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Concentration and Photochemistry of PAHs, NPAHs, and OPAHs and Toxicity of PM_{2.5} during the Beijing Olympic Games

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Abstract

Atmospheric particulate matter with diameter <2.5 μm (PM_{2.5}) was collected at Peking University (PKU) in Beijing, China before, during, and after the 2008 Olympics and analyzed for black carbon (BC), organic carbon (OC), lower molecular weight (MW<300) and MW302 Polycyclic Aromatic Hydrocarbons (PAHs), nitrated PAHs (NPAHs) and oxygenated PAHs (OPAHs). In addition, the direct and indirect acting mutagenicity of the PM_{2.5} and the potential for DNA damage to human lung cells were also measured. Significant reductions in BC (45%), OC (31%), MW< 300 PAH (26% – 73%), MW 302 PAH (22% – 77%), NPAH (15% – 68%) and OPAH (25% – 53%) concentrations were measured during the source control and Olympic Olympic period. However, the mutagenicity of the PM_{2.5} was significantly reduced only during the Olympic period. The PAH, NPAH, and OPAH composition of the PM_{2.5} was similar throughout the study, suggesting similar sources during the different periods. During the source control period, the parent PAH concentrations were correlated with NO, CO, and SO₂ concentrations, indicating that these PAHs were associated with both local and regional emissions. However, the NPAH and OPAH concentrations were only correlated with the NO concentrations, indicating that the NPAH and OPAH were primarily associated with local emissions. The relatively high 2-nitrofluoranthene/1-nitropyrene ratio (25 – 46) and 2-nitrofluoranthene/2-nitropyrene ratio (3.4 – 4.8), suggested a predominance of photochemical formation of NPAHs through OH-radical-initiated reactions in the atmosphere. On average, the Σ NPAH and Σ OPAH concentrations were 8% of the parent PAH concentrations, while the direct-acting mutagenicity (due to the NPAH and OPAH) was 200% higher than the indirect-acting mutagenicity (due to the PAH). This suggests that NPAH and OPAH make up a significant portion of the overall mutagenicity of PM_{2.5} in Beijing.

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Supporting Information Available Details of the concentrations during the different periods, correlation analysis, and temporal variation in concentrations with meteorological parameters are provided in the Supporting Information. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Introduction

There was an unprecedented effort by the Beijing municipal government to improve air quality for the 2008 Beijing Olympics. A wide range of combustion sources, including vehicles, trucks, factories, and coal combustion for power generation, were controlled leading up to the Olympics, with the most stringent combustion source control measures occurring during the Olympic period (August 8–24, 2008) (14). From July 20–August 7, 2008, traffic was reduced, with license plates ending in even (odd) numbers allowed on the roads on even (odd) numbered calendar days, construction sites were closed, and the operation of coal fired power plants were strictly limited (1–3). From August 8–24, 2008 (the Olympic period), additional restrictions on coal-combustion were implemented (1–4). It has been estimated that the traffic volume of typical roads in Beijing was reduced by ~32% during the Olympic period (4–6). From August 25–September 20, 2008 the source control measures were less strictly implemented (1). As a result, significant reductions in carbon monoxide (CO), nitrogen oxide (NO_x), sulfur dioxide (SO₂), ozone (O₃) volatile organic compound (VOC), and particulate matter (PM) emissions and concentrations have been reported in Beijing, particularly during August 8–24, 2008 (1–17). In addition to source control measures, the fluctuations in Beijing PM concentration during the source control period have also been attributed to regional transport and meteorology (1, 4, 7, 9, 10).

Black carbon (BC) and organic carbon (OC) are emitted into the atmosphere on fine, respirable PM, including PM_{2.5}, during incomplete combustion and contribute to climate change, decreased visibility, and health effects (18, 19). Polycyclic aromatic hydrocarbons (PAHs) are components of OC on PM_{2.5} and are mutagenic products of incomplete combustion (20, 21). China is currently the world's largest emitter of BC (19), OC (19) and PAHs (20) and human exposure to these air pollutants has been predicted to be a major health concern in China now and in the future (17, 18, 21).

The significant effort to reduce combustion emissions in Beijing during the Olympic period provided a unique opportunity to study the PM_{2.5}-bound PAH, NPAH and OPAH concentrations, as well as the associated toxicity, in correspondence with the implementation and removal of source control measures. The objectives of this study were to: 1) measure the PM_{2.5}-bound PAH, NPAH, and OPAH concentrations and toxicity in Beijing before, during, and after the Olympics; 2) characterize the influence of photochemistry on the formation of NPAH and OPAH; and 3) assess the influence of sources and source control measures on the PM_{2.5}-bound PAH, NPAH, and OPAH concentrations and, in turn, mutagenicity and potential for DNA damage.

