SIZE DISTRIBUTIONS OF METALS, PAHs, AND PARTICLE-EXTRACT-INDUCED CYTOTOXITY OF TRAFFIC-RELATED NANO/ULTRA-FINE PARTICLES

Shui-Jen Chen,Department of Environmental Engineering and Science

1. Characteristics of Traffic-Related Particles

Several epidemiologic studies have demonstrated the association of ambient ultra-fine particles (UFPs) with adverse respiratory and cardiovascular effects, resulting in morbidity and mortality in susceptible parts of the population. Coarse inhalable particles may be deposited in the upper respiratory tract whereas fine particles travel deeper into the lungs. The finest particles can reach the alveolar regions. Several works were conducted using in vitro simulations of the respiratory environment or in vivo simulations using animal models;these studies suggested that particulate metals are correlated to pulmonary toxicity. The particle size and chemical composition of inhalable particles importantly govern their potential impacts on human health because the efficiencies of both inhalation and respiratory deposition depend on particle size, while the chemical composition mediates toxicity by the presence of specific toxic elements and influences of nonspecific particle toxicity.

At a given mass, UFPs have 10^2 to 10^3 times more surface area than particles with diameters in the 0.1~2.5 μm range and approximately 10^5 times more surface area than coarse particles (2.5 μm < diameter < 10 μm). This surface area-to-mass effect may affect the relative toxicity of particles to respiratory systems because smaller particles have a higher surface area-to-mass ratio and the deposition efficiency of ultrafine particles is higher in the alveolar region. UFPs were found to cause a stronger toxic effect than coarse particles at the same mass level. Hence, researchers have paid increasing attention to nano-particles in the atmosphere or from vehicular emissions, but they have focused more strongly on the number concentration of nano-particles.

Ultrafine particles can more efficiently transfer catalytic metals to the surface of irritant particles into the lungs than can an equal mass of larger particles. Variable amounts of Pb, Fe, Cu, Zn, Ni, and Cd were found in the exhaust from gasoline and diesel-fueled vehicles. The abrasion of tire rubber is another source of Zn. Ba, Cu, Fe, Mn, Pb, and Zn have been found to be the main metal pollutants emitted by vehicles in Florence (Italy). In fact, toxic anthropogenic elements (Ag, Ba, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Sr, Ti, V, and Zn), while having much less mass than crustal elements, are typically present in gasoline/diesel fuels and gasoline/diesel engine exhausts. Traffic-related particulate Cu, Mo, Sb, and Zn may partially attributed to abraded tire materials and rubbed-off brake-linings.

Traffic emissions are regarded as one of the major contributors to the high concentrations of polycyclic aromatic hydrocarbons (PAHs) in urban air. Diesel and gasoline-powered engines emit large amounts of PAHs that partition between gas and particle phases. Particle-bound semivolatile PAHs and nonvolatile four- to six-ring PAHs may dominate the submicrometer particles collected using 0.1~0.18, 0.18~0.32, and 0.32~0.56 μm MOUDI stages. Significant amounts of some PAH species may be present in the ultrafine mode (0.05~0.12 μm) in tunnels. Recently, heavy-duty diesel vehicles were found to produce quantifiable emissions of 3- to 6-ring PAHs (including coronene) under light driving cycles whereas heavier driving cycles produced only 3- and 4- ring PAHs in quantifiable amounts.

Alveolar macrophages (AMs) play an important role in the local regulation of inflammatory responses within the alveolar space. They may clear infected pathogens or exogenous particle extracts by binding to and ingesting bacteria and environmental particles through specific receptors. Atmospheric particles reaching the alveoli are ingested by AMs. Ultrafine carbon particle aggregates may be ingested by human or rat AMs. AMs obtained from children have been found to contain carbonaceous UFPs. UFPs may also be found in AMs from healthy nonsmoking adults. Therefore, AMs are relevant target cells for UFPs.

2. Most Recent Research on Traffic-Related Particles in Our Lab

Investigations into ultra-fine and nano-particle metal components/concentrations from vehicle emissions are very few and little attention has been paid to the carcinogenic potencies and cytotoxicities of traffic-related nano-particle bound PAHs (10~ 56 nm, with health significance). Accordingly, the author applied and obtained projects supported by the National Science Council in Taiwan(under grant numbers NSC 93 - 2211 - E - 020 - 008 , NSC 94 - 2211-E-020-001 , and NSC 94 - 2211 - E - 020-008) to perform such investigations. This project was conducted by Mr. Chih-Chung Lin (doctoral student at the Department of Environmental Engineering and Science (EES)) who was also co-advised by Professor Kuo-Lin Huang at the Department of EES and Professor Hso-Chi Chaung at the Department of Veterinary Medicine. In our work, we used a MOUDI and a Nano-MOUDI to collected sized particles near a major road with busy traffic. The mass concentrations in nano, ultrafine, fine, and coarse particles, and the metal components/concentrations (including 7 crustal elements and 12 toxic anthropogenic metals) and 15 PAHs were determined. The carcinogenic potencies (as equivalent BaP concentrations (BaP_{eq})) induced by PAHs in the size-resolved particles were also determined and compared. The particle extracts (containing PAHs) were tested using an AM assay to examine the cytotoxicities derived from the corresponding reduction in cell viability of AMs. The cytotoxicities induced by the particle extracts were statistically compared.

