

Responses of Human Lung Cells to Synthetic and Engine Emitted PM

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ABSTRACT

The cytotoxicity of airborne particles was investigated by employing a direct *in-vitro* exposure approach of human lung cells to particles. An apparatus was constructed to enable deposition of airborne particles to the lung cells. Cytokine (IL-8) production by the cells was employed as the measure of acute particle toxicity. The toxicity of particles made from a binary mixture (e.g., NiCl₂-H₂SO₄) exceeds that caused by super-micrometer nickel-laden particles by several folds. This result is inconsistent with the common view that response is proportional to dose; i.e., the mass of the target agent. Ultrafine particles emitted from internal combustion engines (i.e., diesel and gasoline) stimulated excessive production of IL-8. The IL-8 production kinetics due to exposure to diesel emission particles was different from exposure gasoline emission particles; delayed cellular responses were found in gasoline particle exposure experiments, whereas an immediate response was observed in the diesel particle exposure experiments. The magnitude of IL-8 production was also found to be much higher in the gasoline exposure than in the diesel particle exposure experiments.

INTRODUCTION

Airborne particles are ubiquitous and originate from both anthropogenic and natural sources. The sizes of environmental particles range from one nanometer to several tens of micrometers. Recent epidemiological evidences suggest that a small increase in PM₁₀ (particles of aerodynamic diameters smaller than or equal to 10 μm) could result in consistent increases in both morbidity and mortality rates due to impacts on the cardiopulmonary systems^{1,2,3}. Of particular concern are the health impacts resulting from exposure to particles produced by anthropogenic activities like internal-combustion (IC) and turbine engines.

Recent laboratory experiments found exposure of tumor necrosis factor (TNF) α-primed and/or control human lung epithelial cells to air particulate matter (e.g., residual oil fly ash and dust quartz) increased cytokines production in a particle mass concentration-dependent manner up to 200 μg/ml. Production of IL-8 cytokines is indicative of subsequent cellular expression and malfunction such as inflammation. Neither primed nor control cells responds to 1-μm size TiO₂. TiO₂ is a chemically inert

material known to be “non-toxic.” Other researchers have used 20- to 30-nm particles made of TiO₂ or Teflon in animal-exposure experiments.

Previous work^{4,5} demonstrated that ultrafine particles made of “chemically inert” materials can cause pulmonary inflammation and death of laboratory animals in some cases. Most (73%) of the particles measured in Eastern Germany⁶ were in the ultrafine (i.e., ≤ 100 nm) fraction. Health effects as measured by increases in coughing, feeling ill, and reduction of peak expiratory flow (PEF) correlate strongly with the number rather than the mass of ultrafine particles.

The A549 cell line has properties of the type II alveolar epithelial cells. Alveolar epithelial cells are primary targets for the toxic effects of particles in the lung, and loss of epithelial integrity is an important event leading to inflammation in the lung. Macrophages, a first mechanism of defense against particles in the lung, produce mitogenic cytokines in response to phagocytosis of particles. Increased cytokine production can cause lung epithelial cell injury leading to fibrosis, one of the most common manifestations of particulate-induced lung injury. Neutrophils recruited to the site of inflammation in the lung by chemokines cause further inflammation of the lung epithelium by release of proteolytic enzymes and toxic oxygen radicals. TNF- α “primed” A549 cells, used as a model for pre-existing lung disease, inflammation or injury, can have greatly increased levels of IL-8 compared to unexposed cells. Thus, TNF- α cells can be used as an indicator of cellular damage by particulate matter *in vitro*⁷.

TECHNICAL APPROACH

In this study, we produced synthetic monodisperse ultrafine particles using an electro spray apparatus⁸. The electro spray apparatus produced particles of an exceptionally narrow size distribution and consistent morphology. We exposed cells to particles produced by the electro spray technique in a manner similar to that we used in the exposure to engine particles.