Materials and Methods

Sampling

The sampling site and sample collection have been previously described in detail (7). In brief, the sampling site was located on the roof of the 7-story Geology Building on the PKU campus, about 25 meters above ground. PKU is located in a primarily residential and commercial area in Northwestern Beijing. Local BC and PAH emission sources, within 1 km of PKU, include vehicular traffic and fuel combustion for cooking. Several 2008 Olympic events took place on or near the PKU campus.

PM_{2.5} was collected using a High Volume Cascade Impactor (Series 230, Tisch Environmental, Cleves, OH) that operated in accordance with procedures established by USEPA (CFR40, Part 50.11, Appendix B, July 1, 1975, pages 12–16) and ASTM Specification D2009 (7). Sixty-three PM_{2.5} samples were collected over 24 h periods (~1500 m³ of air) from July 28 to September 3, 2008 and from September 13 to October 7, 2008.

The source control period includes samples from July 28–September 20, 2008, while the non-source control period includes samples from September 21–October 7, 2008 (7). The Olympic period includes samples from August 8–August 24, 2008, while the non-Olympic period includes samples from July 28–August 7, 2008 and August 26–October 7, 2008 (7).

Six field blanks were also collected during these periods. Samples were not collected from September 4 to 12, 2008 because of sampler motor failure. Pre-baked (350°C) quartz fiber filters (No.1851-865, Tisch Environmental, Cleves, OH) were used for sample collection. The filters were weighed before and after sample collection for PM_{2.5} mass (7).

Black and Organic Carbon Measurement

The black carbon (BC) and organic carbon (OC) concentrations were measured using a Sunset EC/OC analyzer (Sunset Lab, USA) (22). There is debate as to whether thermal-optically measured elemental carbon (EC) should be referred to as BC. However, based on previous studies (19, 23, 24), we refer to the measured EC concentration as BC concentration.

Chemicals

All of the MW<300 parent PAHs, MW 302 PAHs, NPAHs and OPAHs are listed in Table 1. Deuterium-labeled PAHs and NPAHs were purchased from CDN Isotopes (Point-Claire, Quebec, Canada) and Cambridge Isotope Laboratories (Andover, MA). The isotopically labeled recovery PAH and NPAH surrogates included d₁₀-fluorene, d₁₀-phenanthrene, d₁₀-pyrene, d₁₂-triphenylene, d₁₂-benzo[a]pyrene, d₁₂-benzo[g,h,i]perylene, d₇-1-nitronaphthalene, d₉-5-nitroacenaphthene, d₉-9-nitroanthracene, d₉-3-nitrofluoranthene, d₉-1-nitropyrene and d₁₁-6-nitrochrysene. The labeled PAH and NPAH internal standards included d₁₀-acenaphthene, d₁₀-fluoranthene, d₁₂-benzo[k]fluoranthene, d₉-2-nitrobiphenyl and d₉-2-nitrofluorene.

Sample extraction and Analysis

Using the extraction method previously described in detail (26–28), the PM_{2.5} filters were extracted twice using pressurized liquid extraction with dichloromethane. The extracts were combined and divided into two halves by weight. One half of the extract was prepared for toxicity testing by evaporating the extract to dryness under a stream of N₂ with a Turbovap II (Caliper Life Sciences, MA). The residue was dissolved in 500 µl of dimethyl sulfoxide (DMSO). For chemical analysis, the other half of the extract was spiked with perdeuterated PAH and NPAH surrogates. It should be noted that the surrogates were spiked after extraction to avoid any interference of the surrogates with the subsequent toxicological testing of the extracts. For chemical analysis, the extracts were then purified using 20-g silica columns (Mega BE-SI, Agilent Technologies, New Castle, DE), eluted with dichloromethane, and spiked with perdeuterated PAH and NPAH internal standards. The analysis of parent PAHs was conducted using gas chromatographic mass spectrometry (Agilent 6890 GC coupled with an Agilent 5973N MSD) in selected ion monitoring mode using electron impact ionization (26, 28, 29), while the analysis of NPAH and OPAHs was conducted using electron capture negative ionization (ECNI) with a programmed temperature vaporization (PTV) inlet (Gerstel, Germany). A 5% phenyl substituted methylpolysiloxane GC column (DB-5MS, 30m×0.25mm I.D., 0.25 µm film thickness, J&W Scientific, USA) was used to measure the MW<300 parent PAHs and the majority of NPAHs and OPAHs. A 50% phenyl substituted methylpolysiloxane GC column (DB-17MS, 30m×0.25mm I.D., 0.25 µm film thickness, J&W Scientific, USA) was used to resolve 2-NF and 3-NF, and a similar column (DB-17MS, 60m×0.25mm I.D., 0.25 µm film thickness, J&W Scientific, USA) was used to measure the MW 302 parent PAHs (17). Additional

information on the analysis and method recovery of MW 302 parent PAH, NPAH, and OPAH is given in the Supporting Information and Table SI 1.

