Review

Environmental Behaviors and Toxicities of Polycyclic Aromatic Hydrocarbons and Nitropolycyclic Aromatic Hydrocarbons

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Airborne particulate matter (PM) has been collected at four cities in Japan starting in the late 1990s, at five or more major cities in China, Korea and Russia starting in 2001 and at the Noto Peninsula starting in 2004. Nine polycyclic aromatic hydrocarbons (PAHs) and eleven nitropolycyclic aromatic hydrocarbons (NPAHs) were determined by HPLC with fluorescence and chemiluminescence detections, respectively. Annual concentrations of PAHs and NPAHs were in the order, China>Russia≫Korea=Japan, with seasonal change (winter>summer). During the observation period, concentrations of PAHs and NPAHs in Japanese cities significantly decreased but the increases in the PAH concentration were observed in Chinese and Russian cities. Concentrations of PAHs and NPAHs were higher in the Northern China than those in the Southern China. At the Noto peninsula, which is in the main path of winter northwest winds and a year-round jet stream that blow from the Asian continent to Japan, the concentrations were high in winter and low in summer every year. A cluster analysis and back trajectory analysis indicated that PAHs and NPAHs were long-range transported from Northeastern China, where coal burning systems such as coal-heating boilers are considered to be the major contributors of PAHs and NPAHs. A dramatic change in atmospheric concentrations of PAHs and NPAHs in East Asia suggests the rapid and large change of $PM_{2.5}$ pollution in East Asia. Considering the adverse health effects of $PM_{2.5}$, continuous monitoring of atmospheric PAHs and NPAHs is necessary in this area.

Key words   polycyclic aromatic hydrocarbon; nitropolycyclic aromatic hydrocarbon; $PM_{2.5}$; East Asia; long-range transport; adverse health effect

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) such as benzo[*a*]-pyrene (BaP) and nitropolycyclic aromatic hydrocarbons (NPAHs) such as 1-nitropyrene (1-NP) are ubiquitous environmental organic pollutants, which mainly originate from imperfect combustion and pyrolysis of organic matters.[1] In addition, several NPAHs, such as 2-NP and 2-nitrofluoranthene (2-NFR), are secondarily formed in the atmosphere *via* reactions of their parent PAHs and NO_2.[2] Structures and abbreviations of typical PAHs and NPAHs observed in the atmosphere are shown in Fig. 1. As sources of these PAHs and NPAHs in East Asian environments, combustions of coal, oil and biomass *etc.* are considered. On the other hand, PAHs and their branched derivatives are main components of petroleum. Oil spill accidents cause damages in marine ecosystems.

Many of PAHs and NPAHs are carcinogenic and/or mutagenic. For example, the International Agency for Research on Cancer ranked BaP in Group 1 (carcinogenic to humans), 1-NP in Group 2A (probably carcinogenic to humans) and several other PAHs and NPAHs in Group 2B (possibly carcinogenic to humans). Additionally, World Health Organization

(WHO) moved $PM_{2.5}$ in Group 1 in 2013, because several PAHs and NPAHs, such as BaP and 1-NP, contained in $PM_{2.5}$ are in Group 1 or Group 2A as above described.[3] On the other hand, several metabolites of PAHs and NPAHs, such as hydroxylated and quinoid derivatives, have estrogenic/antiestrogenic or antiandrogenic activities.[4–6] These oxidative metabolites also produce reactive oxygen species.[7,8] It is important to estimate not only the carcinogenicity/mutagenicity but also activities of endocrine disruption and production of reactive oxygen species of PAHs and NPAHs from the view point of toxicity markers of $PM_{2.5}$.

In East Asia, the economics and industrial outputs of Japan, China, Korea and Russia have developed rapidly in recent decades. Together, these countries account for more than a quarter of the world energy consumption. Especially, the primary energy consumption of China increased drastically and has reached to 12% of the total world consumption in 2014.[9] The main energy sources in these countries are oil in Japan and Korea, coal in China and natural gas in Russia (but coal for Far Eastern Russia). The burning of these fuels and biomass releases many pollutants including gases and particulates into
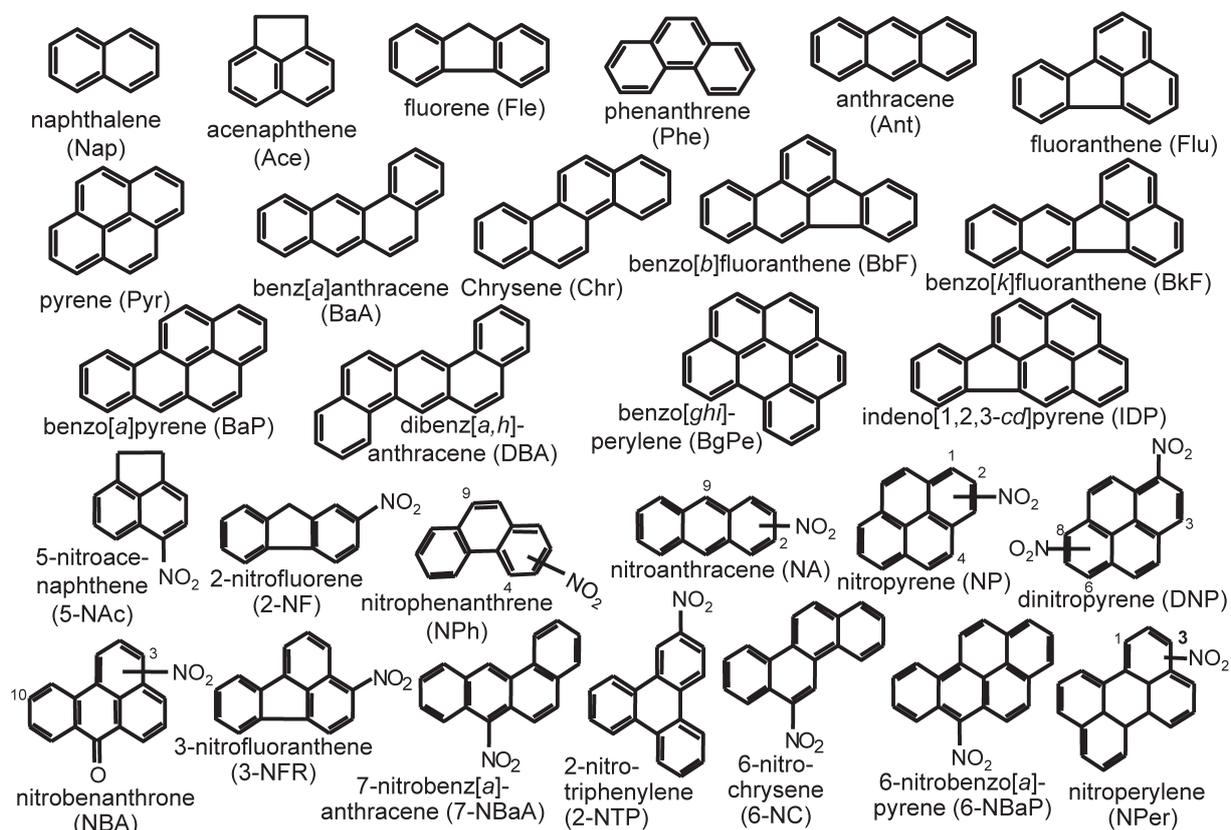
Fig. 1.   Typical PAHs and NPAHs Observed in the Atmosphere

the atmosphere. Recent increases in the consumption of coal and petroleum in China have led to heavy air pollution. One class of small atmospheric pollutants is refereed to as "atmospheric particulate matters whose diameter are $2.5\,\mu$m or less ($PM_{2.5}$)." High concentration levels of atmospheric $PM_{2.5}$ in Beijing have been reported since January 2013.[10] These facts suggest that the exposure to high levels of $PM_{2.5}$ might cause respiratory, cardiac and lung diseases such as asthma and lung cancer in human.

