D05-069

Improve Understanding the Risks of Nanomaterials through Integrated Research

OAK RIDGE National Laboratory

Understanding Risks of Nanoscale Materials and Structures

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Key Scientific Questions

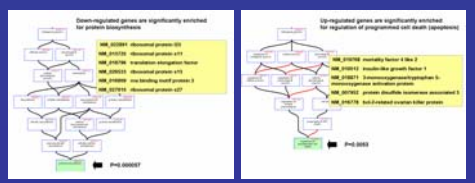
- What attributes of nanomaterials/ nanostructures contribute to biological effects?
- How would these attributes change in the environment and/or physiological fluids?
- What are the health consequences of surface modification on nanomaterials (e.g., material synthesis, decoration, air chemistry, environmental processing, and so on)?
- What are the cellular and physiological mechanisms of health effects of the nanomaterials (biological responses)?

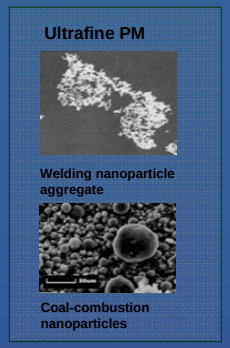
Focuses of this Project

- Survey current techniques for nanotoxicology evaluation and risk assessment
- Physiological, immunological, and genetic responses to exposure of precisely fabricated metal oxides and carbon nanoparticles
- Method development for controlled generation of aerosol aggregates for inhalation exposure experiments
- To improved understandings of carbon nanoparticles formation during production

Take Home Messages

- Limited toxicological knowledge hampers distinction of ultrafine nanoparticles (as in waste products) from engineered nanoparticles (as in commodity), which is a major problem.
- Nanoparticles aggregation hinders precise exposure experiments, but the problem is mitigated with newly developed exposure method by this LDRD project.
- Aggregates can be engineered via aerosol route for precision inhalation exposure experiments using either ultrasonic and or electrospray generation techniques in conditions suitable to cell or animal studies.
- Benign nanomaterials could trigger severe biological responses, if modified properly as shown by this project. *Conversely (not studied in this work), a toxic nanomaterial can be biocompatible if we know what to treat it.*
- Aggregated carbon nanohorns (CNHs) appear to be biocompatible; however, nanohorn singlets do exist and their toxicological properties wait to be evaluated. Carbon nanotubes however can be oxidized and appear to retain oxidants (measured by ROS) on their surface. Inhibition of cellular responses was achieved by using antioxidants.
- Genomic profiling shows multiple active regulations (up or down) of mouse cell lines upon short-term exposure to undecorated CNH aggregates; however, link to phenotype is yet to be established.

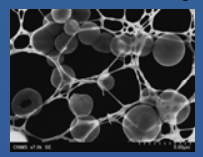




The images show that the primary units in each photo are all ≤ 100 nm in size/diameter, but the ultrafine PM group has ***NOTHING IN COMMON*** to those in the "engineered group" except their size.

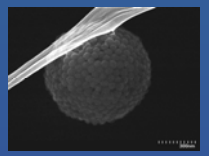
"Nature uses only the longest threads to weave her patterns, so each small piece of her fabric reveals the organization of the entire tapestry." - Richard Feynman

Ultrasonically Generated PSL

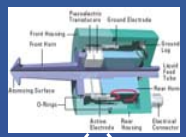


Large aggregates - in agreement with SMPS/APS data

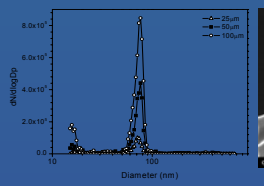
Zoom in on one aggregate



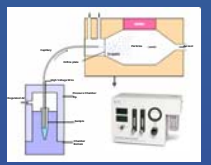
Aggregates composed of individual PSL nanoparticles. Particles are not completely fused together.

