



## **SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety**

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

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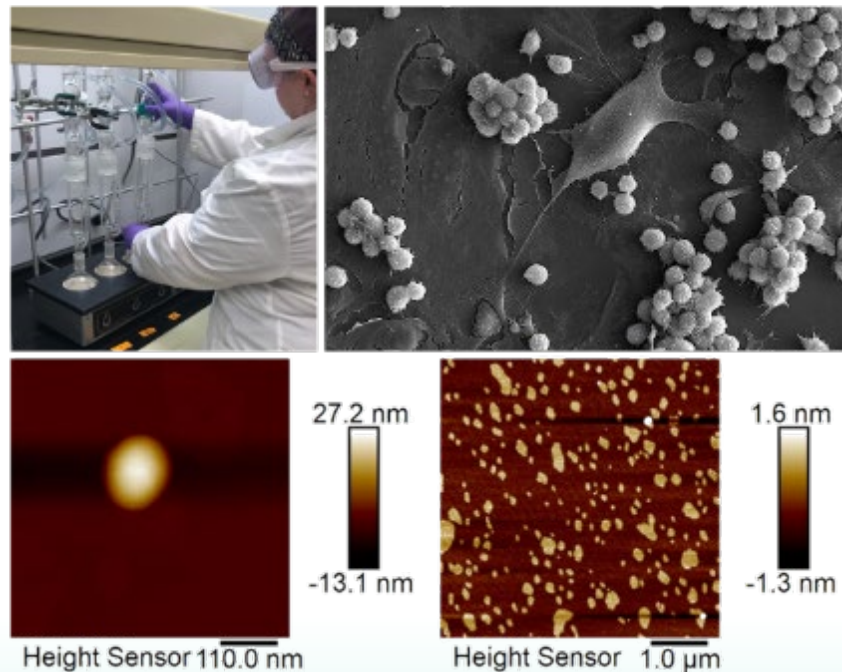
# Conflict of Interest Statement

- I have no conflicts to declare.



# Objectives

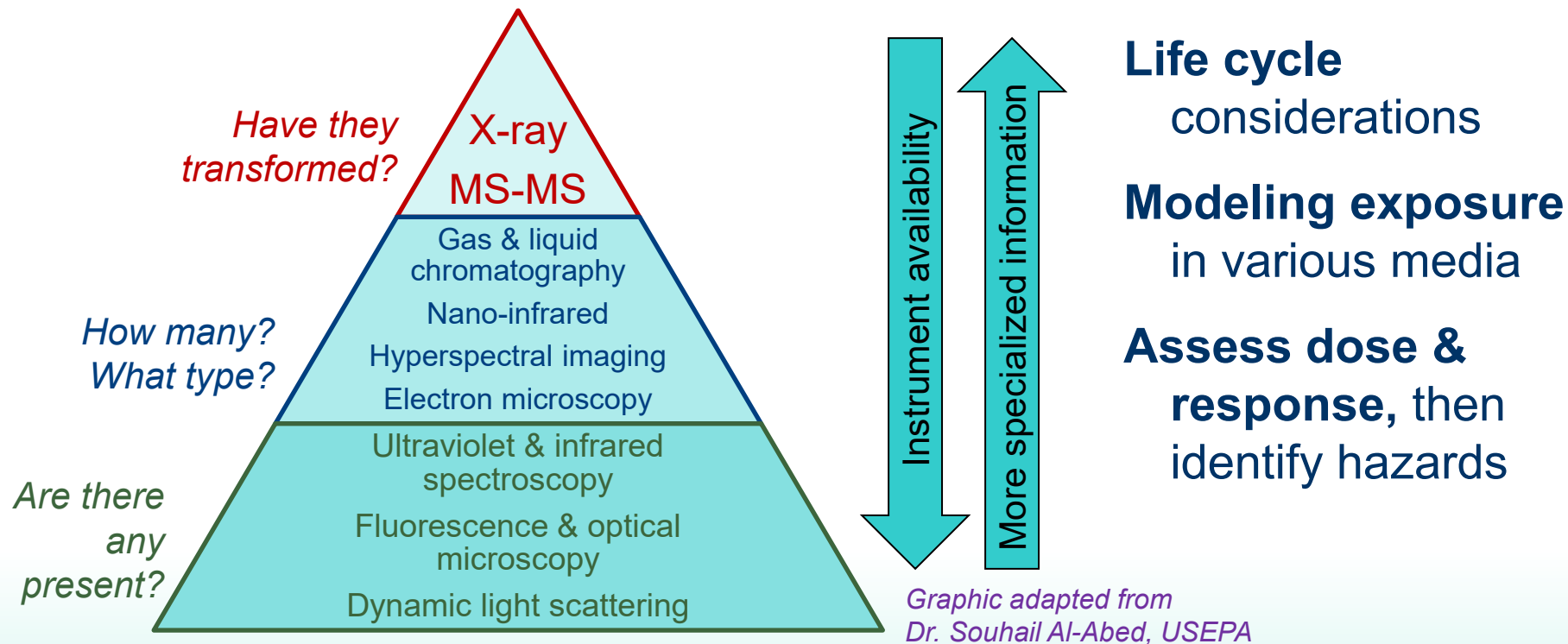
1. **Introduce** analytical methods to assess exposures
2. **Present** challenges associated with relating nanomaterial dose (*in toxicology*) to nanomaterial concentration (*in products*)
3. **Justify** using a “mixtures toxicology approach” in nanotoxicological research