My reserch group started to collect airborne particulates at four cities from Hokkaido to Kyushu of Japan from the late 1990s. After then, the sampling area was expanded to major cities in Pan-Japan Sea countries (China, Korea and Russia) starting in 2001. This network has continued the international collaboration of airborne particulate sampling up to now with the addition of several cities such as Shanghai in China and backround sites such as the Noto peninsula in Japan.

2.   Development of Analytical Methods of PAHs and NPAHs

High-performance liquid chromatography with fluorescence detection (HPLC-FLD) is populary used for analyzing PAHs in environmental samples, because of strong fluorescent activity of PAHs. Gas chromatography/mass spectrometry (GC/MS) is also used for the determination of PAHs in environmental samples. In our experiments, HPLC-FLD was mainly used, because HPLC-FLD showed higher sensitivity to PAHs having 6 rings or more than GC/MS. Concentrations of NPAHs are much lower than those of corresponding PAHs in environmental samples. GC/MS is not sensitive enough for determin-

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1. Reducation
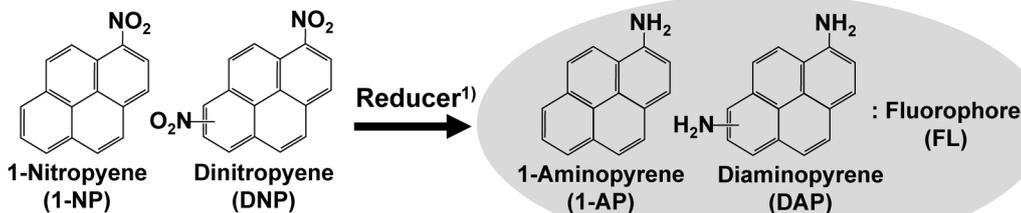
1) Examples of reducer: NaSH, Zn/HCl, Pt/Rh (Catalyst)
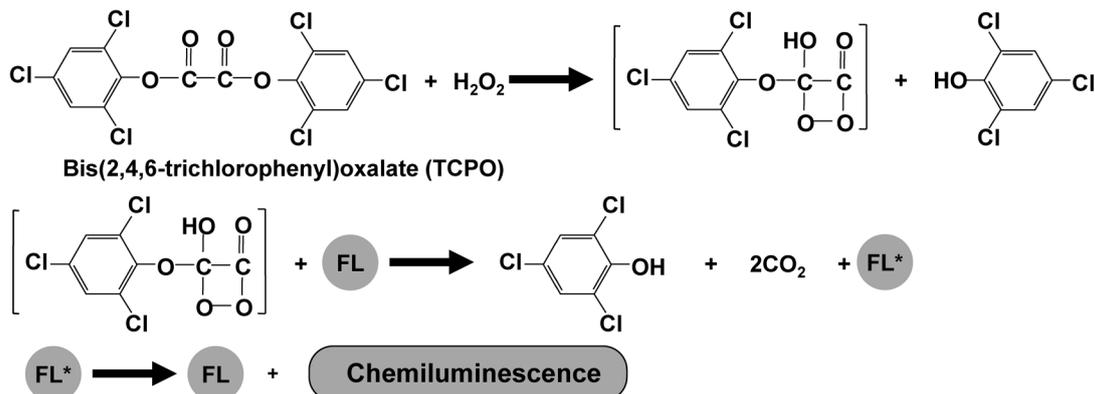
2. Chemiluminescence

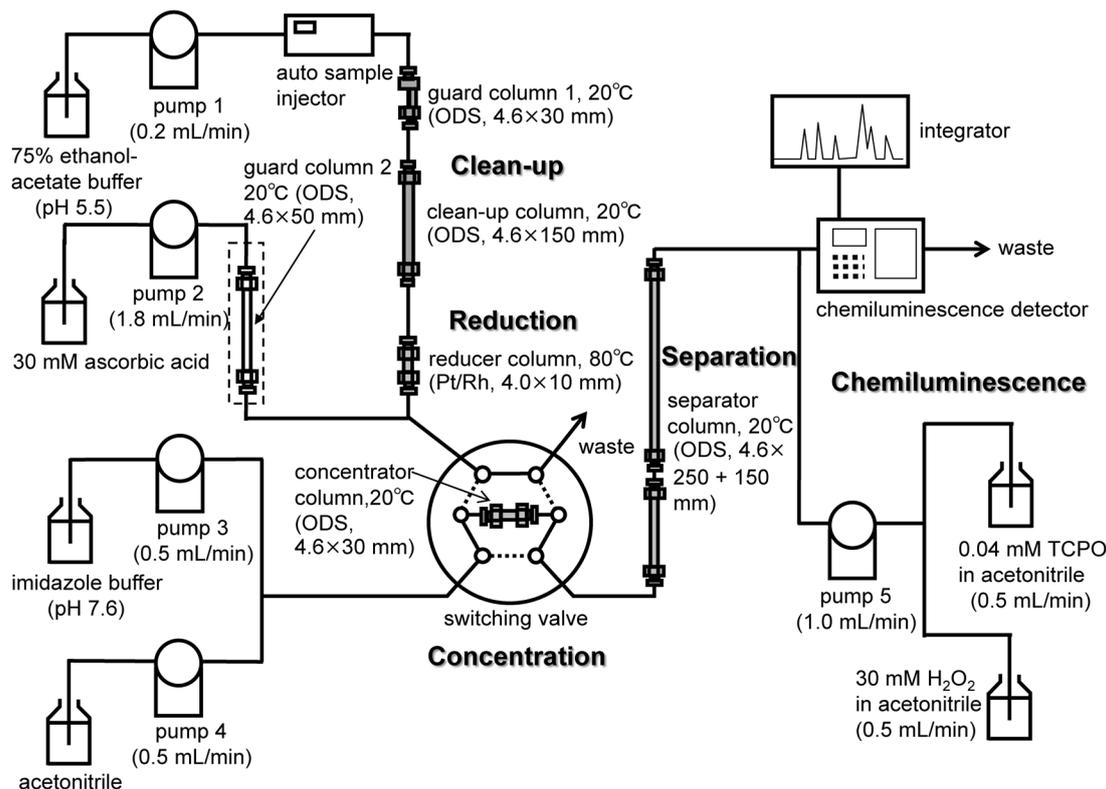Fig. 2. Chemiluminescence Detection Mechanism of NPAHs

