

In Vitro Lecture and Luncheon for Students

Supported by the Colgate-Palmolive Company

Society of
Toxicology
54th
Annual
Meeting
and ToxExpo



2015



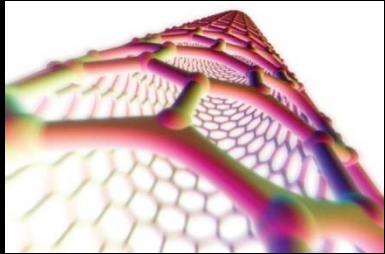
Alternative *In Vitro* Approaches for Predicting the Health Impact of Nanomaterials

James C. Bonner

North Carolina State University



Colgate-Palmolive *In Vitro* Toxicology Lecture



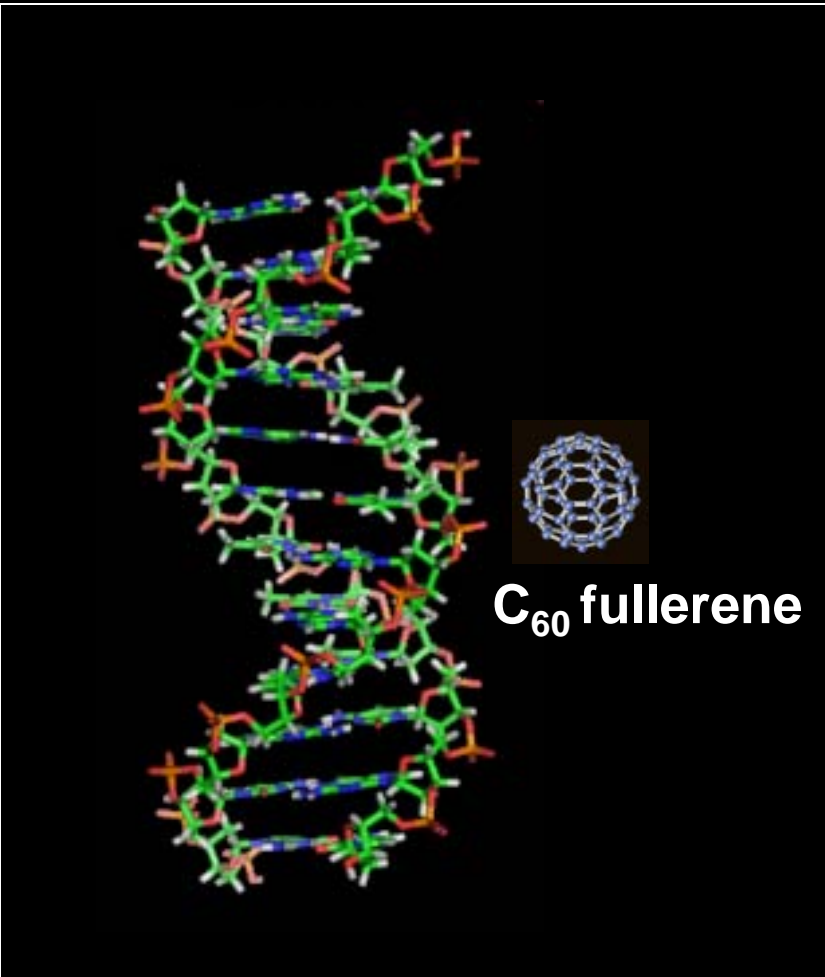
“Alternative *In vitro* Approaches for Predicting the Health Impacts of Nanomaterials”

James Bonner, PhD

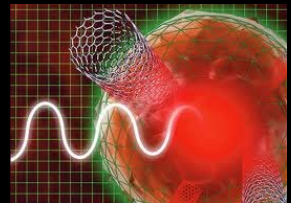
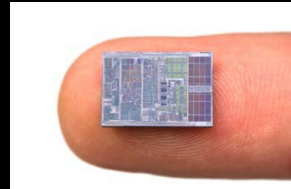
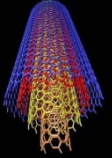
North Carolina State University, Raleigh, NC