*Sayes CM, et al; unpublished*



# 1. Analytical Methods Used to Assess Exposures



# 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*

Sayes CM, Rothrock GD,  
et al. (2013) InNSTI-  
Nanotech 3:742



Electron micrographs of  
pristine TiO<sub>2</sub> NPs

Paint formulation process

“Wear-and-tear”  
process

Electron micrographs of  
powders “worn-and-torn”

# 1b. Example of Modeling Exposure in Various Media

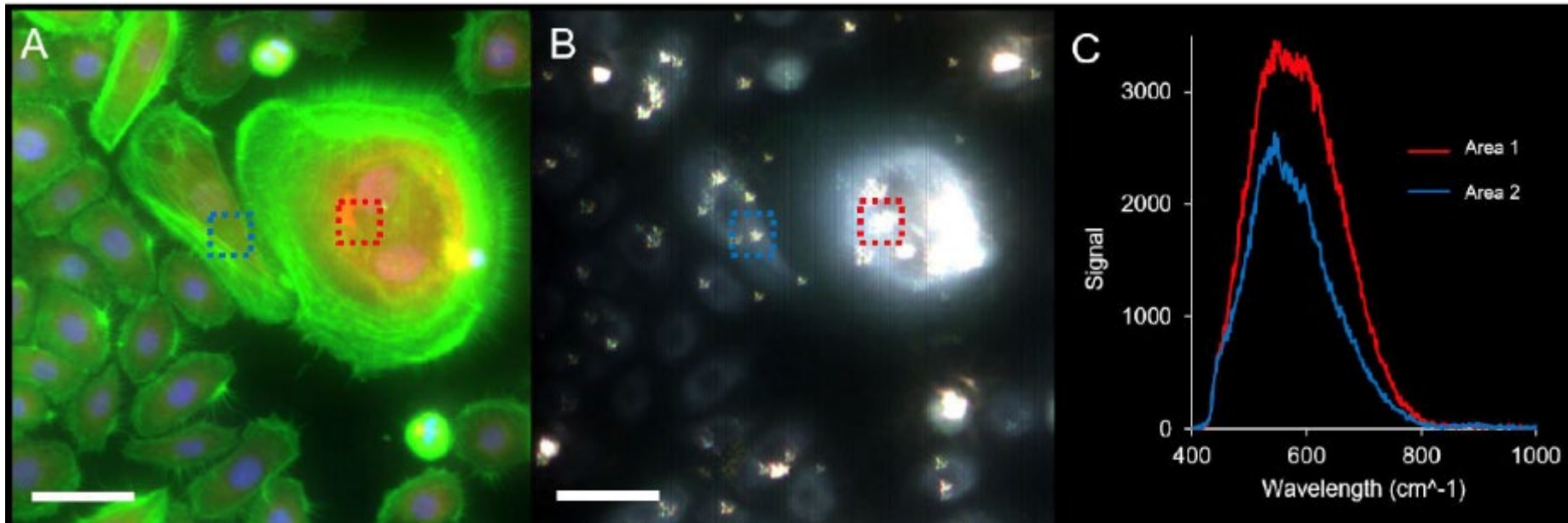
- Studies are designed to ask ***how many (and what type of) nanoparticles*** might be in sample
  - *The literature has many examples of measuring (or re-measuring) physiochemical properties in physiological & environmental matrices*
- Usually, ***multiple instruments are used in tandem***
- Data collected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

George MM, et al  
(2020). *Microscopy &  
Microanalysis*  
26(2):2748.