The MW<300 parent PAH concentrations are reported as individual PAH concentrations as well as the sum of NAP, 2-MNAP, 1-MNAP, 2,6-DMNAP, and 1,3-DMNAP, ACY, FLO and DBT concentrations ($\Sigma\text{PAH}_{2\text{ring}}$), the sum of PHE, ANT, 2-MPHE, 2-MANT, 1-MPHE, and 3,6-DMPHE concentrations ($\Sigma\text{PAH}_{3\text{ring}}$), the sum of FLA, PYR, RET, 1-MPYR, BaA, CHR+TRI, and 6-MCHR concentrations ($\Sigma\text{PAH}_{4\text{ring}}$), the sum of BbF, BkF, BeP, BaP, IcdP, DahA, and BghiP concentrations ($\Sigma\text{PAH}_{5\text{ring}}$), the sum of NAP, ACY, FLO, PHE, ANT, FLA, PYR, BaA, CHR, BbF, BkF, BaP, IcdP, DahA, and BghiP concentrations ($\Sigma\text{PAHUS-Priority}$), and the sum of all 28 individual MW<300 parent PAH concentrations (ΣPAH_{28}). The MW 302 parent PAH concentrations are reported as individual PAH concentrations as well as the sum of DBaEP, DBaIP, DBaLP, and DBaHP (ΣDBP), the sum of N12kF, N23bF, DBaEF/DBbkF, DBaKF, DBaLP, N23eP, DBaEP, DBeLP, N23aP, DBaIP, DBaHP, DBbeF, and N21aP that have been reported as human mutagens in literature (30–32) ($\Sigma\text{302PAH}_{\text{mut}}$), and the sum of all individual MW 302 PAHs (Σ302PAH). The NPAH and OPAH concentrations are reported as individual PAH concentration as well as the sum of all individual NPAH concentrations (ΣNPAH) and the sum of all individual OPAH concentrations (ΣOPAH). The sum of ΣPAH_{28} and Σ302PAH are reported as ΣPAH_{51} .

Toxicology Studies

Bacteria for Ames Assays

The basic method follows that reported by Maron and Ames(33). Salmonella strains TA98 were used in the study. Salmonella tester strain TA98 was originally purchased from Xenometrix, Inc.

Ames Assays

Briefly, 2 ml molten top agar (45°C), 30 μl samples in DMSO, 0.5 ml of phosphate buffered saline or rat S9 mix (an exogenous metabolic activation system based on rat liver enzymes), and 0.1 ml of bacteria were quickly mixed in a sterile disposable tube and the mixture was poured onto a Vogel-Bonner minimal agar plate. After the bacteria-containing agar was solidified, the plates were incubated at 37°C in inverse position for 48 hr. The histidine revertant colonies were counted with a Sorcerer Colony Counter (Perceptive Instruments, Haverhill, Suffolk, UK). All air samples were tested in triplicate. The positive control (4-nitro-1,2-phenylenediamine) and negative control (DMSO) doses were 20 μg and 50 μl , respectively. With respect to cytotoxicity, no adverse effects were seen on the background lawn.

Human A549 lung carcinoma cells and treatment for Comet Assays

Human A549 lung carcinoma cells were originally purchased from American Type Culture Collection (ATCC, Manassas, VA). ATCC protocols were followed for cell culture and maintenance. The cell line was maintained in F-12K medium supplemented with 10% fetal bovine serum in a 5% CO_2 incubator at 37°C. On the day of Comet treatment, cells in a T25 flask (~90% confluence) were trypsinized and re-suspended in growth medium. Approximately 20,000 cells were treated with the 10 μl air sample extract in DMSO and with 990 μl growth medium for 1 hr at 37°C.