SOT March 23rd, Noon – 1:20 pm, San Diego, CA

Engineered Nanomaterials: Small Stuff

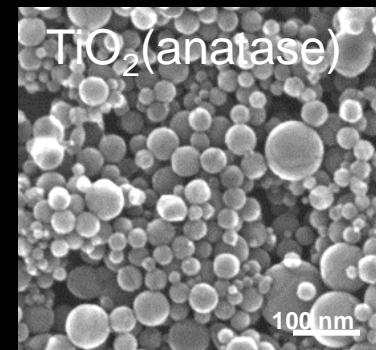
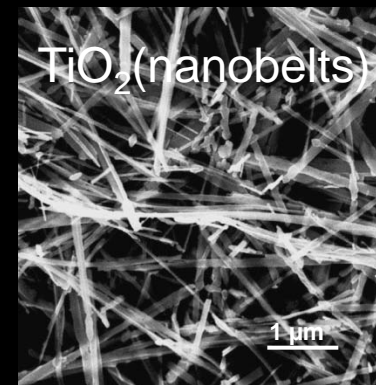
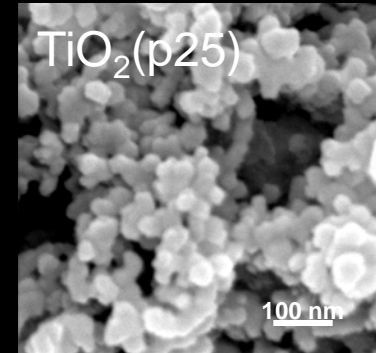


- 1 nanometer (nm) = one billionth (10^{-9}) of a meter
- Nanomaterials have one or more dimension in nanoscale ($\sim 1-100$ nm)
- Numerous elemental and chemical forms (C, Ag, ZnO, TiO₂, Fe₃O₄)
- Many uses: Electronics, Coatings, Structural Materials, Medicine.



Why *In Vitro* is Needed to Refine, Reduce, Replace (3R's) *In Vivo* Testing of Engineered Nanomaterials (ENMs)

- 1) The number and variety of ENMs is rapidly growing with over 500 consumer products containing ENMs and an expected market value of \$1 trillion by 2015.
- 2) ENMs of the same chemical composition can be designed with different physico-chemical characteristics: (e.g., shape, size, charge), increasing the complexity of the issue.
- 3) Ethical considerations and high costs of standard 2-year rodent bioassays limits the number of ENMs that can be tested for chronic diseases.