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

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

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

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

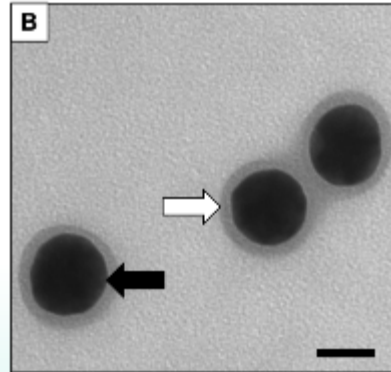
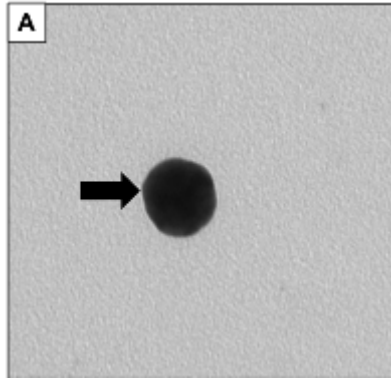
In **Area 1**, the cell shows signs of increased reactive oxygen species (ROS)

Scale bars represent 100  $\mu\text{m}$

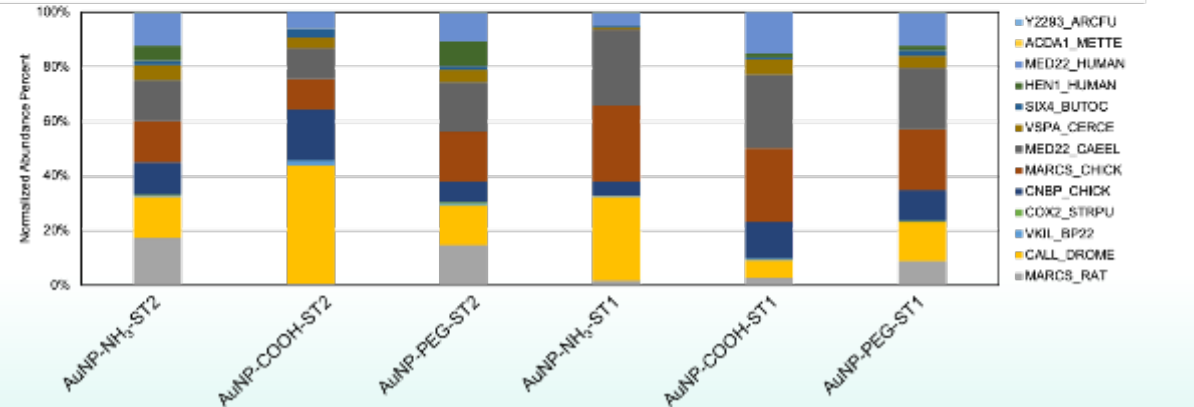
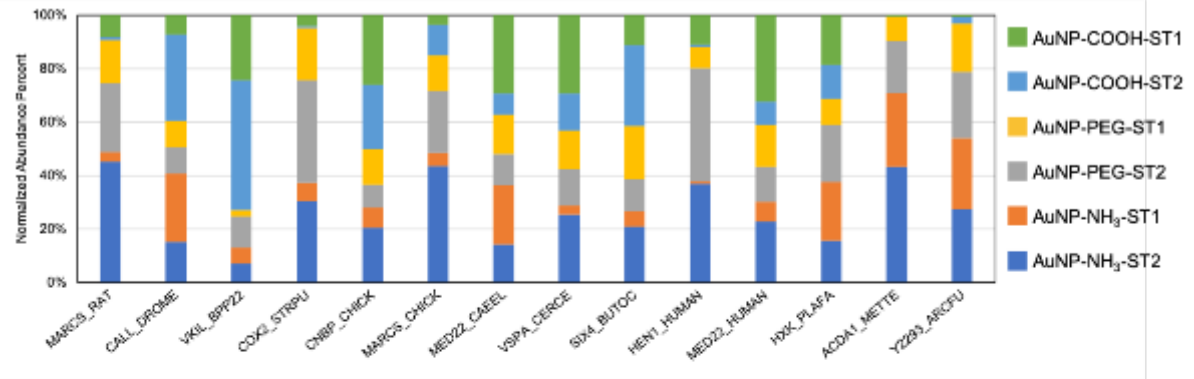
# 1b. Example of Modeling Exposure in Media:

*NP protein corona formation varies depending on surface charge*

Stewart M, et al  
(2018). *Applied Sciences* 8:2669-2684.