Comet Assays

The single cell gel electrophoresis ('comet') assay was used to assess levels of DNA damage in A549 cells. The assay was modified based on the protocol of Singh et al.(34). Briefly, cells in 60 μl of 0.5% low melting point agarose (LMPA) were spread onto a dry, pre-coated

slide (with 1% normal melting point agarose) with a coverglass, and then placed onto a 4°C surface for 20 min. The coverglass was removed and another layer of cell-free LMPA (70- μ l) was spread over the cell-containing layer using a second coverglass. After the layer of agarose had hardened for 15 min, the coverglass was removed and the slide was immersed overnight in cold lysing solution (2.5 M NaCl, 100 mM EDTA disodium salt, 10 mM Tris, pH 10, containing 1% Triton X-100 and 10% DMSO, added just before use). Slides were rinsed in cold deionized water and placed in a horizontal gel electrophoresis tank containing fresh cold electrophoresis solution (300 mM NaOH and 1 mM Na EDTA, pH >13) for 30 min, followed by electrophoresis at 0.8 V/cm for 30 min. Upon completion of the electrophoresis, slides were rinsed briefly in deionized water and neutralized using 0.4 M Tris-HCl buffer, pH 7.4. The slide was stained with 60 μ l of 10 μ g/ml ethidium bromide, covered with a coverglass, and 25 randomly chosen nuclei per duplicate slide were analyzed using a Nikon E400 fluorescence microscope linked to Comet Assay III software (Perspective Instruments, Suffolk, UK), as reported elsewhere (35). Statistical analyses were performed for 'Percent DNA in the Tail' (the percentage of DNA in the "Comet" tail area in the assay and an indicator of the degree of DNA damage in the cells). With regard to cytotoxicity, none of the treatments reduced cell viability below 90%, as measured by the trypan blue exclusion assay.

Gas Pollutant Data

The NO, NO₂, NO_x, SO₂, CO and O₃ concentrations were measured by Zhang et al (1) at the China Meteorological Administration (CMA) in Beijing. This site was at approximately 20 m above ground and 5 km south of Peking University where the PAH samples were collected.

Results and Discussion

Effect of Source Control Measures on BC, OC, and Parent PAH Concentrations

Because PAHs are an important part of the OC, it is useful to understand the relationship between BC, OC and PAH concentrations. The mean $\pm$ standard deviation of the BC, OC, MW<300 parent PAH, and MW 302 parent PAH concentrations during the non-source control and source control periods are given in Table SI.4, while their concentrations during the non-Olympic and Olympic periods are given in Table SI.5. In addition, Figure 1A shows the temporal variation in the PM_{2.5}, OC, and BC concentrations, as well as the OC/BC ratio, while Figure 1B shows the variation in Σ PAH₅₁ concentration.

The mean BC and OC concentrations were statistically different ($p < 0.05$) during the non-source control and source control periods and the non-Olympic and Olympic periods (Table SI.4 and Table SI.5). The BC and OC concentrations ranged from 0.8–6.4 μ g/m³ and 4.9–25.6 μ g/m³, respectively, during the non-source control period and from 0.7–3.7 μ g/m³ and 4.9–16.3 μ g/m³, respectively, during the source control period, with a mean reduction in concentrations of 1.4 μ g/m³ (45.5%) and 4.5 μ g/m³ (31.1%), respectively. During the non-Olympic and Olympic periods, the mean BC and OC concentrations were reduced by 1.1 μ g/m³ (44.8%) and 3.8 μ g/m³ (31.5%), respectively. Other authors have reported BC concentrations in the range of 2–6 μ g/m³ and a reduction of 12–50% during the Olympic period (Table SI.6) (1–3). In addition, we measured relatively high OC to BC ratios (up to 9) during all periods, with a mean ratio of 5.88 ± 1.28 for all periods (Table SI.6).

Twenty-five of the 28 individual MW<300 parent PAH concentrations were significantly different between non-source control and source control periods, with concentration reductions of 26.6% to 77.9% ($p < 0.05$) during the source control period (Table SI.4). Only 1-MNAP, 2,6-DMNAP, and 1,3-DMNAP concentrations were not significantly different. Similarly, 22 of the 28 individual PAH concentrations were statistically different between

the non-Olympic and Olympic periods, with concentration (ng/m^3) reductions of 26.0% to 72.4% ($p < 0.05$) during the Olympic period (Table SI.5). In addition to 1-MNAP, 2,6-DMNAP, and 1,3-DMNAP concentrations, NAP, 2-MNAP, and ANT concentrations were not significantly different. This is likely because naphthalenes are emitted from a wide variety of consumer products (including personal care products, household products, adhesives, sealants, pesticides, and coatings) (20), as well as incomplete combustion, and emissions from consumer products were not controlled in Beijing during this time period. In addition, the lower molecular weight PAHs, including naphthalenes, exist primarily in the atmospheric gas phase. Because only the particulate-phase was sampled, their total concentration in the atmosphere was significantly (but consistently) underestimated. Like the majority of the individual PAHs, ΣPAH_{28} , $\Sigma\text{PAH}_{2\text{ring}}$, $\Sigma\text{PAH}_{3\text{ring}}$, $\Sigma\text{PAH}_{4\text{ring}}$, $\Sigma\text{PAH}_{5\text{ring}}$ and $\Sigma\text{PAH}_{16\text{-US priority}}$ concentrations were all significantly different between non-source control and source control periods and between non-Olympic and Olympic periods, with concentration reductions of 32.4% to 60.0% and 22.8% to 58.3% ($p < 0.05$), respectively.