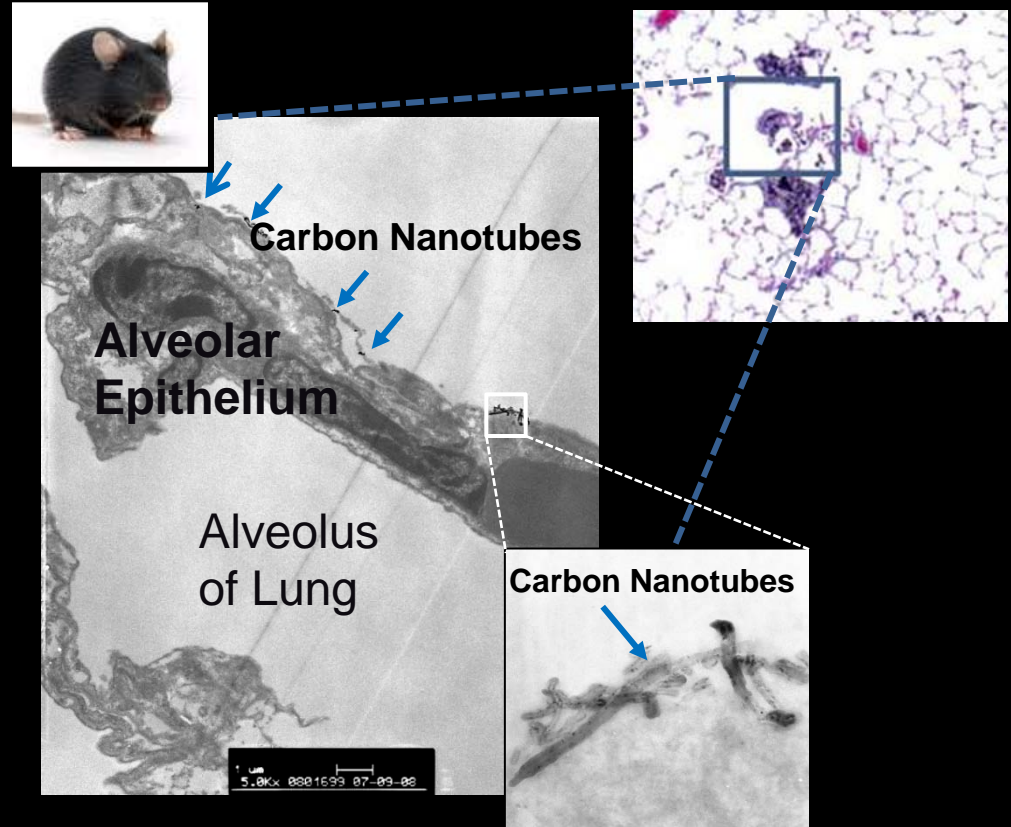
POLL QUESTION #2

Which of the Following Most Accurately Sums Up the Current State of *in vitro* Alternative Testing Strategies for Predicting Nanomaterial Toxicity and Safety?

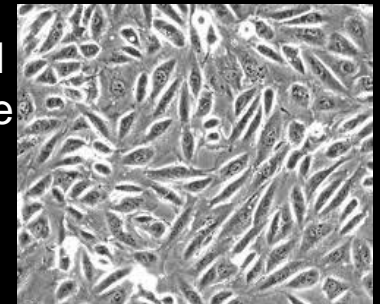
Response	%
<i>In vitro</i> testing is useful for predicting some <i>in vivo</i> outcomes	90.5%
Neither <i>in vitro</i> nor <i>in vivo</i> testing is useful for regulatory risk management and protecting human health	7.4%
The use of <i>in vitro</i> testing is well-developed and to a stage where <i>in vivo</i> testing for regulatory risk assessment and protecting human health is no longer necessary	1.1%
The use of <i>in vitro</i> testing holds little or no value and only <i>in vivo</i> testing can provide useful information	1.1%
	N=95

Cell Types to Use for *In vitro* Testing

‘Particle Deposition Site Determines Local Injury...