Fig. 3. A Schematic Diagram of NPAH Analysis System

ing trace levels of NPAHs. Chemiluminescence reaction using peroxyoxalate esters, such as (2,4,6-trichlorophenyl)oxalate, and hydrogen peroxide as chemiluminescence reagents was developed as a highly sensitive determination method for several fluorescent compounds.[11] We found that amino PAHs were highly sensitive to this chemiluminescence reaction and that NPAHs could be reduced to their corresponding amino PAHs (APAHs) in the presence of chemical reductants such as hydrosulfide (Fig. 2). Although APAHs were fluorescent, HPLC-FLD was not sensitive enough. Then, we developed
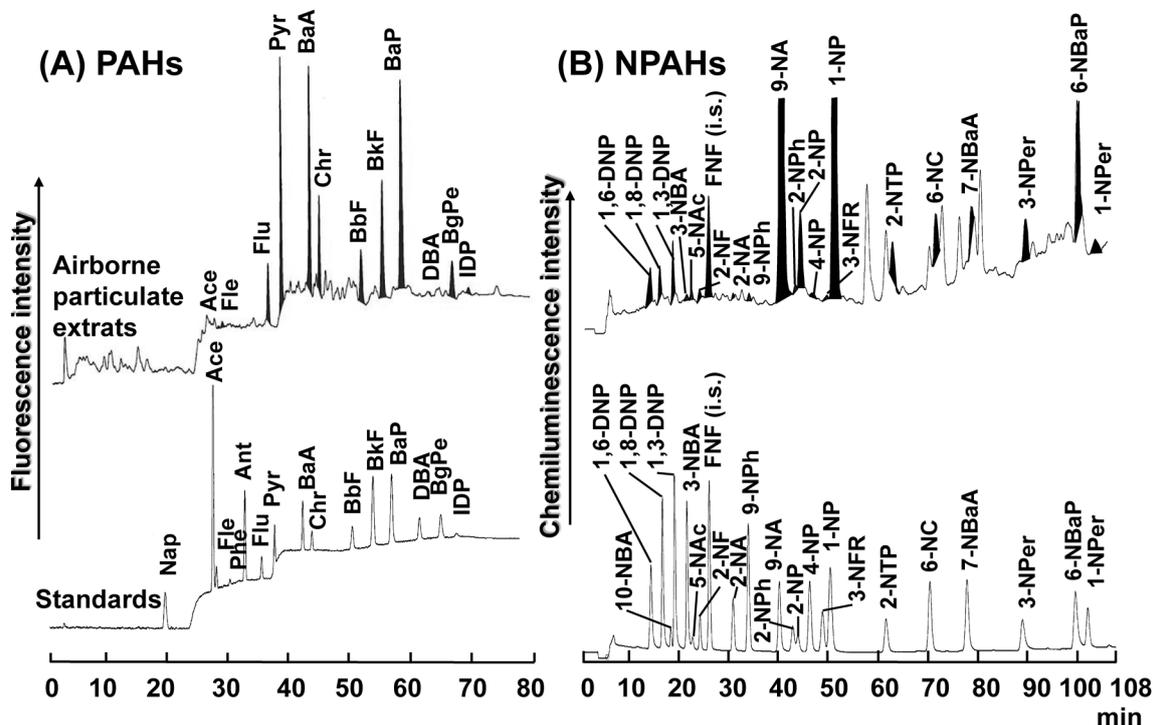
Fig. 4. Typical Chromatograms of (A) PAHs and (B) NPAHs in Extracts from Airborne Particulates by HPLC-FLD and CLD, Respectively
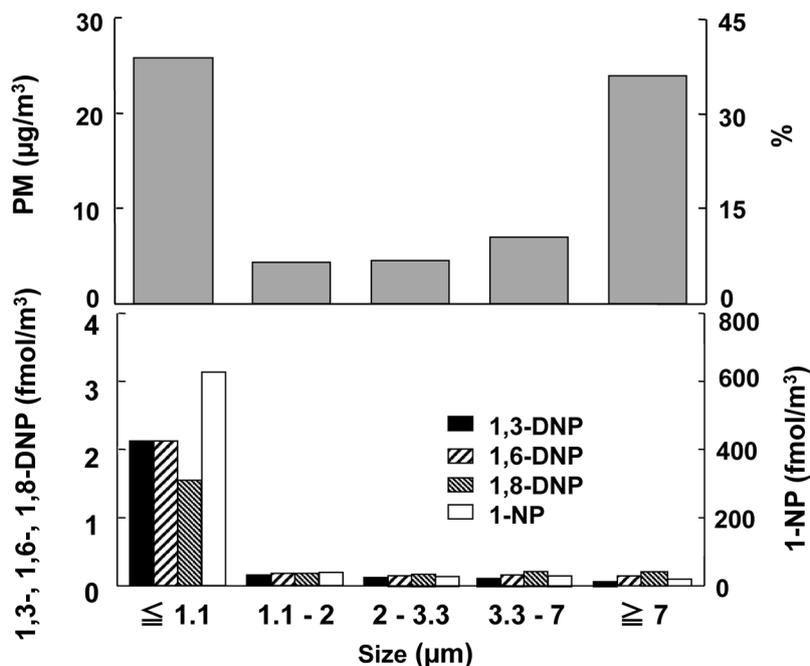

Fig. 5. Distribution of Particulates and NPAHs in Five Particulate Size Fractions by Andersen High-Volume Air Sampler

HPLC with chemiluminescence detection (CLD) for highly sensitive determination of NPAHs. After treating NPAHs with hydrosulfide, APAHs were separetely determined by the HPLC-CLD system. The sensitivity of the HPLC-CLD was about 100 times higher than the sensitivities of previous methods such as HPLC-FLD and GC/MS.[12,13]

However, the above CLD method needed the reduction of NPAHs before injecting into the HPLC system. We found that NPAHs could be also converted to APAHs in the presence of catalysts such as Pt/Ph in hot alchohol. After several other

improvemnets, we developed an HPLC system with CLD unit equipped with a catalytic column packed with Pt/Ph.[14–16] The improved HPLC system time-programed the following five steps: (1) separation of NPAHs from PAHs in a clean-up column, (2) reduction of NPAHs to APAHs in a reduction column packed with Pt/Rh, (3) concentration of APAHs on a concentrator column, (4) separation of APAHs in a separation column and (5) detection of APAHs chemilumigenically[17] (Fig. 3). Both PAHs and NPAHs in extracts from only 1 mg of airborne particulates could be separately determined by

Fig. 6.   Airborne Particulate Sampling Cities in the World in Our Research Network

HPLC-FLD and HPLC-CLD, respectively (Fig. 4).

Airborne particulates were extracted with benzene/ethanol and the extracts were cleaned up with alkaline solution, acidic solutions and water. Then PAHs were determined by the HPLC-CLD system above described. The United States Environmental Protection Agency has listed 16 PAHs having 2 to 6 rings as priority pollutants. We mainly measured nine of these PAHs having 4 to 6 rings, by using pyrene-d_{10} (Pyr-d_{10}) and benzo[a]pyrene-d_{12} (BaP-d_{12}) as internal standards. The nine PAHs, fluoranthene (FR), pyrene (Pyr), benz[a]-anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), BaP, benzo[ghi]perylene (BghiPe) and indeno[1,2,3-cd]pyrene (IDP), were separately detemined at each optimum detection wavelength (Fig. 4A). Using the same extracts that were used for the PAH analysis, eleven NPAHs were measured by the HPLC-CLD system using 2-fluoro-7-nitrofluorene (FNF) and/or deuterated 1-nitropyrene-d_{10} (1-NP-d_9) as internal standards. The eleven NPAHs were 1,3-, 1,6-, 1,8-dinitropyrenes (1,3-, 1,6-, 1,8-DNPs), 9-nitroanthracene (9-NA), 1-, 2-NPs, 2-nitrofluoranthene (2-NFR), 6-nitrochrysene (6-NC), 7-nitrobenz[a]anthracene (7-NBaA), 6-nitrobenzo[a]pyrene (6-NBaP) and 3-nitroperylene (3-NPer) (Fig. 4B). We collected PM by air samplers and determined PAHs and NPAHs in extracts. In the atmosphere, 2 to 3-ring PAHs exist in the gas phase, 4-ring PAHs in the both gas phase and PM and PAHs having 5 rings or more exist in PM. The particulate phase PAHs and NPAHs existed mainly fine particulate fraction less than 2.1 μm (Fig. 5), suggesting that PM$_{2.5}$ is important for these organic pollutants.

