



MODEL FOR ASSESSING CARBON NANOPARTICLE INHALATION EXPOSURE RISK:

A Protocol for Combustion Emission Ultrafine Particulate Testing

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Nanoparticle Characterization

I. Morphology: nanoparticle size & shape, uniformity, surface defects
Microscopy: TEM, HRTEM
Field Emission SEM

II. Nanoparticle Dispersion/Agglomeration/Aggregation
Microscopy, UV-Vis, dynamic light scattering

III. Surface Reactive groups & Charge: FT-IR, Zeta analysis

IV. Elemental Composition & Concentration:
X-ray microanalysis, UV-Vis

Correlate specific nanoparticle physicochemical characteristics with human cell exposure responses, to reveal specific nanoparticle characteristics associated with exposure risks via inhalation.

Tox Data

Human Cell Cytotoxicity Testing of Nanomaterials

Cell Monolayers: Grown on coverslips in shell vials. Used at 95-98% confluency

Characterized Nanoparticles added to monolayers.

Vials incubated 37°C with rotary mixing (swirling to distribute nanoparticles).

LM & SEM to verify cell structure & confluency

Media removed. 2 PBS washes

Media analyzed for cells, nanoparticles & debris

Lung Monolayers removed

Glutaraldehyde fixation → TEM

SEM

Critical Point dried Lung control (normal) monolayer cells.

Human Airway Cells exposed 3 hr to Cleaned/Cut Carbon nanotubes (SWCNTs)

Viability studies

Light Microscopy Histochemistry: vital staining (apoptosis, necrosis...)

Simulated Aqueous Environmental Exposure Testing:

Nanoparticle (SWCNTs) characterization following aqueous exposure to:

Natural Organic Matter (NOM)

Phosphate buffered saline (PBS)

Same SWCNTs aqueously aged in ultrapure water (~18MΩ.)

HRTEM images showing SWCNTs embedded in NOM, on cell surface and within cells (red arrows).

At 3 hrs exposure cleaned nanotube ropes were seen within phagosomes and cells.

Carbon nanoropes (red).

Cell Surface

Nano

Cell Surface

CNTs within cells (arrows).

Acid/peroxide Cleaned SWCNTs produced less Cell Death Following Exposure to pH Neutral Saline (PBS) and Natural Organic Matter (NOM)

Lung Cell Viability following 3.5 hr exposure to Acid/Peroxide cleaned Carbon Nanotubes

Legend: Saline (S)-AIP CNT, Only NOM, NOM-treated AIP CNT, AIP CNT Only (CNT), Normal cells (C, Control)

FTIR of acid/peroxide cleaned SWCNTs: After 1 month exposure of acid/peroxide cleaned SWCNTs in sterile phosphate buffered saline (PBS), the red trace (f) shows the presence of hydroxyl and other surface groups. However these PBS-treated SWCNTs produced no increase in cellular necrosis (death) compared to normal control values.

Testing Biomass Combustion Emission Ultrafine Particulates:

Portable Thermal Precipitator (Bang & Murr, 2006)

Figure 1. Thermal precipitator design and calibration. (a) A schematic diagram showing general features of operation. (b) TP with cover. (c) A standard demonstration grid for burning paper smoke particle collection.

Field collection and testing of UFPs will provide answers to:

- How similar are UFPs released into the environment to those released within the burner?
- Do UFPs collected in the environment show altered morphology? agglomeration/aggregation
- Do environmentally released UFPs show reduced surface reactive groups? Does this reduce risk?
- Following exposure to surface waters containing NOM, salt, etc... do UFPs exhibit different characteristics, or reveal altered interactions with lung airway cells reducing risk?

- Collect / Characterize Ultrafine Particulates (UFPs) from within the biomass burner flue, as well as UFPs released at the burner exit flue. Compare UFPs released from these two sites [morphology, surface reactive groups, elemental & amorphous composition, dispersion/aggregation, etc...].
- Collect sufficient UFPs (μg) for rapid *in vitro* human airway cell testing, to determine viability, binding and intracellular UFP fate.
- Collect UFPs from different advanced technology burners to compare type, quantity and biological airway cell risk potential of emissions.
- Collect UFBs from the same advanced technology burner during combustion of different biomass fuels to evaluate physico-chemical and cytotoxicity characterization of each type of biomass-composition UFB emission.