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

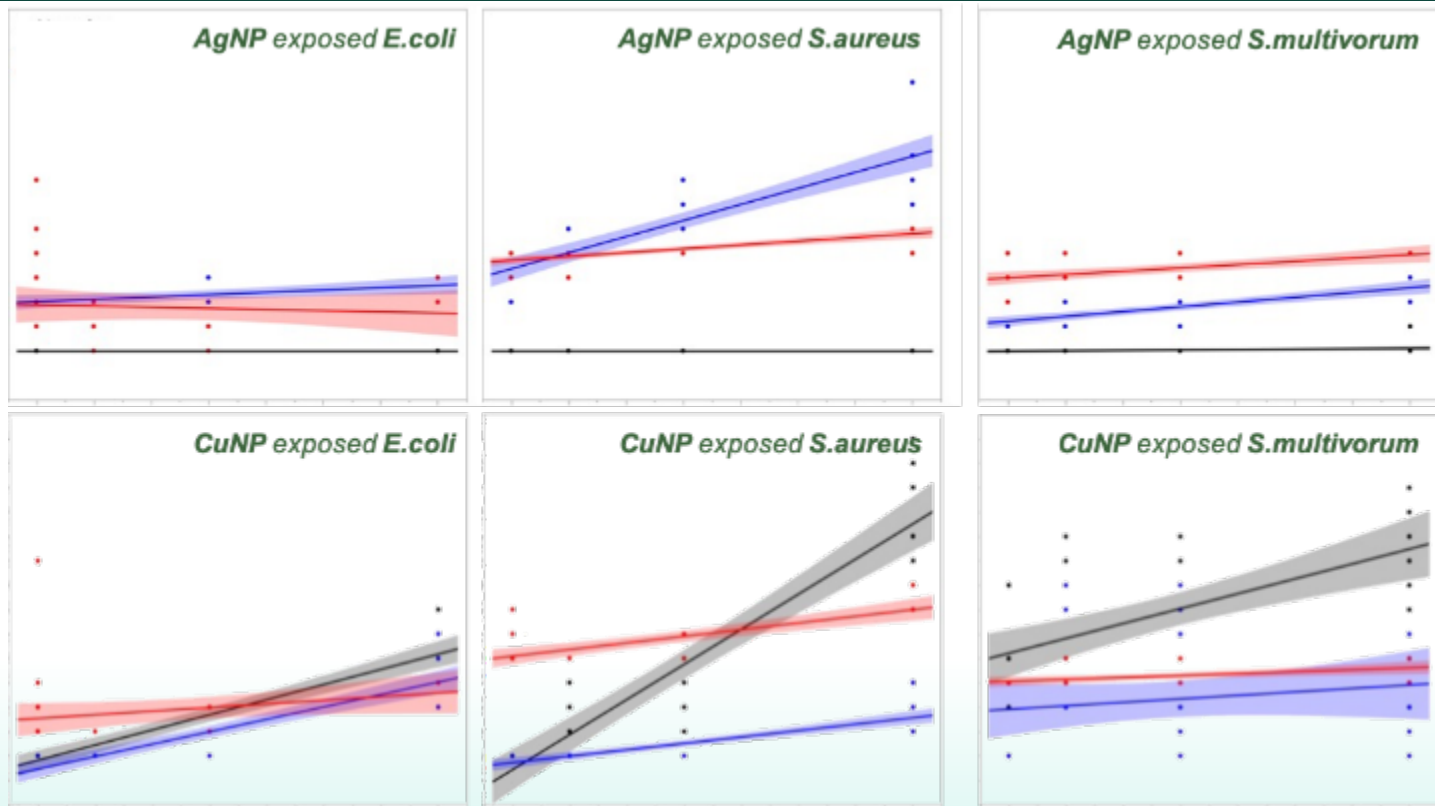


# 1c. Example of Assessing Dose-Response, Then Identify Hazards:

*Degree of bacterial growth inhibition after nanoparticle treatment, over dose*

Sayes CM, et al;  
unpublished

Bacterial Inhibition; scale 1-14 mm



Exposure Concentration; scale 1-8 μM

**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.