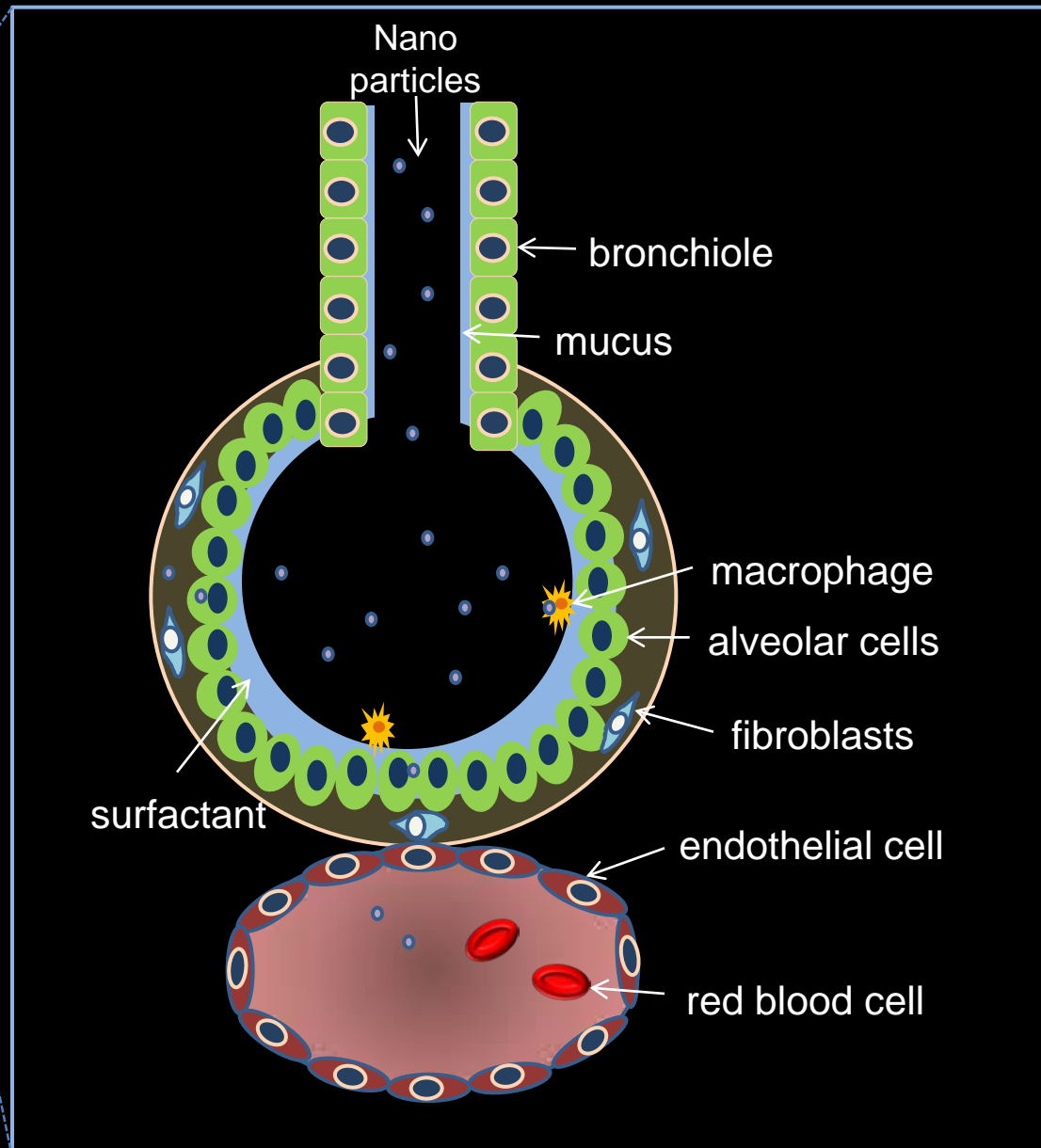
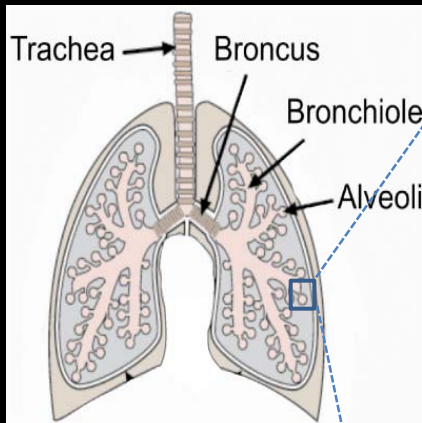


Lung Epithelial
Cells in Culture



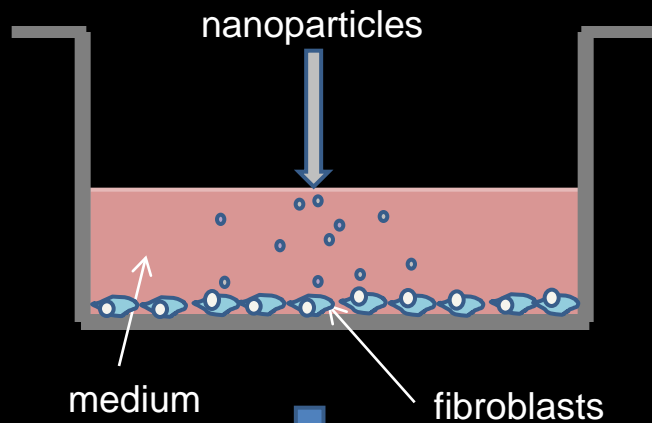
...and the logical choice for cell type selection.’