A diesel and a spark-ignited gasoline engine available at the Advanced Propulsion Technology Center (APTC) at Oak Ridge National Laboratory (ORNL)/Engineering Science and Technology Division were used for generating engine exhausts. In addition, different fuel and fuel blends were used that included synthetic fuels of low sulfur content and reference fuels. For all the experiments, exhaust from a heavy-duty diesel engine or a light-duty gasoline engine was conditioned with dilution air in a micro-dilution tunnel.

We used a commercial ELISA kit for analyzing human IL-8 cytokine. The OptEIA™ Human IL-8 Set was purchased from Pharmingen in San Diego, CA (catalog #2654KI). The kit contains (1) capture antibody of anti-human IL-8, (2) detection antibody of biotinylate anti-human IL-8, (3) enzyme reagent of avidin-horseradish peroxidase conjugate, and (4) standards, recombinant human IL-8. The 50th percentile values were within 3% of the calibration data provided by the vendor in the range from 3 to 200 pg/ml. IL-8 levels in samples from our exposure experiments generally exceeded 12 pg/ml. When IL-8 concentration exceeds 200 pg/ml in a sample, we would dilute the

sample to bring the concentration down to the calibrated range. The final value for the original, pre-diluted, sample then was obtained by multiplying the measured value by the dilution ratio. The bias and great uncertainty at the lower end did not impact our ELISA analysis. The regression curve using the median values has the following form:

$$\ln(OD) = (-3.78 \pm 0.064) + (0.853 \pm 0.018) * \ln(IL - 8) \quad (1)$$

The R² value for this regression line shown in [Eq. (1)] was 0.998 indicating an excellent linear relationship between OD and IL-8 values in their logarithmic form.

RESULTS AND ACCOMPLISHMENTS

Exposure to Diesel Engine Particles (DEP)

The diesel exhaust experiments were conducted on a heavy-duty bus engine that meets 1998 regulations for particulate matter and NO_x of 0.067 g/kW·hr and 5.33 g/kW·hr, respectively. The engine has turbo-charging, intercooling and direct injection. The fuel used was a special low-sulfur fuel (Chevron Specialty Chemicals, Bartlesville, OK) that was first used in the Diesel Emissions Control–Sulfur Effects (DECSE) program for a number of studies. This fuel was augmented with additional fuel sulfur compounds to bring the fuel from a nominal 3 ppm to 40 ppm. The engine was also equipped with a catalyzed diesel particulate filter (CDPF) of the type used extensively in the DECSE program. The CDPF removes over 99% of the PM mass from the exhaust. The system is sampled upstream and downstream of the CDPF. A heated transfer line is used between the exhaust and the dilution device. The exhaust is diluted between 10:1

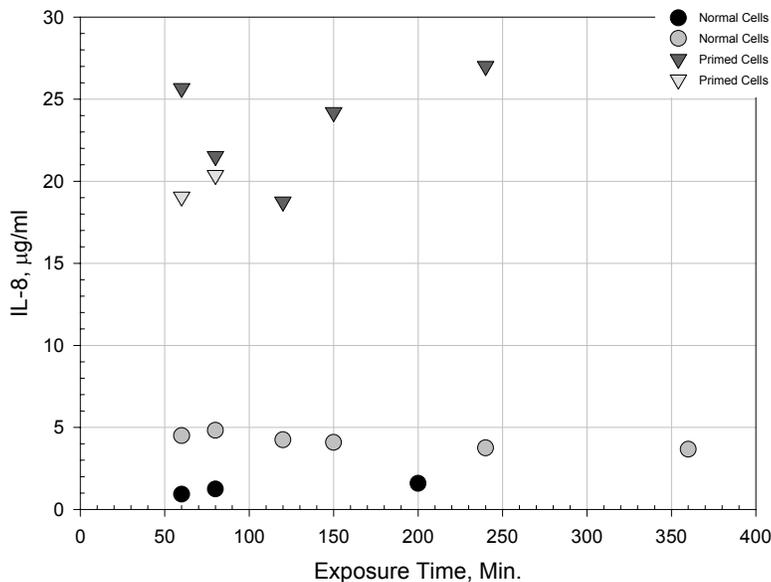


Fig. 1. IL-8 production as a result of DEP exposure

and 15:1 to reduce its dewpoint and temperature before introduction to the cell exposure apparatus.