3.   Atmospheric Concentrations of PAHs and NPAHs in Japan and East Asia

Figure 6 shows airborne particulate sampling cities in the world in our research network. We started to collect TSP at four Japanese cities, Kanazawa, Tokyo, Sapporo and Kitakyushu in Japanese since 1990s and determined PAHs and NPAHs. Since 2001, monitoring sites were additionally set at foreign cities in Pan-Japan Sea countries, Shenyang, Tieling, Fushun, Beijing and Shanghai (China), Busan (Korea) and Vladivostok (Russia) from 2001. The atmospheric concentrations of naphthalene (Nap) and 3-ring PAHs such as phenanthrene (Phe) are higher than those of PAHs having 4 rings or more.[18] However, the latter PAHs show stronger toxicities such as carcinogenicity and mutagenicity. This is the reason why both PAHs and NPAHs in mainly PM were determined in our atmospheric study.

Annual mean atmospheric concentrations of total PAHs and NPAHs showed a big difference among the East Asian cities. The PAH concentrations were in the order; Fushun>Beijing>Tieling>Shenyang≫Vladivostok>Tokyo>Sapporo>Kitakyushu>Busan>Kanazawa. The NPAH concentrations were in the order; Fushun>Beijing>Tieling>Shenyang>Tokyo>Vladivostok=Kitakyushu>Busan>Sapporo>Kanazawa. Although there were a small difference in the order of cities, the PAH and NPAH concentrations in the four countries were in the order, China>Russia>Korea=Japan. It must be emphasized that the atmospheric PAH concentrations in China were much higher than those in Japan, suggesting that the urban atmosphere in China was very much polluted with PM-containing PAHs[19–23] (Fig. 7).

Moreover, Fig. 7 showed that the concentration ratios of NPAHs to PAHs ([PAHs]/[PAHs]) were smaller at Chinese cities (0.014–0.048) than those at Japanese cities (0.055–0.407). This chemical composition difference suggested that the sources were different between the two counties.[24,25] This will be discussed in detail in the next session.

4.   [NPAH]/[PAH] Ratio as a Marker for Identifying Sources of Combustion PM and Main Sources in East Asia

The formation of NO$_x$ depends on the combustion temperature and the formation of NPAHs from corresponding PAHs in the presence of NO$_x$ also depends on combustion temperature. Therefore, the formation yield of NPAHs from PAHs
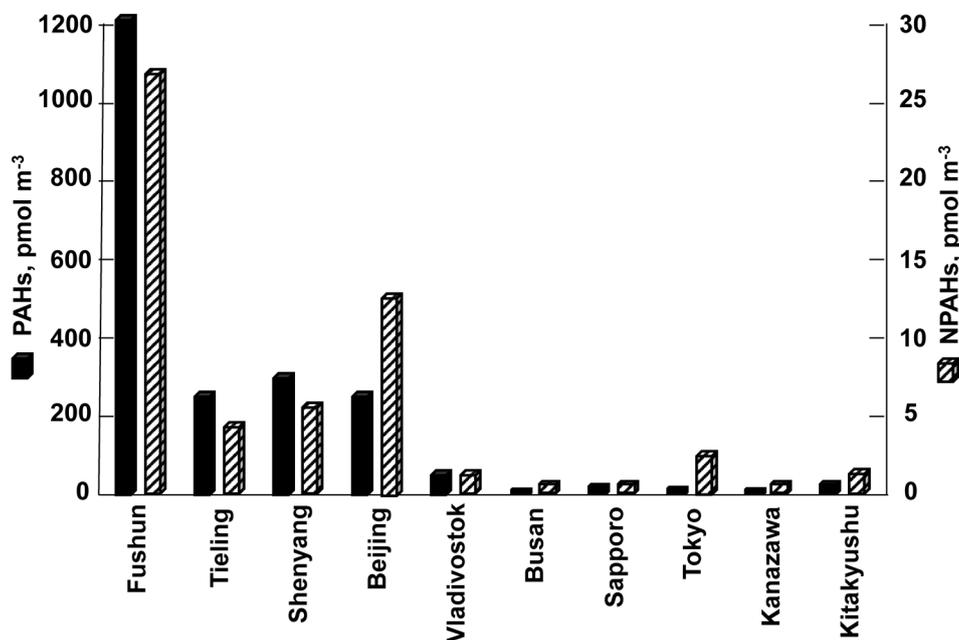
Fig. 7. Annual Concentrations of PAHs and NPAHs in East Asian Cities

PAHs=pyrrene+benz[*a*]anthracene+chrysene+benzo[*b*]fluoranthene+benzo[*k*]fluoranthene+benzo[*a*]pyrene; NPAHs=1,3-dinitropyrene+1,6-dinitropyrene+1,8-dinitropyrene+9-nitroanthracene+1-nitropyrne+2-nitrofluoranthene+2-nitropyrne+6-nitrochrysene+7-nitrobenz[*a*]anthracene+6-nitrobenz[*a*]pyrene+3-nitroperyrne; Sampling period: 2001–2002 (Shenyang); 2002–2003 (Fushung); 2003–2004 (Tieling); 2004–2005 (Beijing, Busan, Vladivostok, Kanazawa, Sapporo, Tokyo, Kitakyushu).
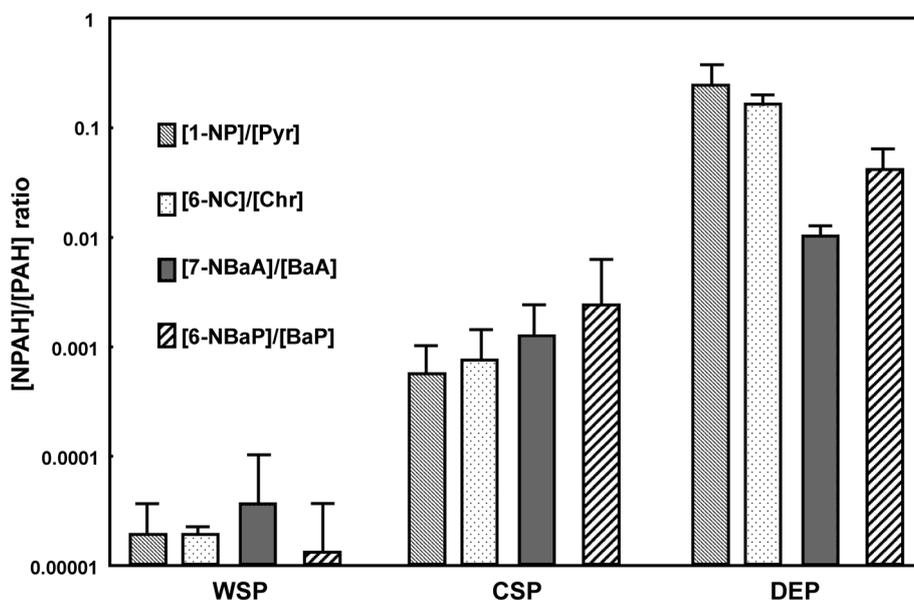

Fig. 8. [NPAH]/[PAH] Ratios of Diesel-Engine Exhaust Particulates (DEP), Coal-Smoke Particulates (CSP) and Wood-Smoke Particulates (WSP)

increased with increasing combustion temperature, suggesting that the [NPAH]/[PAH] concentration ratios in PM might also depend on combustion temperature. To test this hypothesis, the [NPAH]/[PAH] concentration ratios were determined in three different types of PM from diesel-engine vehicles (combustion temperature was about 2700–3000°C), coal-burning stoves (about 900–1200°C), and wood burning stoves (about 500–600°C).