Significant reductions were also observed for all measured MW 302 PAH isomers during the source control period compared to the non-source control period, ranging from 22% to 77% (Table SI.4 and Table SI.5) ($p < 0.05$). Concentrations of $\Sigma 302\text{PAH}$, $\Sigma 302\text{PAH}_{\text{mut}}$, and ΣDBP were reduced by 32% (4.6 ± 2.3 to $3.2 \pm 1.2 \text{ ng}/\text{m}^3$, $p < 0.001$), 31% (2.9 ± 1.4 to $2.0 \pm 0.8 \text{ ng}/\text{m}^3$, $p = 0.001$), and 39% (0.44 ± 0.22 to $0.27 \pm 0.11 \text{ ng}/\text{m}^3$, $p < 0.001$), respectively. Similar and further reductions were observed during the Olympic period compared to the non-Olympic period, with individual MW302 PAH isomers reduced by 32% to 67%, and $\Sigma 302\text{PAH}$, $\Sigma 302\text{PAH}_{\text{mut}}$, and ΣDBP reduced by 43–44% ($p < 0.001$). The significant reductions in the MW 302 PAH concentrations were consistent with our findings for the majority of the lower molecular weight parent PAHs.

In general, for the $\text{MW} < 300$ parent PAHs, the individual PAH concentrations were strongly positively correlated with the concentrations of other individual PAHs and with the ΣPAH_{28} and $\Sigma\text{PAH}_{16\text{-US priority}}$ concentrations ($p < 0.01$) (Table SI.7). However, 1-MNAP, 2,6-DMNAP, and 1,3-DMNAP concentrations had less or no correlation with the other individual PAH, σPAH_{28} , and 3 $\Sigma\text{PAH}_{16\text{-US priority}}$ concentrations. However, these individual NAP concentrations were highly correlated with each other. This suggests that the naphthalenes are coming from a different source than the other individual PAHs, including consumer products (20). Concentrations of ANT, one of the most photoreactive PAHs, were not as highly correlated with other individual PAH, ΣPAH_{28} , or $\Sigma\text{PAH}_{16\text{-US priority}}$ concentrations. This may suggest that PAHs, especially ANT, undergo photodegradation enroute from regional and local sources to our sampling site.

Effect of Source Control Measures on NPAH and OPAH Concentrations

The mean $\pm$ standard deviation of the individual NPAHs and OPAHs detected during the non-source control and source control periods, and during the non-Olympic and Olympic periods, are given in Table SI.4 and Table SI.5, respectively. Figure 1B shows the temporal variation in the ΣNPAH and ΣOPAH concentrations. Five of the 11 individual NPAH concentrations, and 2 of the 5 OPAH concentrations were significantly different between the source control and non-source control periods, with concentration reductions of 15.1% to 56.6% and 24.8% to 46.6%, respectively. During the Olympic period, 3-NBP, 3-NBF, 5-NAc and 1-NP concentrations were detectable, but below the limit of quantitation. Excluding these compounds, six of the 11 individual NPAH and all of the individual OPAH concentrations were statistically different between the Olympic and non-Olympic periods, with concentration reductions of 28.0% to 68.1% and 36.5% to 49.7%, respectively.

Except for 3-NBP, 3-NBF and 5-NAC, the individual NPAH and OPAH concentrations were strongly positively correlated to other NPAH and OPAH concentrations (Table SI.9 and Table SI.10).

Parent PAH, NPAH and OPAH Sources

Table SI.11 shows the correlation of individual parent PAH, NPAH, OPAH, Σ PAH28, Σ PAH2ring, Σ PAH3ring, Σ PAH4ring, Σ PAH56ring, Σ PAH16-US priority, Σ 302PAH, Σ 302PAHmut, σ NPAH and Σ OPAH with NO, NO₂, NO_x, CO, SO₂, and O₃ concentrations measured on the same days, during the source control period, when the PAH and gas-phase pollutant sampling overlapped (1). Most of the individual parent PAH, NPAH and OPAH concentrations were positively correlated with NO and NO₂ concentrations. NO is a short-lived species, with atmospheric residence time of 1 day (36), and is an effective tracer for local traffic emissions (37). However, only the individual parent PAH concentrations, and not the NPAH and OPAH concentrations, were correlated with CO and SO₂ concentrations. CO has an atmospheric residence time of 37 days (36), and has been reported to undergo long-range transport (38). This suggests that the parent PAHs are associated with both regional and local emissions, while the NPAH and OPAHs are primarily associated with local emissions and local photochemical formation. In addition, the CO and PM_{2.5} mass concentrations were correlated with air masses from the south of Beijing ($p < 0.001$) (7), where there are significant regional combustion sources, while the NO, NO₂ and NO_x concentrations were not.