The dilution device was a homemade micro-diluter that has been characterized in detail⁹. All of the experiments were carried out at low engine load (10% of rated torque) or idle to maximize the number count of particles in the range of 10–20 nm measured by using a TSI scanning electrical mobility spectrometer (SMPS®). The SMPS is equipped with a nano-differential mobility analyzer and an ultrafine condensation particle counter. The results of DEP exposure are shown in Fig. 1. Un-primed cells stimulated by DEP from the engine burning high-sulfur fuel produced more IL-8 (gray closed circles), by approximately 5 times, than DEP from the engine burning low-sulfur fuel (black closed circle). The IL-8 production was substantially higher in the primed cell cases (inverted triangles). The diesel engine broke down after 80 minutes of operation causing a premature termination of the second DEP experiment; data from this experiment are shown by the 2 gray inverted triangles. The IL-8 values detected after 60-min exposure to the primed cells were between 20 and 26 $\mu\text{g}/\text{ml}$, while that for normal cells was around 1-5 $\mu\text{g}/\text{ml}$ for particles produced under similar conditions. We also noted that the variation of IL-8 values in these experiments was approximately $\pm 20\%$.

The SMPS measurements in these experiments show that DEP were polydisperse. The peak diameters of the distributions were between 10 and 20 nm. An example of DEP size distribution is shown in Fig. 2. The total number concentration of DEP particles was

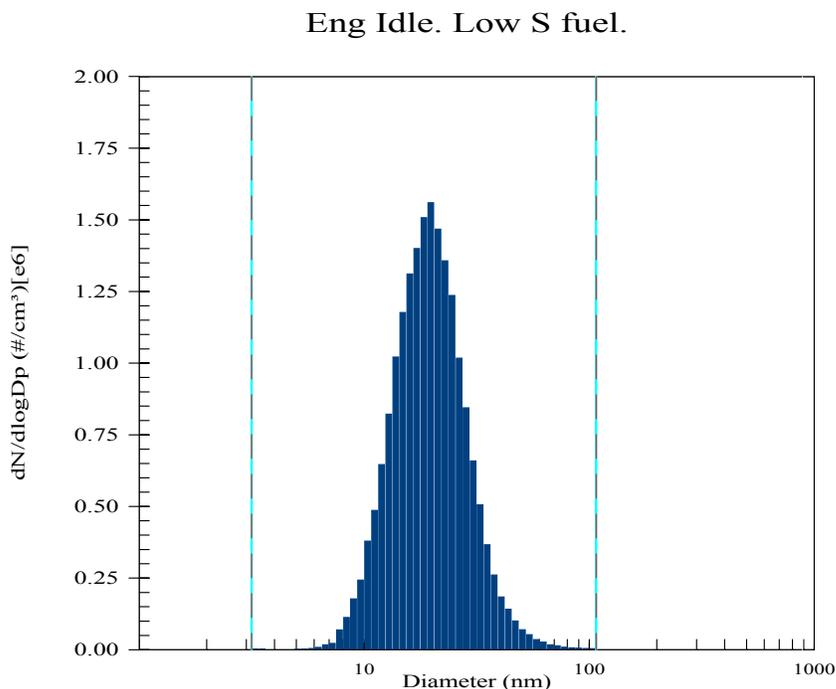


Fig. 2. An example of DEP distribution obtained at engine idle condition and burning low sulfur fuel.

greater than 10^8 per ml in each experiment. The size distribution spans from 7 nm to 640

nm. The fluctuation of IL-8 over time in the experiments was about 1 $\mu\text{g}/\text{ml}$ for normal cells and 5 for primed cells. Variation in engine operations could have resulted in the variation of IL-8 ($\pm 20\%$) as the size distributions taken every 30-min during the experiments have approximately $\pm 10\%$ fluctuation in the total number concentrations of particles.

