rate of PAHs observed in winter is probably due to less solar radiation intensity as well as lower ambient temperature. It can be concluded that (1) PAHs associated with air particles disappear more slowly in the dark, probably due to the presence of oxidizing materials inside the particles and/or in ambient air, (2) sunlight has much more pronounced effects on their losses, and (3) degradation of PAHs occurs more easily in summer than does in winter. With respect to mutagenicity, the reduction of the indirectly active mutagenicity by sunlight irradiation in summer was coincident with the disappearance of PAHs, while in winter it did not parallel the change in PAH content. These results suggest that indirect mutagens which are resistant to in-winter sunlight irradiation are present in the particles and that the contribution of PAHs determined to the whole indirect mutagenicity is very small. In addition, it was found that the photochemical reaction of PAHs indeed occurs on the air particles, however, ovbious evidence for the photoconversion of them into direct mutagenic compounds was not obtained. At present, we can have only general explanations such that (1) photoconversed-direct mutagens are very small in quantity, (2) there are alternative pathways to non-mutagenic substances on the particles, and (3) formation/degradation of direct ones is balanced in quantity and/or in quality.

Our experimental system simulates actual atmospheric conditions with respect to chemical nature of aerosol, because "natural" airborne particles, already collected on a glass fiber filter, was exposed to the actual atmosphere including sunlight without enrichment of PAHs. However, physical parameters such as effective surface area or the degree of freedom of the particle are limited, as they are "fixed" on and inside the filter. Consequently, chemical reactivity of organic compounds associated with the actual "suspended" particles toward other atmospheric constituents (O_x, NO_2, oxygen or photon) would expect to increase particularly in summer, as compared with that of the "fixed" one. In spite of these limitations, the findings reported here are of considerable environmental and biological significance.

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