Figure 2A shows the mean ($\pm$ standard deviation) profile of the 28 individual MW<300 parent PAHs (percent of total 28 MW<300 parent PAH concentration) during the source control, non-source control, Olympic, and non-Olympic periods. For all periods, the general trend in concentrations was: BbF > BeP $\approx$ IcdP $\approx$ BghiP > FLA > PYR $\sim$ BkF $\sim$ BaP > CHR + TRI $\sim$ BaA. The lower molecular weight PAHs, those with 2 or 3 rings, had lower concentrations on PM_{2.5} because they exist primarily in the atmospheric gas phase. However, the 4-ring PAHs, such as fluoranthene and pyrene, are distributed between the gas- and particulate-phases and the 5-ring (and higher) PAHs exist primarily in the particulate-phase. Because only the particulate-phase was measured in this study, the 5-ring PAHs, such as BbF and BeP were most abundant. Most individual MW<300 parent PAHs made up a similar percentage of the total MW<300 parent PAH concentration during the different periods. However, the 1-DMNAP, 2,6-DMNAP, 1,3-DMNAP, 3,6-DMNAP, and DahA concentrations were slightly enhanced in both the source control and Olympic periods, relative to the other MW<300 parent PAHs. In contrast, the CHR+TRI, BkF, and BaP concentrations were slightly enhanced in both the non-source control and non-Olympic periods. Furthermore, the FLA/ (FLA + PYR); IcdP/ (IcdP + BghiP); BeP/ (BeP + BaP); and IcdP/ (IcdP + BeP) concentration ratios are consistent with local traffic emissions (Table SI.12) (39).

The mean profile of the MW 302 parent PAHs, NPAHs, and OPAHs was similar between the source control and non-source control periods and between the Olympic and non-Olympic periods (Figure 2B and 2C). This indicates that the combustion sources of these PAHs were similar among the different periods. For the MW 302 parent PAHs, N12bF, N23jF/N12kF, DBaEF/DBbkF, and DBelP were the most abundant species, accounting for 34% to 57% of the total measured MW 302 parent PAH concentration. Together, 2-NF and 9-NAN were the most abundant NPAHs, accounting for 74% to 80% of the total NPAH concentration, while ANQ and BaAD were the most abundant OPAHs, accounting for 63% – 68% of the total OPAH concentration.

Role of PAH Photochemistry

To assess the contribution of primary emission (direct emission) and secondary emission (photochemical formation) of NPAH and OPAH, the 2-NF/1-NP concentration ratio was calculated. 2-NF is formed photochemically from the reaction of FLA with OH radical and NO₃ radical, while 1-NP is emitted from primary emissions (40, 41). A 2-NF/1-NP ratio of 5 or greater indicates a dominance of photochemical formation, while a ratio of less than 5 indicates a dominance of direct emissions (42, 43). The mean 2-NF/1-NP ratios during the source control, non-source control, non-Olympic periods were greater than 5 and ranged from 25–46 (Table SI.4 and Table SI.5). This suggests that there was a dominance of photochemical formation during all periods. There was also a statistical difference in the 2-NF/1-NP ratio between the source control and non-source control periods and Olympic and non-Olympic periods, with lower ratios measured during the source control (38.7 ± 15.2) and Olympic periods (25.2). 1-NP was near the limit of quantitation on some of the source control and Olympic days because of the reduced direct emissions. Combined, these ratios indicate that there was greater photochemical formation of NPAHs during the non-source control and non-Olympic periods. This was the result of both meteorological conditions (7) and increased traffic emissions.

The 2-NF/2-NP concentration ratio has been used to estimate the relative importance of OH radical initiated reaction vs. NO₃ radical initiated reaction in the photochemical formation of NPAHs in the atmosphere (42, 44). During daytime, fluoranthene and pyrene react with OH radical and pyrene react with NO₃ radicals to form predominantly 2-NF and negligible amounts of 2-NP (41, 44). A 2-NF/2-NP concentration ratio close to 10 indicates the OH radical-initiated reaction is dominant, while a ratio closer to 100 indicates the NO₃ radical-initiated reaction is dominant (44). During all periods, the mean 2-NF/2-NP concentration ratio was consistently below 10 (Table SI.4 and Table SI.5), ranging from 3.4 to 4.8, suggesting a dominance of daytime OH radical-initiated reaction for NPAH formation. This observation is consistent with previous ambient measurements (42–43).