3. Important Results

3.1 Size Distributions of Metals of Traffic-Related Particles

Our study attempted to characterize the metals in nano (0.010 < Dp < 0.056 μm), ultrafine (Dp < 0.1 μm), fine (Dp < 2.5 μm), and coarse (2.5 < Dp < 10 μm) particles collected near a busy road using a micro-orifice uniform deposition impactor (MOUDI) and a Nano-MOUDI. The nano particles were found to contain more of traffic-related metals (Pb, Cd, Cu, Zn, Ba, and Ni) than particles of other sizes, although crustal metals accounted for over 90% of all the particulate metals. Most crustal metals, Ba, Ni, Pb, and Zn in ultra-fine particles displayed Aitken modes due to their local origins. The Ag, Cd, Cr, Ni, Pb, Sb, V, and Zn were 37, 50, 28, 30, 24, 64, 38, and 22% by mass, respectively, in < 0.1 μm particles, with the submicron mass median diameters (MMDs) in $PM_{0.01\text{-}18}$ (except Zn) (particularly the < 0.1 μm MMDs for Cd and Sb). These levels raise potential health issues. Particle-bound Zn was more abundant in the accumulation mode than in the nucleation/condensation mode, but the opposite was true for Ag, Cd, and Sb. The Ag, Ba, Cd, Pb, Sb, V, and Zn contents in nano particles were strongly associated with diesel fuel, while the Cu, Mn, and Sr in particles < 0.1 μm were more strongly associated with gasoline. The high Si content in nano particles, associated more with diesel soot than with gasoline exhaust, is another health concern.

3.2 Size Distributions of PAHs, and Particle-Extract-Induced Cytotoxity of Traffic-Related Particles

The average particulate total-PAHs in five samplings displayed a trimodal distribution with a major peak in the Aitken mode (0.032~0.056 μm). About half of the particulate total-PAHs were in the ultra-fine size range. The BaP_{eq} sums of BaP, IND, and DBA (with toxic equivalence factors ≧ 0.1) accounted for ~90% of the total-BaP_{eq} in the nano/ultra-fine particles, although these three compounds contributed little to the mass of the sampled particles. The mean content of the particle-bound total-PAHs/-BaP_{eq}s and the PAH/BaP_{eq}-derived carcinogenic potency followed the order nano > ultrafine > fine > coarse. Figure 1 shows the cytotoxicity responses (expressed as reduction in cell viability (RCV in % control) induced by particle extracts (containing PAHs). The cells treated with extracts from 0.018~0.18 and 5.6~18 μm particles had greater mean RCV values than those from 0.18~5.6 μm particles ($p < 0.05$). The mean RCV values for the 0.18~0.32, 0.32~0.56, 0.56~1.0, 1.0~1.8, 1.8~3.2, and 3.2~5.6 μm particles were not statistically different. A similar trend was also observed among the mean RCV values for the 0.018~0.032, 0.032~0.056, 0.056~0.1, and 0.1~0.18 μm particles. It is interesting to find that the mean RCV value (128 ± 21%) for bleomycin (BLM) was statistically similar to those for the 0.032~0.056, 0.056~0.1, and 0.1~0.18 μm particles. Among the extracts of tested size-resolved particles, one of the 0.01~0.018 μm (10~18 nm) nano-particles exhibited the greatest mean RCV value (381 ± 75%), which was also statistically higher than that of BLM ($p < 0.05$). Accordingly, it is inferred that among the tested particle extracts containing PAHs, the one from the 10~18 nm nano-particles displayed the highest AM cytotoxicity (also higher than the BLM-induced one); furthermore, those from the 0.032~0.056, 0.056~0.1, and 0.1~0.18 μm particles were similar to that from BLM. The cytotoxicity of particle extracts (using 1:1 (v/v) n-hexane/dichloromethane) was significantly higher ($p < 0.05$) for the nano (particularly the 10~18 nm)/ultra-fine particles than for the coarser particles and bleomycin. Therefore, traffic-related nano and ultra-fine particles are possibly cytotoxic.

