1663 Alternative Testing Strategies for Nanomaterials and Ultrafine Particles

M. Sharma. *PETA International Science Consortium Ltd, London, United Kingdom.*

Inhalation represents the primary route of exposure to aerosolized nanomaterials (NMs) and ultrafine particles in humans. The increasing use of NMs in consumer-based products warrants a thorough evaluation of their biological impacts and a need to test a large number of different types of NMs. Due to the substantive time, cost, and animals required to conduct traditional *in vivo* toxicity tests, there is much interest in developing human-relevant strategies that are less reliant on the use of animals to assess the toxicity of these materials for various risk assessment applications. This session will include presentations on *in vitro* systems that are currently being used to assess the inhalation toxicity of nanomaterials and ultrafine particles. Additionally, presenters will discuss the parameters that are critical to consider while designing *in vitro* systems and which facilitate their interpretation and application in risk assessment, including the following: dosimetry, aerosol generation and exposure, appropriate cell types, and identification of relevant endpoints. Contribution of adverse outcome pathways (AOPs) to experimental and regulatory toxicology of NMs and strategies for the development of AOPs, as well as associated issues and limitations, also will be discussed. By discussing the aforementioned parameters, this session will provide an insight into the factors that should be considered to increase the ability of *in vitro* methods to predict human outcomes eventually leading to their use in regulatory decision making.

1664 Integrated *In Vitro-In Vivo* Models for Nanomaterial and Ultrafine Particle Toxicity Testing: Moving from a Screening Hazard Tool to Predictive Models for *In Vivo* Adverse Effects

T. A. Stueckle[1], D. C. Davidson[1], V. Kodali[1], T. Kornberg[1], Y. Rojanasakul[2], and L. W. Rojanasakul[1]. [1]*NIOSH, Morgantown, WV; and* [2]*West Virginia University, Morgantown, WV.*

Engineered nanomaterial and ultrafine materials (ENMs) are revolutionizing a diverse spectrum of commercial and industrial sectors with improved or novel technologies. Animal inhalation studies report ENM deposition in the deep lung with distinct disease risks; however, they are time- and resource- consuming and cannot be used to assess each ENM. With the large diversity and complicated life cycles of ENMs, there is no consensus on how to rapidly screen associated hazards and define risks for occupational and public exposures. Several regulatory bodies have urged researchers to develop alternative approaches and integrated *in vitro-in vivo* effect models to press the question: Can *in vitro* models predict *in vivo* effects? Here, we examine several *in vitro* models that show evidence for predictability of *in vivo* damage, fibrosis, and tumorigenesis responses using scaled, realistic pulmonary exposure doses for several ENMs. Single- and multi-walled carbon nanotubes (CNT), and cerium oxide are known to penetrate into lung interstitium and induce interstitial fibrosis. Using scaled mass dose per alveolar surface area, these ENMs stimulate fibroblast proliferation, collagen production, and a fibroblast stem cell-like phenotype that correlate with *in vivo* effects. Furthermore, the use of co-culture models has improved the understanding of how the inflammatory response mediates fibrosis development. Since some CNTs possess properties similar to known carcinogens, long term, continuous exposures were tested *in vitro* and resulted in neoplastic or malignant transformation that correlated well with *in vivo* effect. CNT-transformed human lung bronchial epithelial cells exhibited elevated cancer hallmarks, proto-oncogene signaling, and evidence of cancer stem-like cells, consistent with known lung cancer signaling and established clinical biomarkers. Recent expansion of this approach to nano-sized metal oxides suggested that *in vitro* human cell models have potential as a useful tumorigenesis screening tool. In summary, development of integrated *in vitro-in vivo* approaches to assess ENM and ultrafine particle toxicity will fill key knowledge gaps and allow development of predictive *in vitro* models. *Disclaimer: The views in this abstract are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.*

1665 Predictive 3D Lung Models to Assess the Toxicity of Inhaled Nanoparticles

B. Rothen-Rutishauser. *University of Fribourg, Fribourg, Switzerland.*

The lung is considered by far the most important portal of entry into the human body for aerosolized nanoparticles (NPs) released into the environment from combustion-derived processes, or, in an occupational setting via the use of NPs-containing consumer products, such as aerosol sprays. Although animal testing is still the most prevalent model used for risk assessment of inhaled nanoparticles, intensified efforts have been made during the last years towards a systematic development and evaluation of innovative and more reliable *in vitro* (human) lung cell models. Such models provide standardized and reproducible tools for high-throughput screening but also allow investigating mechanistic studies of inhaled nanoparticles at the single cell level. There are numerous *in vitro* lung models described to evaluate the human pulmonary epithelial tissue barrier but the choice of the model and the mode of exposure depends on the relevant scenario to be studied. These models range from simple mono-cultures to highly sophisticated 3D models, involving a combination of relevant cell types. Furthermore, air-liquid interface (ALI) exposure is more realistic towards mimicking *in vivo* conditions in the lung than the suspension exposure. A dose-controlled deposition of various nanomaterials at the ALI of cultured lung cells is therefore preferred. The advantage of such an approach is that the material characteristics can be fully controlled by monitoring the mass deposition on the lung cell surface on-line, allowing to produce a dose-effect correlation. Current research is ongoing to optimize such *in vitro* tests combining 3D lung models with ALI systems to predict the development of pulmonary diseases such as fibrosis following long-term exposures to aerosolized carbon nanotubes. Such an approach will help to address the regulatory safety testing requirements for inhaled nanomaterials while reducing the use of animals for this purpose.