Toxicity of the PM extracts

We have previously reported the estimated reduction in PAH-related inhalation cancer risk due to source control measures during the Beijing Olympics using a point-estimate approach based on relative potency factors (17). In this study, the PM_{2.5} crude extracts were assayed by the Ames test using the *Salmonella typhimurium* TA98 strain with and without S9 mix. The mutagen density (revertants per volume of air) and the corresponding Σ PAH₂₈, Σ 302PAH, Σ NPAH, and Σ OPAH concentrations are reported in Figure SI.1A. Because the crude extracts were not fractionated, the direct-acting NPAH mutagenicity may have been suppressed by the presence of indirect-acting parent PAHs (45). Nonetheless, the crude extracts are representative of the PAH, NPAH, and OPAH mixture that Beijing residents are exposed to in ambient air.

The correlations of Σ PAH₂₈, Σ 302PAH and sum of Σ NPAH and Σ OPAH concentrations with direct-acting and indirect-acting mutagen densities are shown in Figure 3. The parent PAHs, including Σ PAH₂₈, Σ 302PAH and the sum of Σ NPAH and Σ OPAH were well correlated with the indirect-acting mutagen density, with R² values of 0.44, 0.61 and 0.57 (p-values < 0.0001), respectively. The correlation of the sum of Σ NPAH and Σ OPAH concentrations with direct-acting mutagen density was less significant (R² of 0.41, p-value < 0.001). This may be due to the presence of parent PAHs in the crude extract (45). On average, the Σ NPAH and Σ OPAH concentrations were 8% of the parent PAH concentrations, while the direct-acting mutagenicity (due to NPAH and OPAH) was 200% higher than the indirect-acting mutagenicity (due to parent PAH). This suggests that NPAH and OPAH make up a significant portion of the overall mutagenicity of PM_{2.5} in Beijing.

The lowest mean mutagen density was associated with the Olympic period, which is consistent with statistically significant decreases in ΣPAH_{28} , $\Sigma 302\text{PAH}$, and sum of ΣNPAH and ΣOPAH concentrations.

Human cell assays were carried out on the $\text{PM}_{2.5}$ crude extracts in order to associate the bacteria-based toxicity with human cell-based toxicity. Figure SI.1B shows the median percent DNA damage of human A549 lung carcinoma cells dosed with the daily PM extracts in the Comet assay. The toxicity of PM, including direct and indirect mutagenicities and percent DNA damage in the Comet assay, were not statistically different between the source control and non-source control periods. However, the mutagenicity of the $\text{PM}_{2.5}$ was significantly reduced during the Olympic period. This suggests that the source control measures did not result in as significant a reduction in PM toxicity as ΣPAH_{28} , $\Sigma 302\text{PAH}$, ΣNPAH and ΣOPAH concentrations. This may be because pollutants other than those measured here contributed to the overall toxicity of the $\text{PM}_{2.5}$ crude extracts and may not have been reduced in concentration due to the source control measures.

Figure SI.2 shows the Spearman correlation between mutagenic activities in the Ames bacterial assays and levels of DNA damage in human cell assays for all of the $\text{PM}_{2.5}$ extracts. There was a strong correlation ($r = 0.77$, $p < 0.0001$) between the results of the two different assays. Previous studies questioned the comparison of mutagenic activities of unsubstituted and substituted PAHs in bacterial assays with human cell assays, calling into question the relevance of bacterial assays (46). However, our results suggest that there is a significant correlation between the bacterial assay results and the human cell assay results.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Temporal variation of the (A) PM_{2.5}, OC, BC, OC/BC and (B) ΣPAH₅₁, ΣNPAH, and ΣOPAH concentrations. The source control, non-source control, and the Olympic periods are indicated by labels and lines.

Figure 2.

Mean ($\pm$ standard deviation) of the percent of (A) total MW<300 PAHs, (B) total MW 302 PAHs, and (C) sum of NPAH and OPAH concentrations during the source control, non-source control, Olympic and non-Olympic periods. Red asterisks indicate a significant difference ($p<0.05$) between the source control and non-source control periods and blue asterisks indicate a significant difference ($p<0.05$) between the Olympic and non-Olympic periods.