For the particles in each of the 13 size ranges, the variation trends in total concentration (or amount) of extracted PAHs and corresponding total-BaP_{eq} were roughly similar, but were quite different from that in the cytotoxicity response. The total-BaP_{eq} values of $PM_{0.18\text{~}0.32}$, $PM_{0.32\text{~}0.56}$, $PM_{0.56\text{~}1.0}$, and $PM_{1.0\text{~}1.8}$ were higher than those of particles in the five individual size ranges in $PM_{0.01\text{~}0.18}$, but the cytotoxicity seemed to be lower for the former PM than for the latter (particularly the $PM_{0.01\text{~}0.018}$). PAHs (especially BaP) may alter the functions (e.g., endocytosis and phagocytosis) of macrophagic cells. On the other hand, ultra-fine particles are more critical than $PM_{2.5\text{~}10}$ for particle toxicity although $PM_{2.5\text{~}10}$ may also be toxic. The phagocytic activity inhibition of macrophagic cells is higher for ultra-fine particles than for coarser particles. If considering both the toxicant and particle size effects on the cytotoxicity response, the inconsistency between total-BaP_{eq} values (from PAHs) and cytotoxicity responses in this study reveals that some nano-particles or extractable toxicants (e.g., some organic metals or other organics) were possibly present in the particle extracts. More research (e.g., more sampling and cytotoxicity tests directly on the size-resolved particles) is necessary to further compare the cytotoxicity of traf-

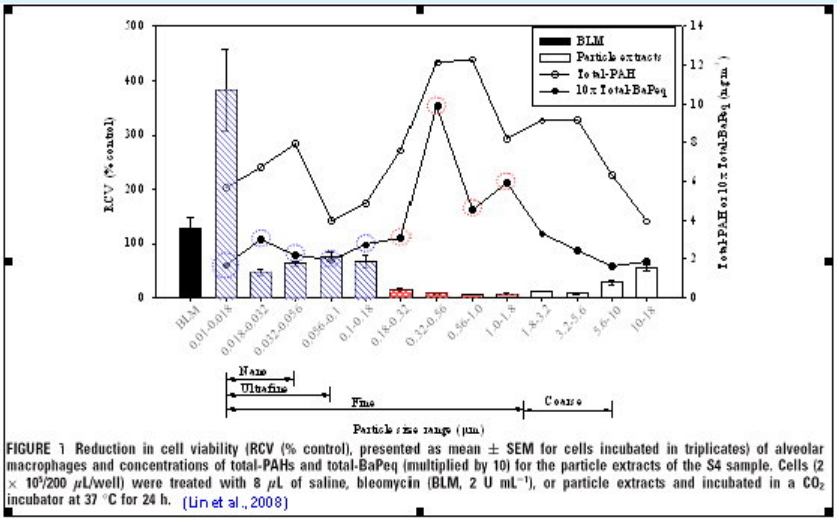

FIGURE 1 Reduction in cell viability (RCV (% control), presented as mean ± SEM for cells incubated in triplicates) of alveolar macrophages and concentrations of total-PAHs and total-BaPeq (multiplied by 10) for the particle extracts of the S4 sample. Cells (2×10^5/200 μL/well) were treated with 8 μL of saline, bleomycin (BLM, 2 U mL^{-1}), or particle extracts and incubated in a CO_2 incubator at 37 °C for 24 h. (Lin et al., 2008)

fic-related particles in these size ranges.

4. Summary

Air pollution related to traffic exhaust is always a public concern. The above statements and discussion are based on our papers published in the issues of November 2005 and June 2008 of Environmental Science and Technology (ACS Publications).

1. Lin, C. C.; Chen, S. J.*; Huang, K. L.; Hwang, W. I.; Chang-Chien, G P.; Lin, W. Y. Characteristics of metals in nano/ultrafine/fine/coarse particles collected beside a heavily-trafficked road. Environ. Sci. Technol. 2005, 39 (21), 8113-8122. [SCI; IF: 4.458 (in 2008); Ranking in Environ. Sci.: 7/163 = 4.3%, in Environ. Eng.: 2/38 = 5.3%; *Corresponding author]

2. Lin, C. C.; Chen, S. J.*; Huang, K. L.; Lee, W. J.; Lin, W. Y.; Tsai, J. H.; Chaung, H. C.* PAHs, PAH-Induced carcinogenic potency, and particle-extract-induced cytotoxicity of traffic-related nano/ultrafine particles. Environ. Sci. Technol. 2008, 42(11), 4229-4235. [SCI; IF: 4.458 (in 2008); Ranking in Environ. Sci.: 7/163 = 4.3%, in Environ. Eng.: 2/38 = 5.3%; *Corresponding author]

The paper (see above) in November 2005 in Environmental Science and Technology was published as the first article in that issue. Since its publication, several researchers from different countries have requested a copy of this paper by mail or email. This paper has been cited 43 times (to December 2009) by international journals, including 3 self-citations. Therefore, this paper is greatly valued by international researchers and helpful for the international reputation of academic research at National Pingtung University. ◆