## 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?

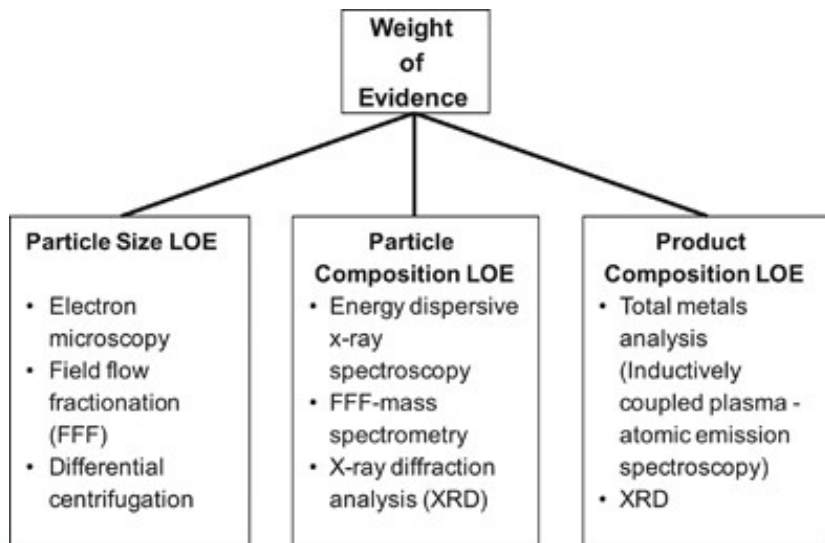


# 2a. Translating Data to “Weight of Evidence”:

## Stress-Induced Mitochondrial Deformation is Predicated on Cell Phenotype

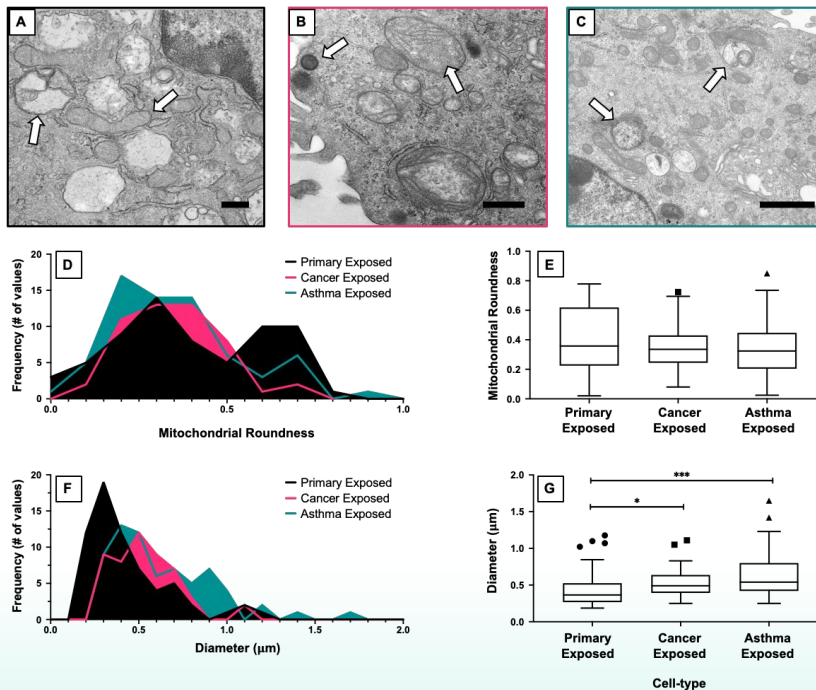
Sayes CM, et al;  
unpublished

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



Cuddy, et al (2016) *J. Exp. Sci. Environ. Epidemiol.* 25:26.

