Standard Dose Measurement for Nanomaterials: What to Include in Exposure and Toxicity so that We Can Bound Dose Estimates for Safety?

Christie M. Sayes, PhD
Baylor University
Email: christie_sayes@baylor.edu
Conflict of Interest Statement

- I have no conflicts to declare.
Objectives

1. **Introduce** analytical methods to assess exposures

2. **Present** challenges associated with relating nanomaterial dose (*in toxicology*) to nanomaterial concentration (*in products*)

3. **Justify** using a “mixtures toxicology approach” in nanotoxicological research

*Sayes CM, et al; unpublished*
1. Analytical Methods Used to Assess Exposures

![Diagram of analytical methods]

- X-ray
- MS-MS
- Gas & liquid chromatography
- Nano-infrared
- Hyperspectral imaging
- Electron microscopy
- Ultraviolet & infrared spectroscopy
- Fluorescence & optical microscopy
- Dynamic light scattering

Life cycle considerations
Modeling exposure in various media
Assess dose & response, then identify hazards

Have they transformed?
How many? What type?
Are there any present?

More specialized information
Instrument availability

Graphic adapted from Dr. Souhail Al-Abed, USEPA
1a. Example of Life Cycle Considerations

- Studies are designed to ask how the nanoparticles have transformed
  - Arguably one of the most challenging questions in nano-EHS to address

- Specialized (and multiple) instruments are used
  - Thus, limiting the amount of data available for dose metrics

- Sample preparation is the key to detect, identify, and quantify
  - We extract, disperse, plate and each of these actions creates a different entity compared to what the model system will see
  - Variation in sample prep can mean different labs can produce different results
1a. Example of Life Cycle Considerations: 
A case study for nano-enabled coatings on drywall

Electron micrographs of pristine TiO$_2$ NPs

Paint formulation process

“Wear-and-tear” process

Electron micrographs of powders “worn-and-torn”

1b. Example of Modeling Exposure in Various Media

- Studies are designed to ask *how many (and what type of) nanoparticles* might be in sample
  - The literature has many examples of measuring (or re-measuring) physiochemical properties in physiological & environmental matrices

- Usually, *multiple instruments are used in tandem*

- Data collected is most relevant for *extrapolating exposure concentration to biological dose*
1b. Example of Modeling Exposure in Media: Hyperspectral imaging of HepG2 cells exposed to gold nanoparticles

Fluorescence imaging of cells stained for nucleus (BLUE), mitochondria (RED) and cytoskeleton (GREEN)

This image was used to find regions of interest (ROIs) in the cells

Two ROIs were acquired to show the spectral difference between the same NP-type penetrating two different cells

In Area 1, the cell shows signs of increased reactive oxygen species (ROS)

In the darkfield view and spectra, the concentration of NPs in Area 1 is higher than that of Area 2

Scale bars represent 100 μm

1b. Example of Modeling Exposure in Media: NP protein corona formation varies depending on surface charge

Electron micrographs of Au NPs

Mass spectrometry analysis of protein-coated Au NPs

1c. Example of Assessing Dose-Response

- How do we ascertain dose?
  - Researchers must ask the question, “Did we really deliver the dose we intended through serial dilutions?”
  - The answer is useful for dose range finding, weight of evidence evaluations, and accurately reporting specific dose-response relationships for a specific nanomaterial
  - More discussion (and teaching and learning) is needed in the community

- Studies are designed to **measure effects after exposure**

- **Requires a known concentration** at the beginning of the study
  - Often, serial dilutions of the known concentration are used to report dose

- **Most studies are incomplete**, but are useful when prioritizing immediate next steps
1c. Example of Assessing Dose-Response, Then Identify Hazards:

Degree of bacterial growth inhibition after nanoparticle treatment, over dose

Blue dots indicate measures of inhibition from negatively charged NPs (Cit-AgNP and AA-CuNP)

Red dots indicate neutrally charged NPs (PVP-AgNP and PVP-CuNP)

Black dots indicate positively charged NPs (CTAB-AgNP and CTAB-CuNP)

Trend lines indicate the best fit regression

Shaded areas represent the 95% confidence interval.

Sayes CM, et al; unpublished
2. Challenges Associated with Relating Nanomaterial Dose (in Toxicology) to Nanomaterial Concentration (in Products)

**Translating data to “Weight of Evidence”**
- WoE is a systematic approach to evaluate the totality of evidence to assess the support of a particular conclusion
- Can scientific data be transformed?

**Read-across studies to decrease uncertainty**
- Read-across is a technique for predicting endpoint information for one substance by using data from the same endpoint from another substance
- Can scientific data be extrapolated?