Exposure to Gasoline Engine Particles (GEP)

The gasoline engine exhaust experiments were performed with a gasoline direct injection engine. This is a new type of engine technology that results in more fuel-efficient operation. Instead of the fuel being injected into the intake, as with conventional modern gasoline engines, the fuel is injected directly into the cylinder and ignited with a spark plug. The fuel used was the California Phase 2 fuel, a reformulated gasoline that is mandated in California for low emissions. One disadvantage of this technology is that there are higher PM emissions than with conventional gasoline engines. Two operating conditions were chosen: idle and a high load (80% of rated torque) at medium speed (50% of red line) condition. As with the diesel, the sample was diluted by the micro-diluter⁹ before introduction to the cell exposure apparatus. This engine was not equipped with aftertreatment. The idle condition resulted in a particle size distribution typically centered at 10 nm, smaller than that of the DEP particles produced at idle, and the high load condition resulted in a particle size distribution centered on 90 nm. The total number concentrations of GEP were between $2\text{-}5 \times 10^6 \text{ cm}^{-3}$, similar to those of DEP.

The results of GEP exposure are shown in Fig. 3. The magnitude of IL-8 production was between 3–5 $\mu\text{g}/\text{ml}$ in the GEP exposure, also similar to those produced by hi-sulfur diesel engine particles. In the primed cell cases, the IL-8 production increased sharply from 3–5 $\mu\text{g}/\text{ml}$, the level of normal-cell response, to 30–34 $\mu\text{g}/\text{ml}$ level after 2 h of exposure. This is a sharp contrast to the results for the DEP exposure in which the IL-8

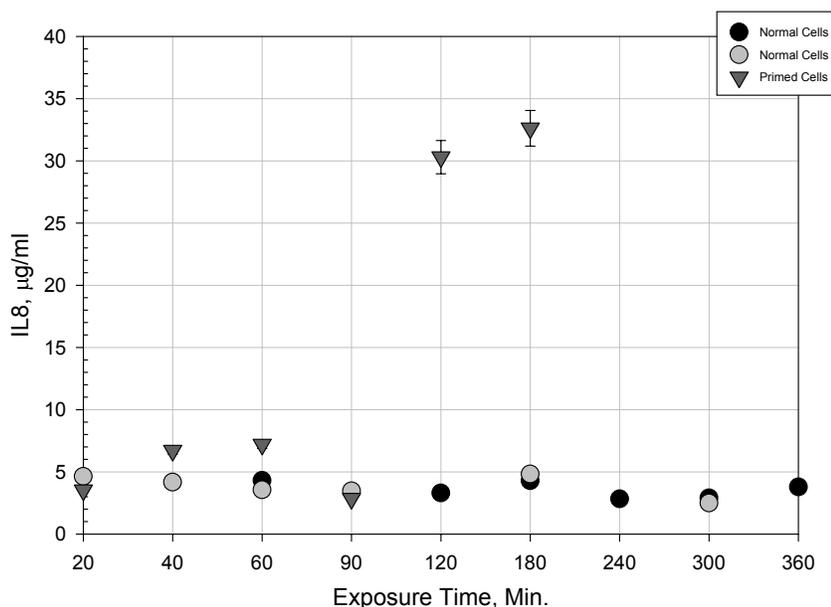


Fig. 3. IL-8 production as a result of the GEP exposure.

level increased immediately after the exposure and remained at 18–27 $\mu\text{g/ml}$ level till the end of the experiments.