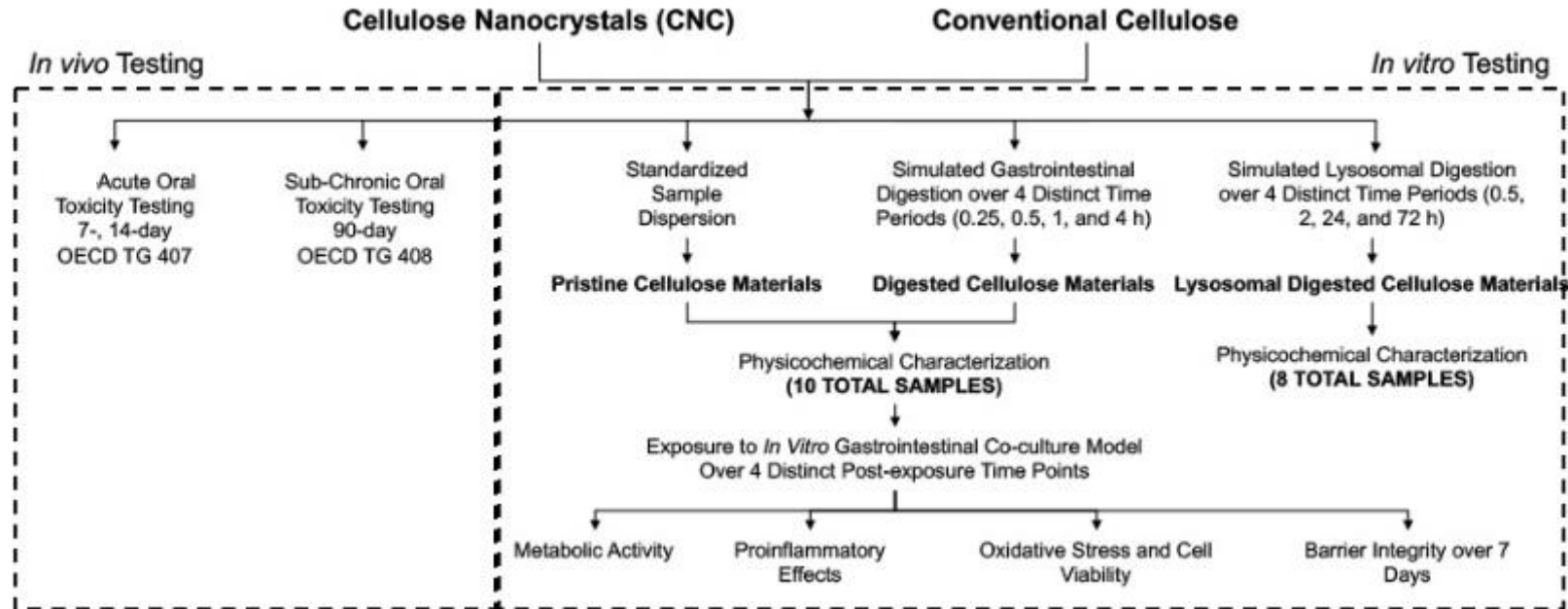
Weight-of-evidence (WOE) approach for nanoparticle exposure using multiple lines of evidence (LOE) to determine mitochondrial effects



# 2b. Read Across Studies to Decrease Uncertainty:

*Physical, chemical, toxicological characterization of sulfated cellulose nanocrystals using in vivo and in vitro strategies*