Figure 8 showed [NPAH]/[PAH] ratios, [1-NP]/[Pyr], [6-NC]/[Chr], [7-NBaA]/[BaA] and [6-NBaP]/[BaP], of diesel-engine particulates (DEP), coal-smoke particulates (CSP) and wood-smoke particulates (WSD). The figure, for example, showed the [1-NP]/[Pyr] ratio of CSP (0.001) was much smaller than the ratio of DEP (0.36).[25] A high concentration of 1-NP in the PM in automobile exhausts has been used as an effective marker of human exposure to automobile exhausts.[26–29] In East Asian cities, DEP and/or CSP were considered as major contributors to combustion PM. The [1-NP]/[Pyr] ratios at Shenyang were 0.003 (winter) and 0.04 (summer), while the values at Tokyo were 0.13 (winter) and 0.09 (summer), respectively. The difference in the values between the two cities in winter (more than 40 times) was larger, probably because of a larger amount of emissions from coal heating in Shenyang.
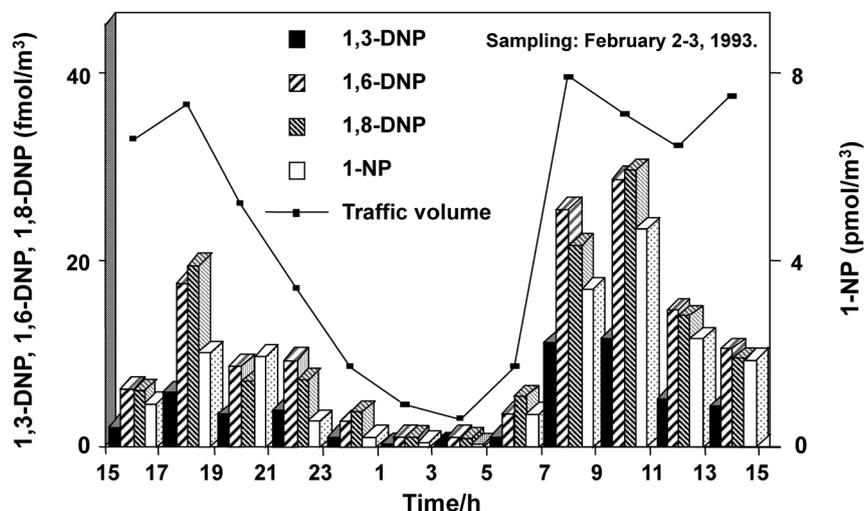
Fig. 9. Diurnal Atmospheric NPAHs Concentrations at Downtown Kanazawa

Identification of source of atmospheric PAHs has been reported.[30–32] Several PAH pairs such as [FR]/[FR+Pyr], [BaA]/[Chr] and [IDP]/[IDP+BghiPe] have been used as markers of the source of the PAHs. However, the differences in those pairs were not large enough to identify the source clearly. Thus, by using the [NPAH]/[PAH] ratios, the major sources of PM containing PAHs and NPAHs of the four countries in Fig. 6 were identified as automobiles in Japan and Korea, coal combustion systems in China and Russia.[24,25] The values for China were typical of a country that exhausts huge amounts of PM from coal combustion systems.

Figure 9 shows diurnal atmospheric concentrations of 1-NP and 1,3-, 1,6-, 1,8-DNPs in downtown Kanazawa, Japan. Total suspended particulates (TSP) were collected by high-volume air sampler every 2 h at roadside. Although the concentrations of 1,3-, 1,6- and 1,8- DNPs were much lower than the concentration of 1-NP, both three DNPs and 1-NP showed very similar patterns. The concentrations of these NPAHs were high in the evening, decreased in the night and reached in the lowest levels in the early morning. Then they increased later in the morning. The pattern of these NPAHs was in parallel with the time course of traffic volume monitored at the nearest intersection, suggesting that these NPAHs were emitted from automobiles.[33] Atmospheric concentrations of PAHs such as BaP and Pyr showed patterns similar to those of the NPAHs in Fig. 9. This is a typical pattern observed in the downtown area of Japanese cities where the major contributors of PAHs and NPAHs were automobiles.[34,35] Both PAHs and NPAHs emitted from automobiles mainly existed in the fine particulate fraction less than $2.1 \mu m$,[36–38] suggesting in $PM_{2.5}$.

In Japan, urban air pollution became serious with the traffic development from the 1970s. In the 1990s, the atmospheric concentrations of PM, which contained PAHs and NPAHs, in city areas as well as along heavy traffic roads, were very high. Urban air pollution was considered as a cause of respiratory and/or cardiovascular diseases. In the early 2000s, the Japanese government strengthened progressive countermeasures (Shin-tanki in 2003, Shin-choki in 2005 and Post shin-choki in 2009) to reduce the urban air pollutions by requiring emission control of PM and NO_x from automobiles and diesel/gasoline quality control (Fig. 10A). Also the quality of fuel has been improved. During the same period, the urban at-
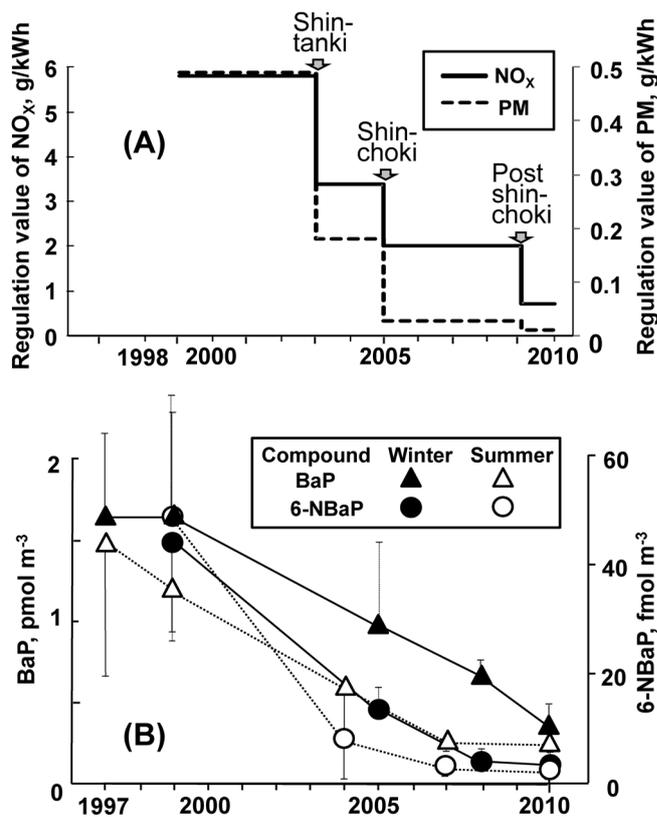