Exposure to Synthetically Made Particles

The responses resulted from exposure to 4 synthetic particles generated by the electrospray technique are shown in Fig. 4. The solution concentration of NiCl_2 was 0.125% v/v, that of sulfuric acid was 0.1 N, and that of vanadium (V)-solution was 100 $\mu\text{g/ml}$ as vanadium. The data showed that the level of IL-8 induced by the nanometer Ni-laden particles was about 1–2 $\mu\text{g/ml}$, and 2 $\mu\text{g/ml}$ for the V-laden particles. The peak sizes for the nickel-laden particles were between 9 and 12 nm, 10 nm for the sulfuric acid particles, while that for the V-laden particles was about 9 nm. It is important to note that the background particles produced by electrospraying the deionized (DI) water were all smaller than 5 nm with single digit number of counts. These particles might have been formed around the residue in the water and from the glassware. In other words, we believe the particles we produced and detected resulted from the chemicals employed. The particle sizes measured also were realistic. Since the sizes of these 4 particles were close, 4–5 nm, the size effect on the IL-8 production was considered to be minimal in these 4 experiments.

Still, when nickel was present in a binary solution mixture such as $\text{NiCl}_2\text{-H}_2\text{SO}_4$, the toxicity of particles made from this binary mixture increased substantially from 1–2 $\mu\text{g/ml}$ to 19 $\mu\text{g/ml}$ after 4 h of exposure as shown in Fig. 4. That was more than a 10-fold

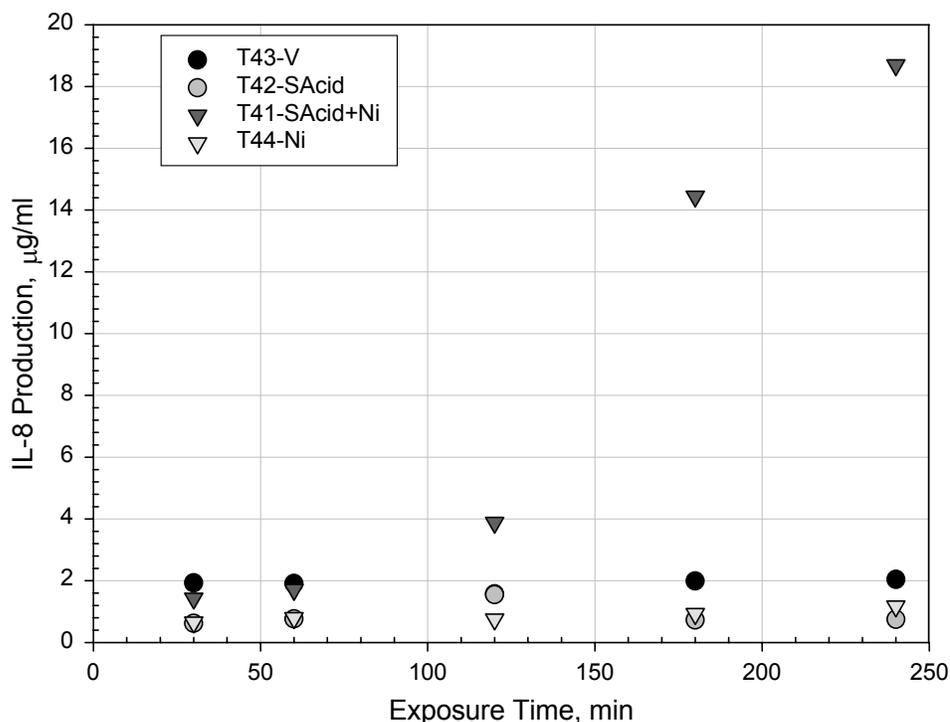


Fig. 4. IL-8 production as a result of exposure to synthetic particles

increase in toxicity measured as the IL-8 production. The IL-8 production by the mixture even exceeded that caused by exposure to 5.8- μm particles made from the same solution strength of NiCl_2 (i.e., 0.125% v/v).