**Concentrations in food and pharmaceuticals**
- For the gut: pH, mechanical forces, mucus layers, are difficult to capture in vitro
- Similarly for the lung: inoculation at the liquid-liquid interface is different than aerosolization at the air-liquid interface
- Does the exposure method induce differing results?
2a. Translating Data to “Weight of Evidence”: Stress-Induced Mitochondrial Deformation is Predicated on Cell Phenotype

Weight-of-evidence (WOE) approach for nanoparticle characterization using multiple lines of evidence (LOE) to determine size and composition


Weight-of-evidence (WOE) approach for nanoparticle exposure using multiple lines of evidence (LOE) to determine mitochondrial effects
2b. Read Across Studies to Decrease Uncertainty:
Physical, chemical, toxicological characterization of sulfated cellulose nanocrystals using in vivo and in vitro strategies


- Strategy includes assessment of materials side-by-side with simulated digestion, mimicking conditions that occur along the gastrointestinal tract as well as intracellularly
- Useful tool to evaluate impact of physical or chemical changes to CNC after oral exposure as future commercial forms are developed and tailored
2c. Concentrations in Food and Pharmaceuticals: Amorphous silica nanoparticle aerosolization for ALI exposures

Does the exposure method induce differing results?
Here, we compare deposited mass of mineral oil aerosols after exposure via gravitational settling (total mass deposited after 15 min = 2,126 ng) vs. gentle impaction (150,000 ng)
A “Mixtures Toxicology” Approach is Relevant for Nanotoxicology: Studies can be designed as equimolar or equipotent ratios and as either a constituent mixture or as part of a formulation.

<table>
<thead>
<tr>
<th>Equimolar ratio</th>
<th>Equipotent ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>50:50 In 50%</td>
<td>50:50 In 50%</td>
</tr>
<tr>
<td>70:30 In 50%</td>
<td>70:30 In 50%</td>
</tr>
<tr>
<td>30:70 In 50%</td>
<td>30:70 In 50%</td>
</tr>
</tbody>
</table>

**Constituent mixture**

- Equimolar ratio: 50µM, 70µM, 30µM
- Equipotent ratio: 65µM, 90µM, 50µM

50% formulation

- Equimolar ratio: 50µM, 25µM, 15µM
- Equipotent ratio: 50µM, 20µM, 5µM
3a. Example of Mixtures Approach:
Synergistic cytotoxicity of disinfection byproducts against human intestinal and neuronal cells

The LC\(_{50}\) measured was compared to predicted using CA model (Berenbaum 1985a; Stalter et al. 2020) where \(n\) refers to the number of components in mixture; \(P_i\) represents the fraction, \((\sum P_i = 1)\)

\[
LC_{50,\text{mixture}} = \frac{1}{\sum_{i=1}^{n} \frac{P_i}{LC_{50,i}}}
\]

The error of prediction \((\sigma LC_{50, \text{mixture}})\) was propagated from experimental StDev of LC\(_{50}\) \((\sigma LC_{50,i})\)

\[
\sigma LC_{50,\text{mixture}} = \sqrt{\sum_{i=1}^{n} \left(\frac{LC_{50,\text{mixture}}^2 \times P_i^2}{LC_{50,i}^2}\right) \times (\sigma LC_{50,i})^2}
\]

- PbNPs exhibited higher cytotoxicity than the other 2 chemicals against human intestinal and neuronal cells
- A ranking can be drawn based on LC\(_{50}\) values calculated from dose-response curves
- PbNPs have different degrees of synergistic effect when co-exposed to cells with the another chemicals

Concentration (mM)
0.001 0.01 0.1 1 10 100
Viability (%)
0 20 40 60 80 100
PbNPs CuNPs Glyphosate

\[
\sigma \frac{LC_{50}}{m,m} = \frac{\text{StDev of } LC_{50}}{\text{StDev of } \sum \sigma P_i}\]

The LC\(_{50}\) measured was compared to predicted using CA model (Berenbaum 1985a; Stalter et al. 2020)

\[
LC_{50,\text{mixture}} = \frac{1}{\sum_{i=1}^{n} \frac{P_i}{LC_{50,i}}}
\]

where \(n\) refers to the number of components in mixture; \(P_i\) represents the fraction, \((\sum P_i = 1)\)
Summary

- There are a variety of analytical methods available to help assess exposures
  - Dosimetry is an important consideration in nanotoxicological research
  - Every study ought to include assessment of dosing concentration and target dose to model system
    - It is critical to compare these doses used in toxicology studies to real-life doses in real-life scenarios

- The challenges associated with relating nanomaterial dose (in toxicology) to nanomaterial concentration (in products) are being addressed
  - Methods, tools, and techniques are available
  - Examples (through specific cases studies) exist in the literature

- A mixtures toxicology approach in nanotoxicological research is needed
  - Engineered nanomaterials for which we are exposed to are inherently mixtures and ought to be considered as such when assessing, hazards, exposures, and risks.
References


Acknowledgements

“Emerging Technologies & Environmental Health” Laboratory

Funding & Support

- Air Force Research Laboratory (AFRL)
- United States Department of Agriculture (USDA)
- Vireo Advisors LLC and National Institute for Standards and Technology (NIST)
- Gus Glasscock, Jr. Endowed Fund for Excellence in Environmental Sciences