Fig. 10. Effect of (A) Japanese Regulation of NO_x and PM Emissions from New Diesel-Engine Vehicle on (B) Atmospheric PAH and NPAH Concentrations in Kanazawa, Japan

mospheric concentrations of PAHs and NPAHs in Japan have been decreased significantly. In downtown Kanazawa, for an example, TPM tended to decrease during the period from 1999 to 2010. The concentrations of BaP more clearly decreased in this period with the decrease in the regulation level of PM from automobiles. Especially, the 6-NBaP concentration decreased significantly by a factor of 30 in summer and decreased by a factor of 13 in winter. The decrease of [6-NBaP] was more significant than that of [BaP] (Fig. 10B). This result can be explained by the nitration formula that the
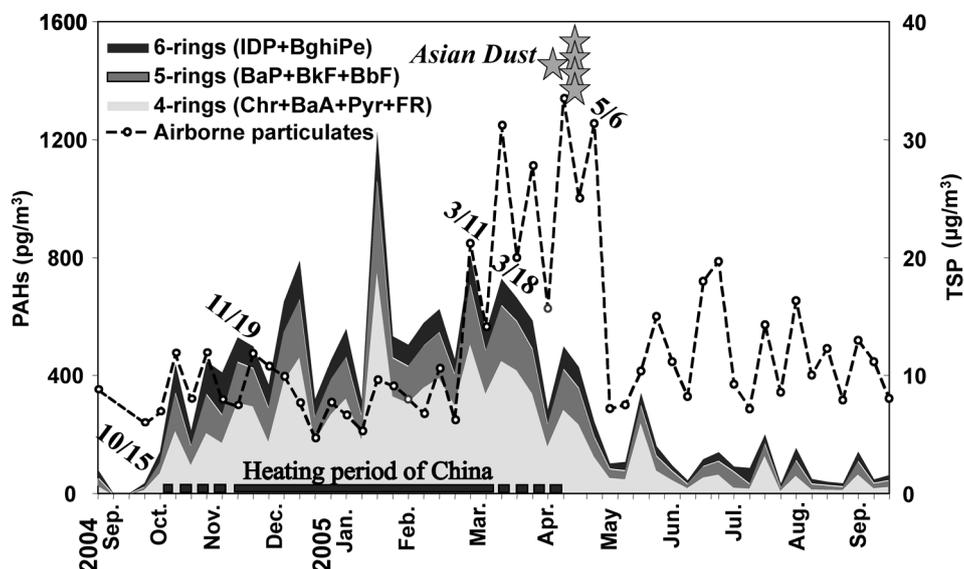
Fig. 11.   Time Courses of Atmospheric PAH and TSP Concentrations at NAMS

nitration rate of [BaP], in proportion with the concentration of TSP, depends on [PM] and [NO$_x$]. Moreover, the [6-NBaP]/[BaP] ratio, which is an effective marker of automobile emission, decreased significantly in the same period, suggesting that the contribution of automobiles to atmospheric PAHs and NPAHs were effectively reduced by the countermeasures. The significant decreases of PAHs and NPAHs were also observed in the other Japanese cities.[39,40]

In China, air pollution became serious with economic growth from the early 1990s. Recently, local governments started several projects to reduce air pollution. However, the effects were not the same as those in Japan. The Shenyang government undertook the Blue Sky Project,[41] in which 100 factories were transferred to the outer city, and 5000 inefficient boilers for domestic heating were removed between 2001 and 2007. The project helped to reduce not only atmospheric PM$_{10}$ but also PAHs concentrations in Shenyang.[42] The Beijing government started to control traffic volume to reduce air pollution for the 2008 Olympic Games. It is important to know the tendency of the concentrations of both PAHs and NPAHs after then.

Thus atmospheric pollution levels in the Pan-Japan Sea countries changes very fast with different tendencies. The follow-up monitoring of these PAHs and NPAHs in East Asian countries is needed to make accurate prediction.

5.   Long-Range Transport of PAHs and NPAHs from the Asian Continent to Japan

Airborne particulate matter was collected at the Noto Air Monitoring Station (NAMS) (Nishi-Futamata-machi, Wajima City, Ishikawa Prefecture, Japan), which is located on the Noto peninsula tip, 2.1 km south of the Japan Sea coast and about 100 km north of Kanazawa. This peninsula is a less-populated area and no major emission sources of atmospheric pollutants are near the station. TSP were collected continuously from September 17, 2004 to the present. This site is in the main path of winter northwest winds and a year-round jet stream that blow from the Asian continent to Japan. The concentrations of PAHs at this site were much higher during the heating period of China (from October 15, 2004 to April 15,

2005) than in other seasons. TSP concentration, on the other hand, was high from March to May, partly caused by Yellow sand storms. The variation of PAHs was different from that of TSP concentration[43] (Fig. 11).

A cluster analysis dendrogram showed that the chemical composition of atmospheric PAHs at the peninsula was closer to the chemical composition at Shenyang, China than to the chemical composition at Kanazawa, Japan, even though Shenyang is much farther away than Kanazawa. Moreover, by analyzing some meteorological conditions, our data showed that the atmospheric PAHs and NPAHs at NAMS were long range transported from Northeast China in cold seasons and were contributed by the Japanese domestic sources in warm seasons.[43,44] In our 10-year observation (from September 2004 to September 2014) showed that the atmospheric concentrations of PAHs as well as NPAHs at NAMS repeated the same seasonal variation (high in winter and low in summer). It is important that the atmospheric PAHs level at NAMS decreased in recent year, although no statistically significant change was found in warm season[45] (Fig. 12). The data observed at NAMS also showed that several events, such as the Beijing Olympic and Paralympic Games and the reconstruction after the Noto Hanto earthquake, possibly largely contributed the yearly variations of atmospheric PAH levels at NAMS from 2004 to 2014. The source control measures implemented by the Chinese and Japanese governments might be effective in decreasing the atmospheric PAH levels at NAMS in recent year.