The interesting point here is that the IL-8 production induced by nanoparticles started to increase after about 2 h of exposure and reached a final level (at the end of our experiment) of more than 9.5 times higher than it started. It is possible that oxidative stress caused by the sulfuric acid component loosened the cell membrane and enhanced the permeability of nickel ions to enter the cells triggering higher IL-8 production in the mixture case. Measurement of reactive oxygen species could help elucidating the cellular response mechanisms in future experiments.

CONCLUSIONS

The cytotoxicity of ultrafine airborne particles to human lung cells has been investigated in this project. The primary goals of this project were to explore the use of an inhalation approach to cells *in-vitro* and investigate the cellular responses to the exposure of particles of well-defined properties as well as from engine emissions using engine dyno-facilities available at the Oak Ridge National Laboratory. The results show that the toxicity of nanoparticles made from a binary mixture exceeded that produced from exposure to supermicrometer particles and from exposure to single-component nanoparticles.

In this study it was found that particles emitted from the diesel and gasoline engines stimulated excessive production of IL-8. We found the levels of IL-8 production by normal cells triggered by GEP or high-sulfur DEP were approximately equal. However, with primed cells, the IL-8 production increased sharply after 2 h of exposure from 3–5 $\mu\text{g}/\text{ml}$ to 30–34 $\mu\text{g}/\text{ml}$ due to the exposure to GEP. This is a sharp contrast to the exposure (to primed cells) results of DEP exposure in which the IL-8 level increased immediately after exposure and remained at 18–27 $\mu\text{g}/\text{ml}$ till the end of the experiment.

The cell exposure technique and apparatus worked reasonably well indicating that the system could be useful for studying the toxicity of mixture such as internal-combustion and atmospheric particles. The technique could also be applied as a rapid screening tool in the assessment of particle toxicity.

PUBLICATIONS AND PRESENTATIONS

¹Pope, C.A., Thun, M.J., Namboodiri, M.M., Dockery, D.W., Evans, J.S., Speizer, F.E., and Heath, C.W. (1995) Particulate air pollution as a predictor of mortality in a prospective study in U.S. adults, *Am. J. Respir. Crit. Care Med.* 151: 669-674.

²Pope, C.A., Dockery, D. and Schwartz, J. (1995) Review of epidemiological evidence of health effects of particulate air pollution, *Inhalation Toxicol.* 7: 1-18.

- ³Schwartz, J. (1993) Air pollution and daily mortality: a review and meta analysis, *Environ. Res.*, 64: 36-52.
- ⁴Oberdorster, G., Ferin, J. and Lehnert, B.E. (1994) Correlation between particle size, in vivo particle persistence and lung injury. *Environ. Health Perspect.* 102:173-179.
- ⁵Oberdorster, G., Gelein, R., Ferin, J. and Weiss, B. (1995) Association of particulate air pollution and acute mortality: involvement of ultrafine particles. *Inhalation Toxicol.* 71:111-124.
- ⁶Peters, A., Wichmann, H.E., Tuch, T., Heinrich, J., and Heyder, J. (1997) Respiratory effects are associated with the number of ultrafine particles, *Am. J. Respir. Crit. Care Med.*, 155:1376-1383.
- ⁷Stringer, B.K., Imrich, A. and Kobzik, L. (1996). Lung epithelial cells (A549) interaction with unopsonized environmental particulates: Quantitation of particle-specific binding and IL-8 production. *Exp. Lung Res.* 22: 495-508.
- ⁸Chen, D. R., Pui, D. Y. H., and Kaufman, S. L. (1995) Electro spraying of conducting liquids for monodisperse aerosol generation in the 4nm to 1.8 μm diameter range. *J. Aerosol Sci.* 26: 963-977.
- ⁹Cheng, M. D., J. M. E. Storey, T. Wainman, and T. Dam (2002) Impacts of Venturi Turbulent mixing on the Size Distributions of Sodium Chloride and Dioctyl-Phthalate Aerosols, *J. Aerosol Sci.*, 33(3): 87-98.

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