Modeling the Alveolar Region of the Lung



In Vitro Cell Systems for Testing Nanoparticle Toxicity

Simple

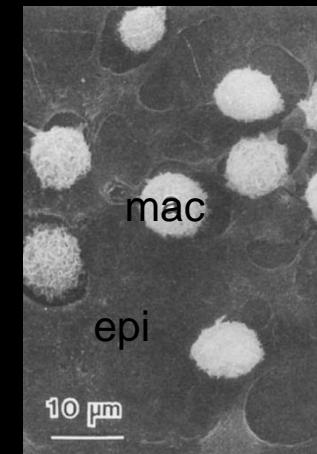
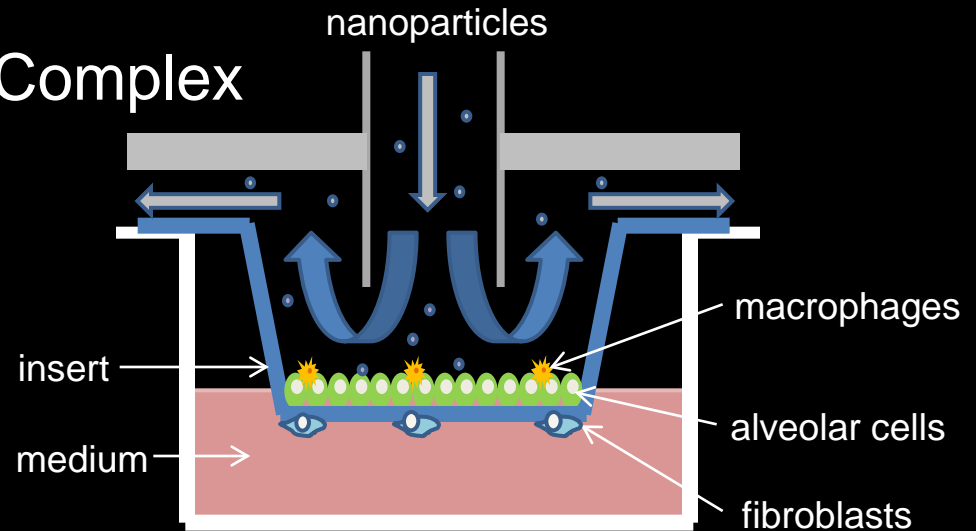


Collect Cells & Media

Measure:

Cytotoxicity, Proliferation,
Collagen, Cytokines,
Lipid Mediators, ROS, etc.