During their atmospheric transport, PAHs and NPAHs were converted to other compounds such as their hydroxylated derivatives in the presence of NO$_x$, sunlight and/or other co-existing compounds such as yellow sand. Some of these compounds were considered to cause respiratory and cardiovascular symptoms.[46–50] Because deterioration of respiratory and cardiovascular symptoms might be caused by yellow sand and PM$_{2.5}$ long-range transported from Asian continent to Japan, as a possible mechanism, continuous monitoring of atmospheric PAHs and NPAHs not only at major cities in Pan-Japan Sea countries but also at remote sites along the Japan Sea coast in Japan is very important. By using recent
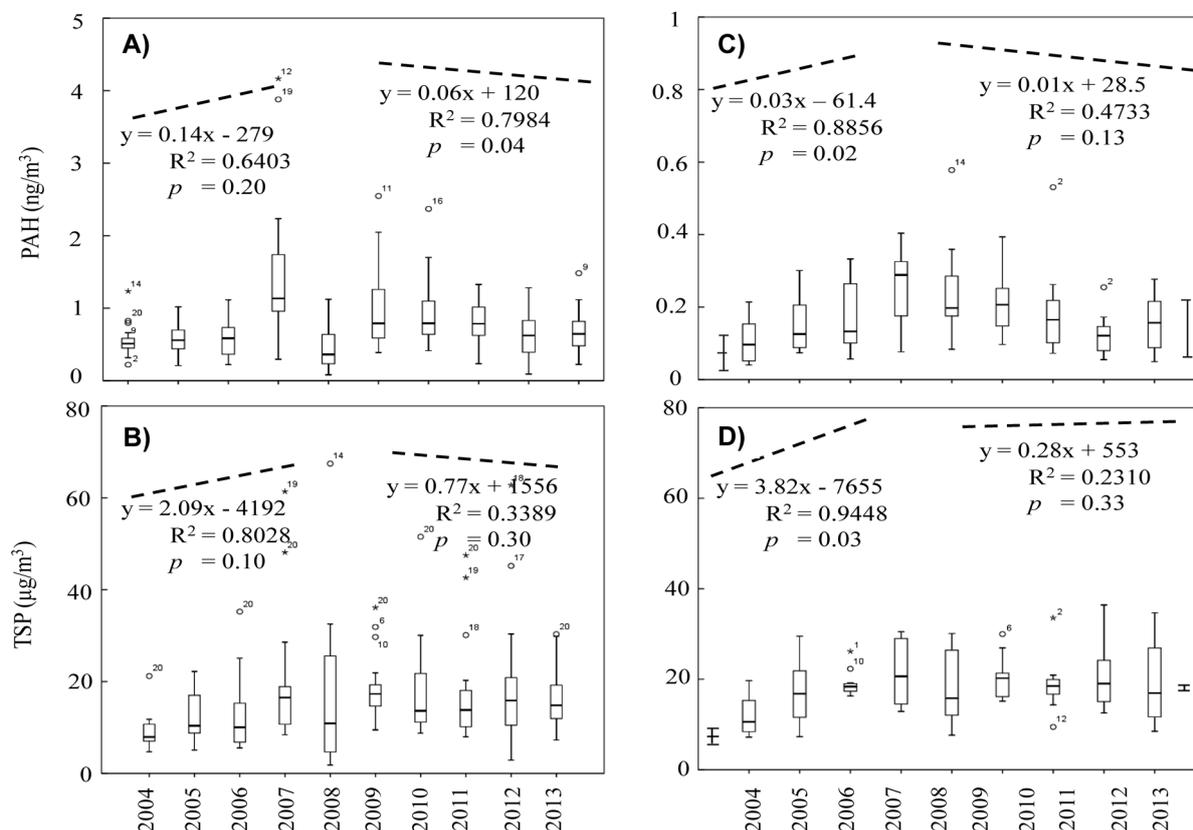
Fig. 12. Yearly Variations of Total Nine PAH Levels (ng/m^3) and TSP Levels (ng/m^3) and Total Nine PAHs in TSP Levels ($ng/\mu g$) at NAMS in Cold and Warm Seasons

(A, C) Total nine PAHs level in cold seasons and in warm seasons, respectively. (B, D) Total TSP level in cold seasons and in warm seasons, respectively. The upper and lower error bars represent the maximum and the minimum concentrations, respectively. The upper and lower edges of box represent the 75th and 25th percentile concentrations, respectively. Within each box, median concentrations are shown as solid lines.
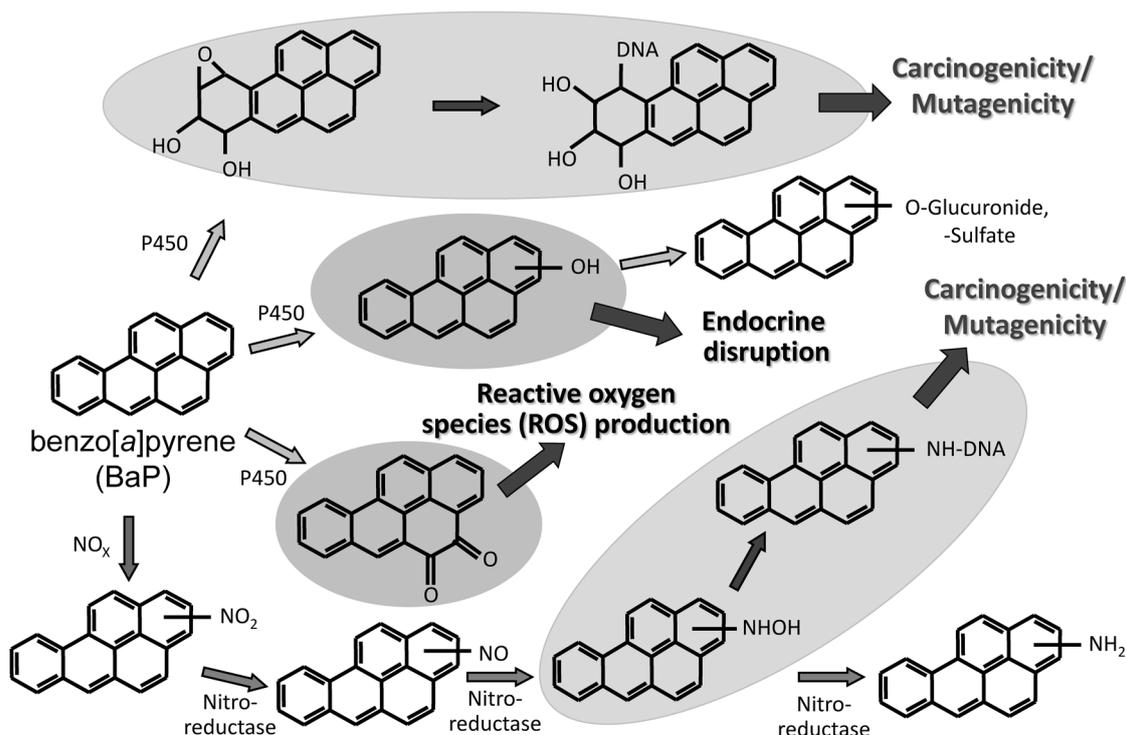