*Ede JD, et al. (2020) Tox. Res. 9(6):808-22.*



- 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

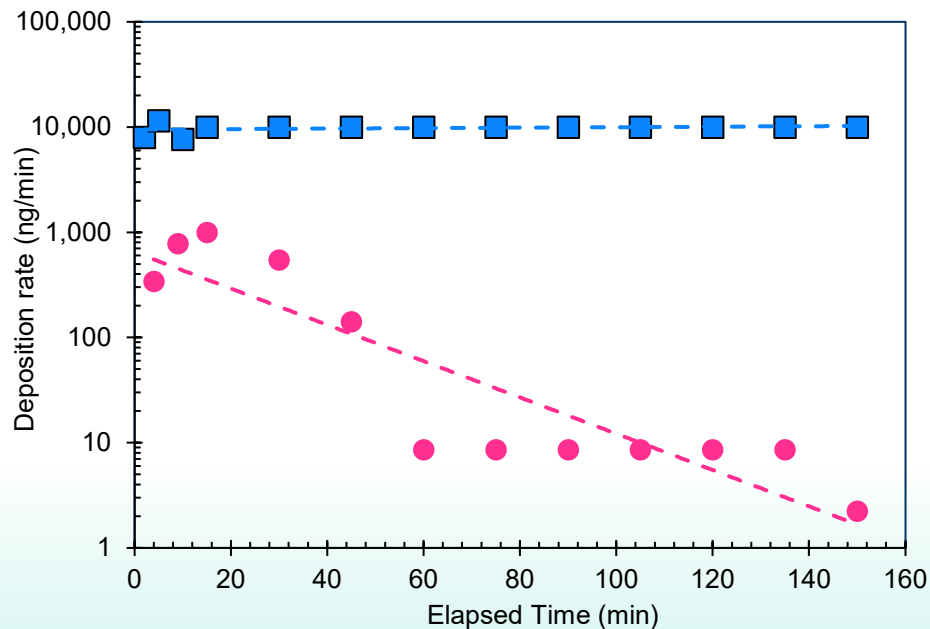
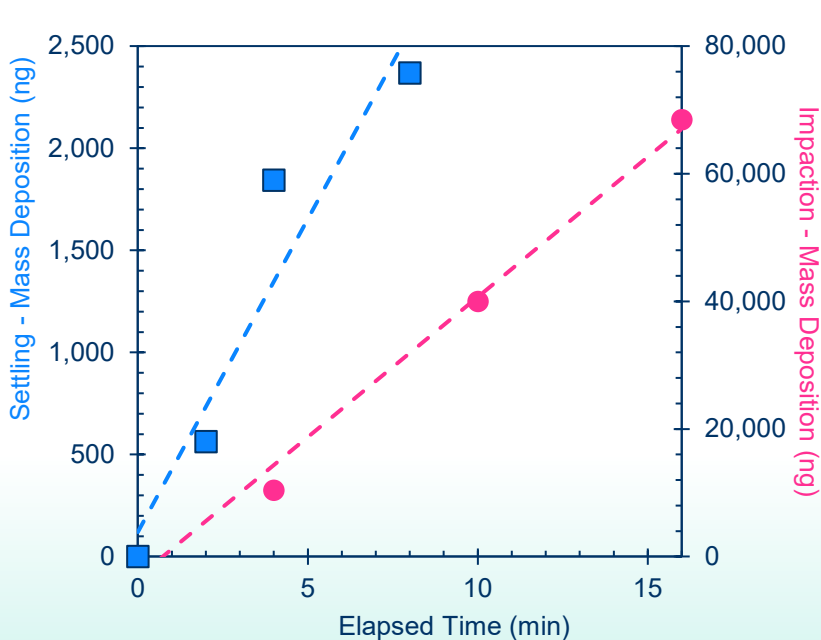
# 2c. Concentrations in Food and Pharmaceuticals:

## Amorphous silica nanoparticle aerosolization for ALI exposures

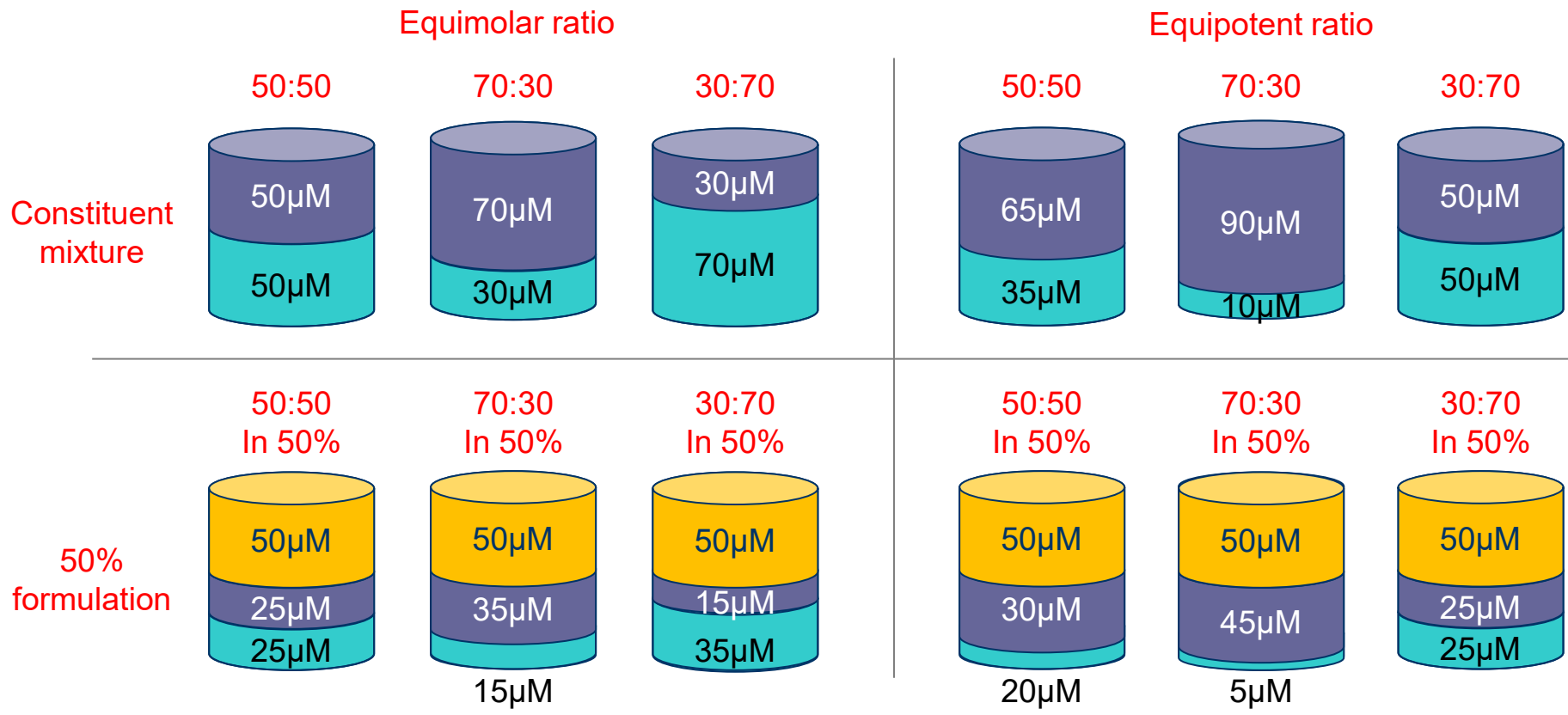
Sayes CM, Singal M. (2021). *Journal of Aerosol Science*. 151:105677.