1666 Contemporary Considerations in Engineered Nanomaterial Characterization, Aerosol Generation, and Exposure

C. M. Sayes. *Baylor University, Waco, TX.*

Traditional hazard testing models using animal inhalation chambers are time and financially expensive. Cell culture models have shown promise as surrogates in screening assays when multiple substances require preliminary information on toxicity. There is also a growing need for development of representative aerosol exposure systems that use a cell-based lung model to simulate inhalation exposure and assess potential hazards associated with aerosolized materials in a rapid and cost-effective manner. This talk presents two aerosol exposure apparati: a settling chamber and a gentle impactor. Both have been demonstrated as a system capable of exposing cells in culture at the air-liquid interface using gravimetric settling and impaction as aerosol delivery mechanisms. These aerosol exposure apparati are composed of two critical parts: (1) an aerosol concentrating chamber that delivers liquid and/or solid aerosols in the size range of 1–3 μm in mass median diameter and (2) an exposure chamber where co-cultured cells are maintained at the air-liquid interface during exposure. The aerosol used in the development of this system was mineral oil mixed with sodium fluorescein that served as a surrogate for known occupational respiratory toxicants. The settling chamber was constructed with simple design specifications but exhibits a low deposition efficiency (8%). It has also proved to be a challenge when increasing dosages of same-sized aerosols by altering aerosol input to the system; this occurred due to aggregation at high aerosol concentration in the chamber. Due to the limitations of the settling chamber an alternate system, the gentle impactor, was developed. The gentle impactor requires a more sophisticated design based on aerosol dynamics of target size for the droplets to be delivered. The delivery is more efficient, which leads to a shorter exposure duration for the same dosage as compared with the settling chamber. It also enables consistent exposure to the same sized aerosols. From these results, depending on the needs of the experiment, either the settling chamber or the gentle impactor show promise as a high-throughput exposure tool that could be used in conjunction with other current cell-based biological test models.

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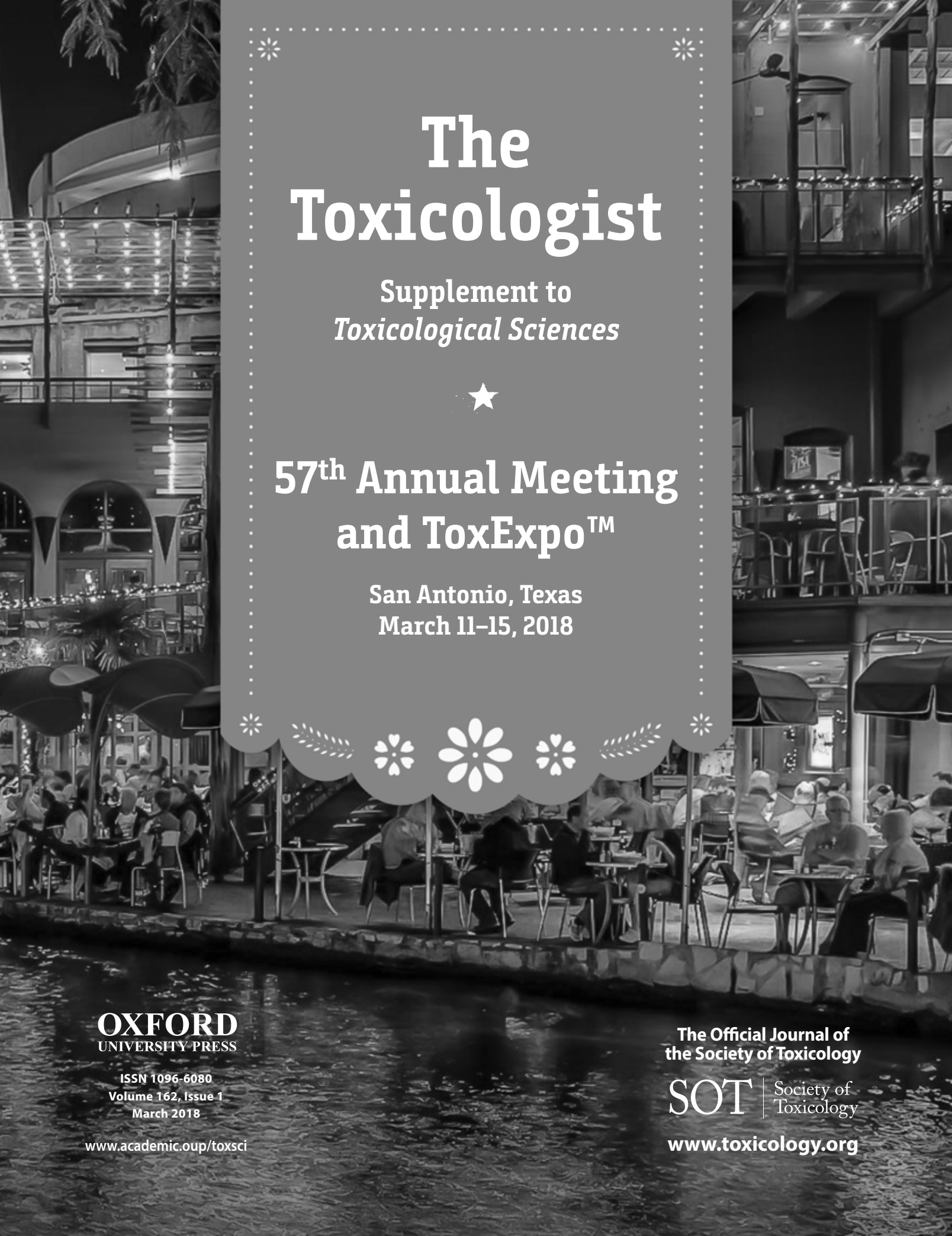

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