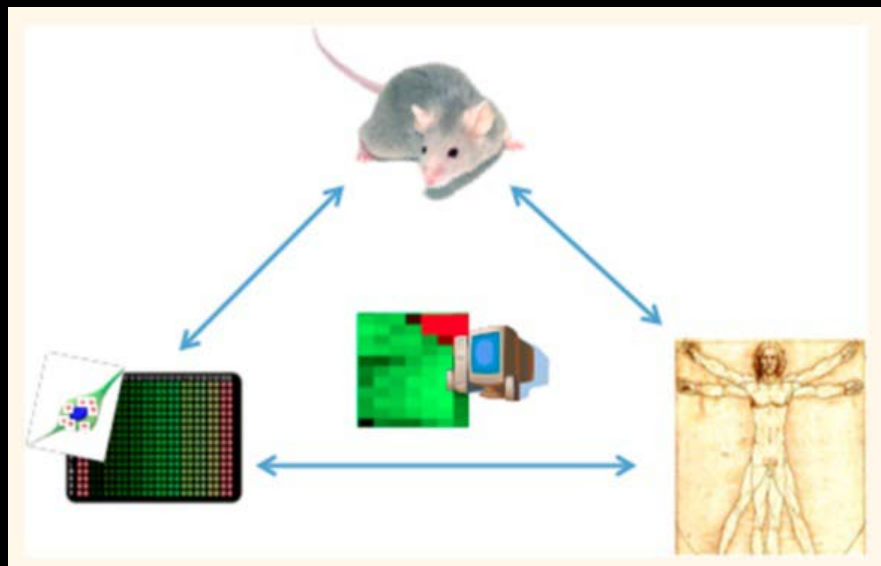
More Complex



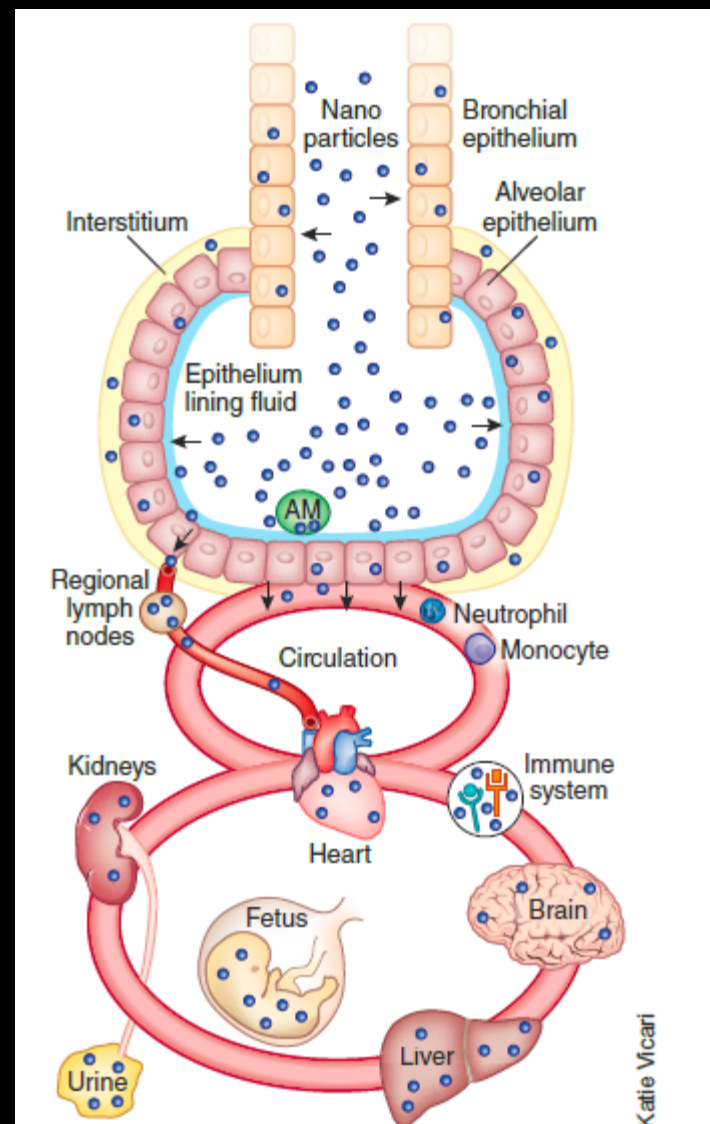
Co-culture of primary rat alveolar type I cells, rat lung fibroblasts, and rat alveolar macrophages.

Challenges to Overcome for *In Vitro* Testing of Nanomaterials

In vitro modeling will need to address complex cellular interactions and systemic effects since some nanomaterials easily cross biological barriers and reach multiple organ systems.

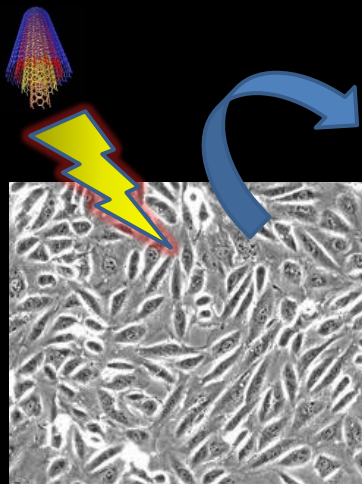


Nel et al., ACS Nano, 2013 7(8): 6422-6433.



Kreyling et al. (2010). *Nat Biotechnol* 28(12): 1275-6.

Missing Gaps in Information



Cells *in vitro*

TGF- β 1,
Collagen?

No



Yes



Normal



Fibrosis

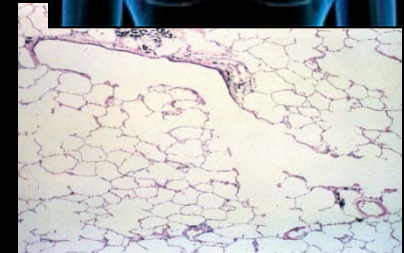
TGF- β 1,
Collagen?