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)



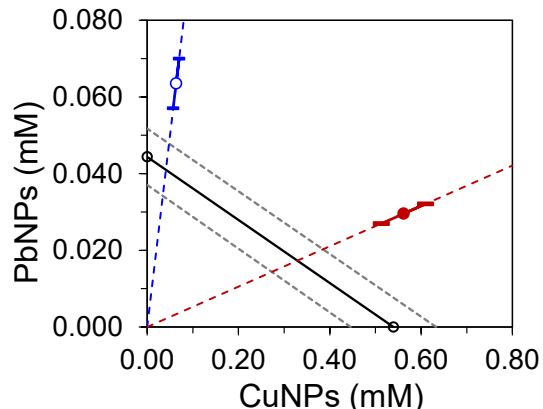
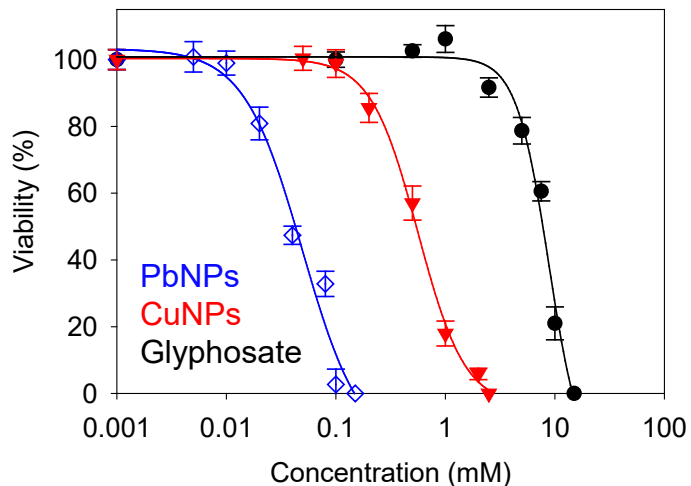
### 3. A “Mixtures Toxicology” Approach is Relevant for Nanotoxicology: Studies can be designed as equimolar or equipotent ratios and as either a constituent mixture or as part of a formulation



# 3a. Example of Mixtures Approach:

## Synergistic cytotoxicity of disinfection byproducts against human intestinal and neuronal cells

Liu J, Sayes CM, et al; unpublished



|                 |            |
|-----------------|------------|
| PbNPs + CuNPs = | Antagonism |
| PbNPs + Gly =   | Additivity |
| CuNPs + Gly =   | Antagonism |

- 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

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

$$LC_{50,mixture} = \frac{1}{\sum_{i=1}^n \frac{P_i}{LC_{50,i}}}$$

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

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

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

# 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.



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