Figure 3. Correlation of ΣPAH_{28} , $\Sigma 302\text{PAH}$ and sum of ΣNPAH and ΣOPAH with direct-acting and indirect-acting mutagen densities during the source control (°) and non-source control periods (•).

Table 1

List of MW<300 parent PAHs, MW 302 PAHs, NPAHs and OPAHs (and their 3 abbreviations) measured in this study.

#	Compound	Abbreviation
<i>MW<300 PAHs¹</i>		
1	naphthalene	NAP
2	2-methylnaphthalene	2-MNAP
3	1-methylnaphthalene	1-MNAP
4	2,6-dimethylnaphthalene	2,6-DMNAP
5	1,3-dimethylnaphthalene	1,3-DMNAP
6	acenaphthylene	ACY
7	fluorene	FLO
8	phenanthrene	PHE
9	anthracene	ANT
10	2-methylphenanthrene	2-MPHE
11	2-methylantracene	2-MANT
12	1-methylphenanthrene	1-MPHE
13	3,6-dimethylphenanthrene	3,6-DMPHE
14	dibenzothiophene	DBT
15	fluoranthene	FLA
16	pyrene	PYR
17	retene	RET
18	1-methylpyrene	1-MPYR
19	benz[a]anthracene	BaA
20	chrysene + triphenylene	CHR+TRI
21	6-methylchrysene	6-MCHR
22	benzo(b)fluoranthene	BbF
23	benzo(k)fluoranthene	BkF
24	benzo[e]pyrene	BeP
25	benzo[a]pyrene	BaP
26	indeno[1,2,3-cd]pyrene	IcdP
27	dibenz[a,h]anthracene	DahA
28	benzo[g,h,i]perylene	BghiP
<i>MW 302 PAH²</i>		
1	naphtho[2,3-a]pyrene	N23aP
2	naphtho[2,3-e]pyrene	N23eP
3	naphtho[1,2-b]fluoranthene	N12bF
4	dibenzo[a,e]fluoranthene	DBaeF
5	dibenzo[b,k]fluoranthene	DBbkF

#	Compound	Abbreviation
6	dibenzo[e,l]pyrene	DBelP
7	dibenzo[a,k]fluoranthene	DBakF
8	dibenzo[j,l]fluoranthene	DBjlF
9	naphtho[2,3-j]fluoranthene	N23jF
10	naphtho[2,3-b]fluoranthene	N23bF
11	naphtho[2,3-k]fluoranthene	N23kF
12	benzo[b]perylene	BbPer
13	dibenzo[a,i]pyrene	DBaiP
14	dibenzo[a,e]pyrene	DBaeP
15	dibenzo[a,l]pyrene	DBalP
16	dibenzo[a,h]pyrene	DBahP
NPAHs³		
1	1-nitronaphthalene	1-NN
2	2-nitronaphthalene	2-NN
3	2-nitrobiphenyl	2-NBP
4	3-nitrobiphenyl	3-NBP
5	4-nitrobiphenyl	4-NBP
6	3-nitrodibenzofuran	3-NBF
7	5-nitroacenaphthalene	5-NAC
8	2-nitrofluorene	2-NFL
9	9-nitroanthracene	9-NAN
10	9-nitrophenanthrene	9-NPH
11	2-nitrodibenzothiophene	2-NDB
12	3-nitrophenanthrene	3-NPH
13	2-nitroanthracene	2-NAN
14	2-nitrofluoranthene	2-NF
15	3-nitrofluoranthene	3-NF
16	1-nitropyrene	1-NP
17	2-nitropyrene	2-NP
18	7-nitrobenz(a)anthracene	7-NBaA
19	2,8-dinitrodibenzothiophene	2,8-DNDB
20	6-nitrochrysene	6-NCH
21	3-nitrobenzathrone	3-NBENZ
22	1,3-dinitropyrene	1,3-DNP
23	1,6-dinitropyrene	1,6-DNP
24	1,8-dinitropyrene	1,8-DNP
25	6-nitrobenzo[a]pyrene	6-NBaP
OPAHs³		
1	9-fluorenone	9-FLU

#	Compound	Abbreviation
2	9,10-anthraquinone	ANQ
3	2-methyl-9,10-anthraquinone	2-MANQ
4	benzanthrone	BEN
5	benz[a]anthracene-7,12-dione	BaAD

¹ Purchased from AccuStandard (New Haven, CT) and Chem Service (West Chester, PA)

² Purchased from Chiron AS (Trondheim, Norway) and AccuStandard (New Haven, CT)

³ Purchased from Chiron AS (Norway), AccuStandard (New Haven, CT), Chem Service (West Chester, PA) and Sigma-Aldrich Corp.