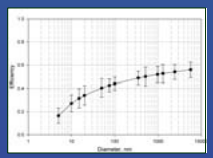


Electrospray of PSL



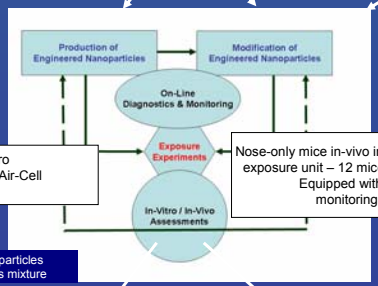
- Electrospray produces primarily single PSL nanoparticles.
- Dispersion is high with little to no aggregation, but counts are low compared to Tri-jet and Ultrasonic methods (possibly due to non-optimal operating conditions).
- This method is useful for studies of biological impact of discrete nanoparticles.
- Key technology for precision particle generation.



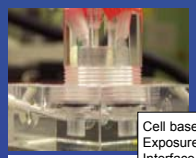


Mass transfer efficiency as a function of monodisperse particle diameter of a single transwell exposure unit

Nano-Toxicology Evaluation



Array of 24 Cellular Exposure Units

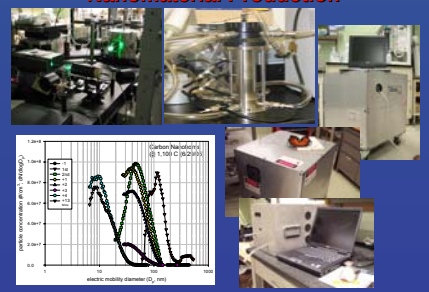


Cell based in-vitro Exposure using Air-Cell Interface Device

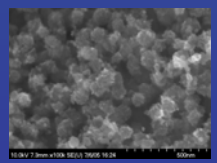
Nose-only mice in-vivo inhalation exposure unit – 12 mice system Equipped with on-line monitoring systems

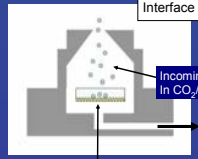
Nose-Only 12-mice Inhalation Apparatus

On-line *In-situ* Characterization of Nanomaterial Production



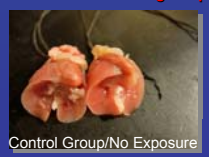
Untreated Carbon Nanohorns



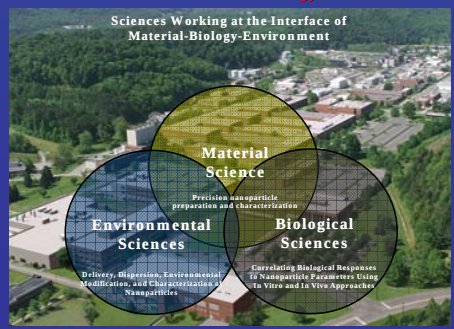


Adherent cells cultured 48 hrs on polycarbonate transwell membrane, 0.4 μm pore size, *prior to* exposure

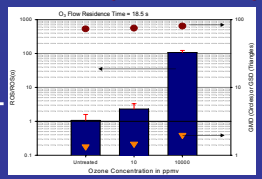
Mice Lungs Exposed to CNHs



Future Center for Nanotoxicology Research

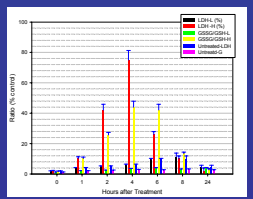


Acellular Assay of Material Change



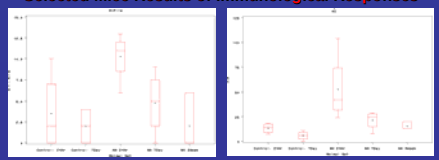
Reactive oxygen species significantly increased, particle peak diameter slightly increased, and size dispersion increased also indicating potential surface, possibly morphological alterations by ozone reaction.

Cellular Detection of CNHs Impacts



Cell membrane breakage peaked after 4 hrs of exposure to treated CNHs. Oxidative damage followed a similar pattern. Ozone was removed by a KI coded denuder sequence during exposure. Ozone-zero CNHs did not cause detectable cellular variations compared to the control (HEPA air exposed) case.

Selected Mice Results of Immunological Responses



A number of proteins and cytokines were assayed. Significant statistical fluctuations were found in the data points taken at different time points after exposure. MIP-1α and KC were shown here to indicate the time courses of expressions in responses to pluronic-coated CNHs exposures.

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