No

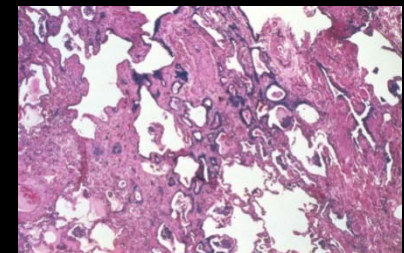


Predict
Risk

Yes



Normal



Fibrosis

POLL QUESTION #3

Alternative *in vitro* tests are not yet available for which of the following adverse health effects?

Response	%
Toxicokinetics (the penetration into, fate within and elimination from the body of a toxic substance, including its absorption, distribution, metabolism and excretion)	43.7%
Immune sensitization (the toxicological impact associated with chemicals that have the intrinsic ability to cause allergy)	5.7%
Carcinogenicity (the ability of substances to cause cancer)	2.3%
All of the above	48.3%
	N=87

Practical Considerations for Moving Forward with *In vitro* Testing

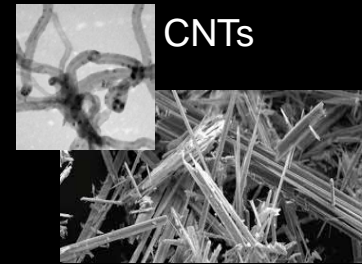
1) Structure-Activity Relationships (SARs) are useful.
“If it looks like a duck, acts like a duck, and swims like a duck...its probably a duck”.

2) Probability of Human Exposure: Which ENMs will represent the highest consumer exposures? (Ag in clothing, ZnO in sunscreens, CNTs as flame-retardants).

3) Dosimetry: Different sedimentation rates for ENMs or different functionalizations of the same ENM in aqueous media in cell culture systems.

4) Inter-Laboratory Reproducibility: Harmonized protocols for *in vitro* assays is key for reliably predicting *in vivo* outcomes.

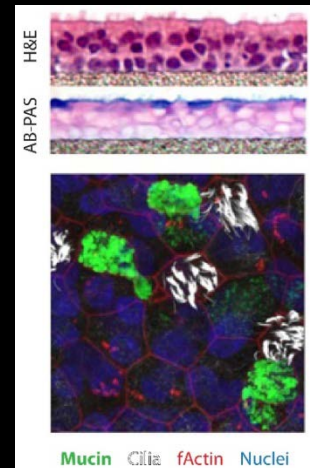
5) Cell Types: Immortalized cell lines are readily available, but often do not behave like cells *in vivo*. Primary cells are superior, but not always available.



Asbestos



CNT f-CNT



Human bronchial epithelial cells in air-liquid interface

POLL QUESTION #4

What is the best way to implement the 3R's for *in vivo* testing of ENMs and protect human health?

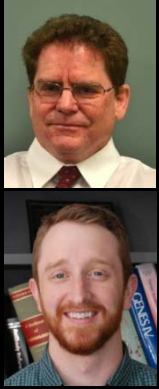
Response	%
Use <i>in vitro</i> testing to narrow down a large number of nanomaterials to a smaller number that can be validated using <i>in vivo</i> assays	80.2%
Do not allow products containing nanomaterials on the market until all have been thoroughly tested with <i>in vitro</i> and <i>in vivo</i> assays	14.6%
Approve all products containing nanomaterials without <i>in vitro</i> or <i>in vivo</i> testing and see if any related disease occurs in the population over the next few decades	3.1%
Ban animal testing of nanomaterials altogether and implement alternative <i>in vitro</i> testing with cell systems	2.1%
	96

Once risks are identified, they can be avoided.



An now, on with the **action exercise**...

You are: The **Scientist**.....
 The **Advocate**.....
 The **Government Regulator**.....



- 1) **READ** the scenario for your table
 - 2) **EVALUATE** the data set
 - 3) **DISCUSS** with the group and table moderator
-
- 1) **PREPARE** answers to the questions
 - 2) **REPORT OUT**