Fig. 13. Possible Metabolic Activation Pathways of PAHs Using BaP as an Example
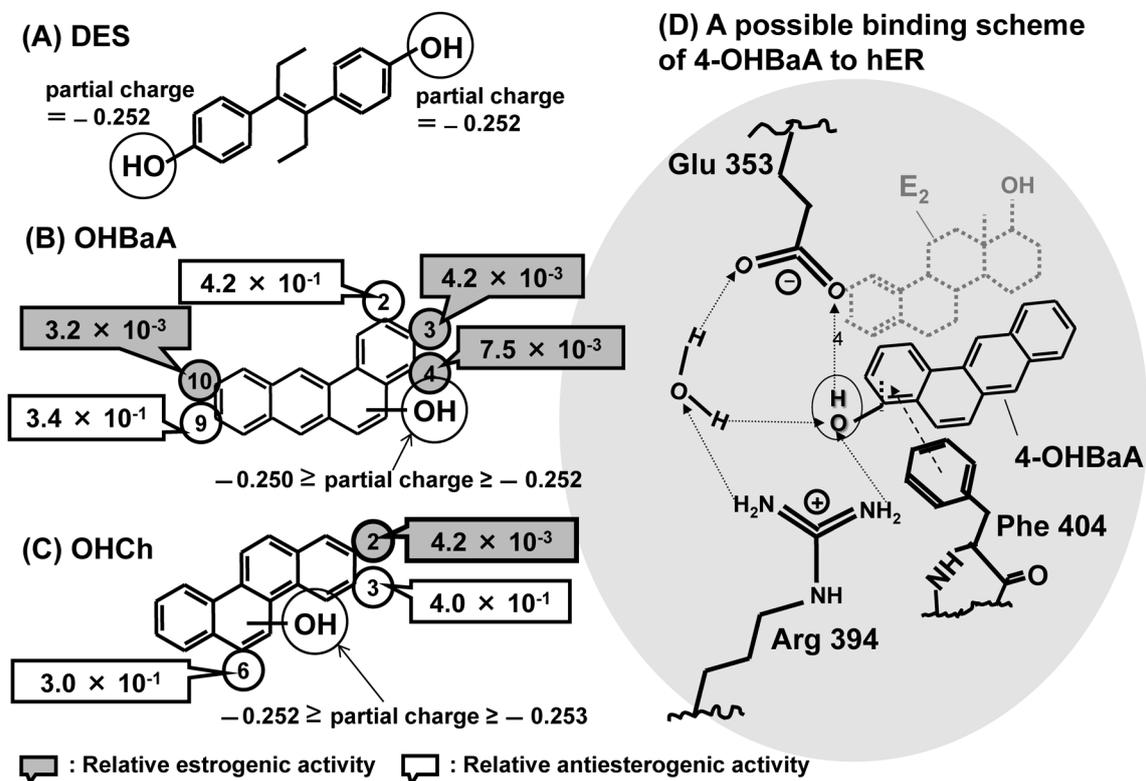
Fig. 14.   Structures of Strongly Estrogenic and Antiestrogenic Hydroxyl PAHs (OHPAHs) and a Possible Binding Scheme to Human Estrogen Receptor (hER)
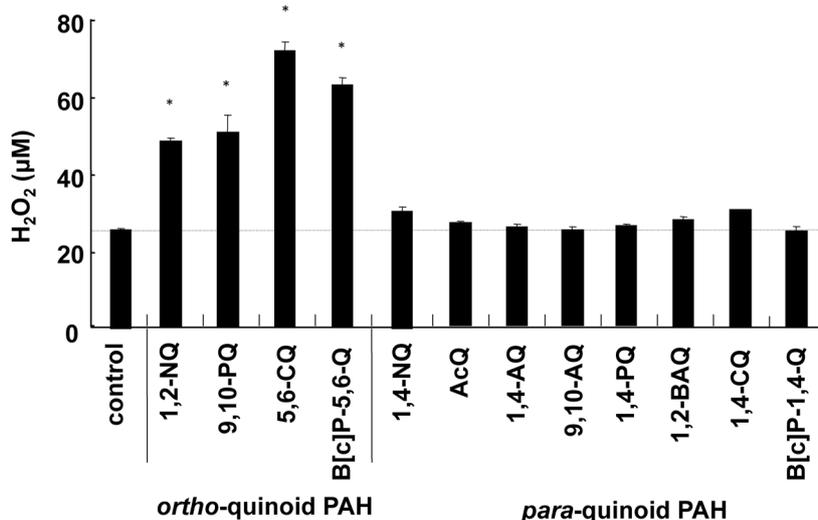

Fig. 15.   Different Effects of *ortho*- and *para*-Quinoid PAHs on H_2O_2 Production in A549 Cells

A549 cells, pre-incubated with BSO and ATZ, were incubated with $10\,\mu M$ quinoid PAH at 37°C for 1h. H_2O_2 levels were determined by FOX assay. Each value is the mean±S.D. of the three determinations. Statistical significance, *$p < 0.001$ *vs.* control.

analytical results, the emission sources, concentrations, and *trans*-boundary transport of particulate PAHs in Northeast Asia could be estimated accurately.[51,52]

6.   Adverse Biological Effects of PAHs and NPAHs from the View Point of Diseases Caused by $PM_{2.5}$

Inhalation of PM including $PM_{2.5}$ causes respiratory, cardiac and lung diseases such as asthma and lung cancer. As the causes, effects of PAHs and NPAHs are considered, because toxic PAHs and NPAHs are major components of $PM_{2.5}$ exhausted from combustion of fossil fuels and biomass. Cytochrome P450 enzymes (CYPs) play an important role to metabolize PAHs and NPAHs to their corresponding hydroxyl, epoxide and quinoid derivatives in human and animal bodies. Some of the above PAH metabolites exhibit adverse biological activities such as carcinogenicity/mutagenicity, endocrine disruption and production of reactive oxygen species. Figure 13 shows possible metabolic activation pathways of PAHs using

BaP as an example PAH.

Toxicity equivalent factor approach by using BaP-equivalent carcinogenicity factor was reported to reflect more accurately the relative potencies of different PAHs.[53] The author calculated the carcinogenic contribution of PAHs in PMs exhausted from motorcycles and automobiles by using the concentrations of PAHs. The largest contributors were BaP and benzo[*b*]-fluoranthene in motorcycles and automobiles, respectively.[54] The author assayed mutagenicity of PMs, PAHs and NPAHs by the Ames test using the *Salmonella typhimurium* strains. PAHs were the main causes of indirect-acting mutagenicity of PMs. Among them, BaP showed a large contribution of PMs exhausted from coal-burning and wood-burning. Recently, several new PAHs have been reported such as benzo[*c*]-fluorene which showed stronger mutagenicity than BaP[55] and they have been determined in airborne particulates.[56,57] NPAHs were the main causes of the direct-acting mutagenicity of PMs. Among NPAHs, 1-NP, 1,3-, 1,6-, and 1,8-DNPs and 3-nitrobenzanthrone (NBA) showed very strong direct-acting mutagenicity.[58,59]

On the other hand, metabolites of PAHs and NPAHs exhibited estrogenic/antiestrogenic activity in the yeast two-hybrid assay system using yeast cells expressing estrogen receptor. Several hydroxyl PAHs (OHPAHs) having 4 rings such as 3-, 4- and 10-hydroxybenz[*a*]anthracenes and 2-hydroxychrycene showed strong estrogenic activity. Several other OHPAHs having 4 rings such as 2- and 3-hydroxybenzo[*c*]phenanthrenes, 2-hydroxybenz[*a*]anthracenes and 3-hydroxybenzo[*c*]phenanthrene showed strong antiestrogenic activity.

There was a structure-binding activity to human estrogen receptor (hER) relationship, suggesting that chemical structures of OHPAHs were similar to those of estradiol (E_2) and diethylstilbestrol (DES) (Fig. 14) and that the compositional flexibility of estrogenic OHPAHs was much smaller than that of antiestrogenic OHPAHs.[60,61] PAHs showed also antiandrogenic activity in LNCaP human prostate carcinoma cells.[62] Oxidative stress induced by quinoid PAHs (PAHQs) was assayed by using A549 cells. Among PAHQs, *ortho*-PAHQs, such as 1,2-naphthoquinone, 9,10-phenenthrenequinone, 5,6-chrysenequinone and benzo[*c*]phenanthrene-5,6-quinone inhibited strongly the viability of the cells. But *meta*- and *para*-PAHQs did not inhibit the viability of the cells.

As a possible mechanism for the cytotoxicity, overproduction of H_2O_2 caused by *ortho*-quinoid PAHs selectively in a redox cycle coupled with the consumption of thiol group was considered[63] (Fig. 15). Thus PAHs and NPAHs are considered to be closely related to several adverse health effects of $PM_{2.5}$.

Conflict of Interest   The author declares no conflict of interest.

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