nanoEHS

Nanomaterials and Human Health & Instrumentation, Metrology, and Analytical Methods

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Real-World Vignette: IANH

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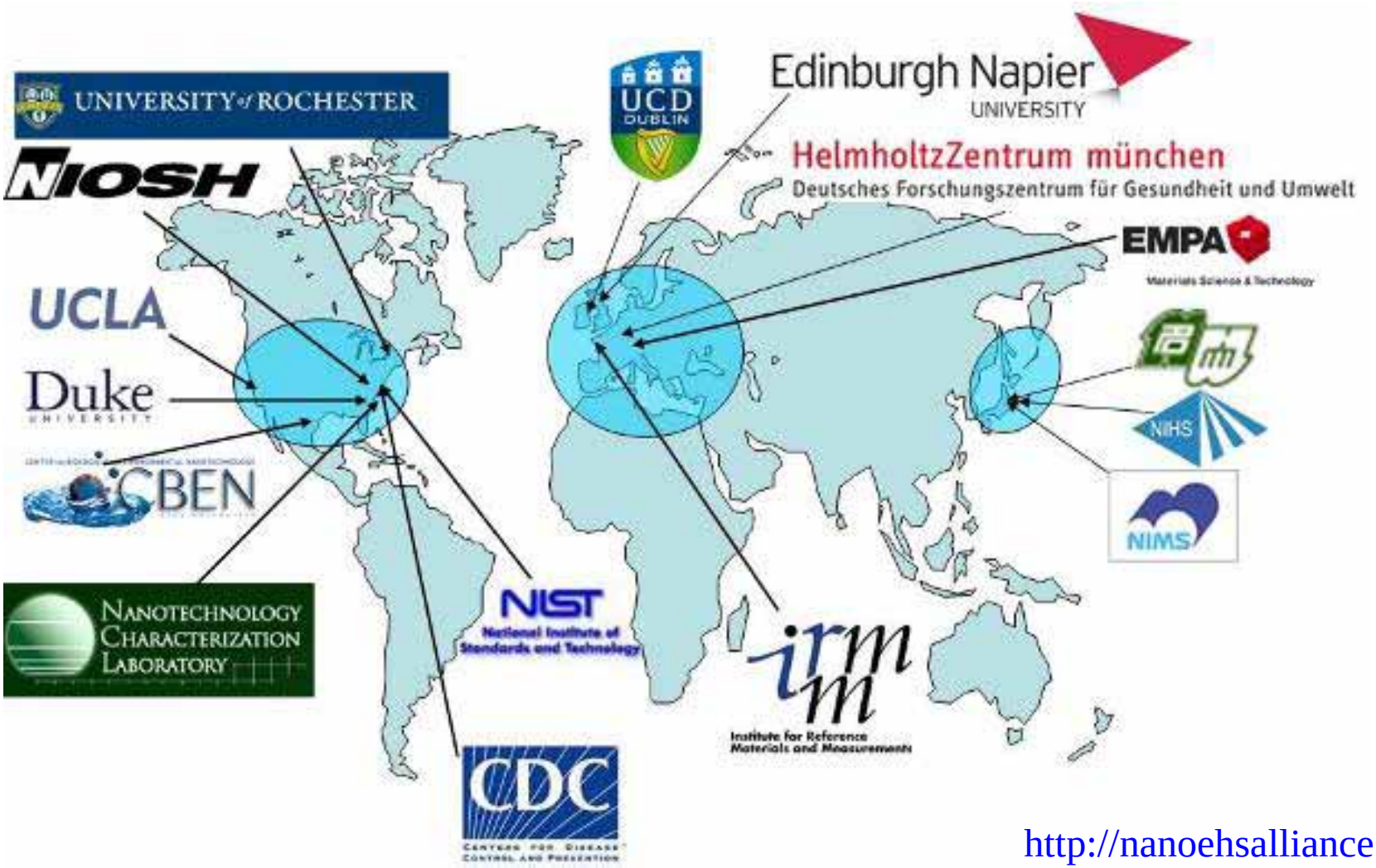
What is the *I*nternational *A*lliance for *N*anoEHS *H*armonization?

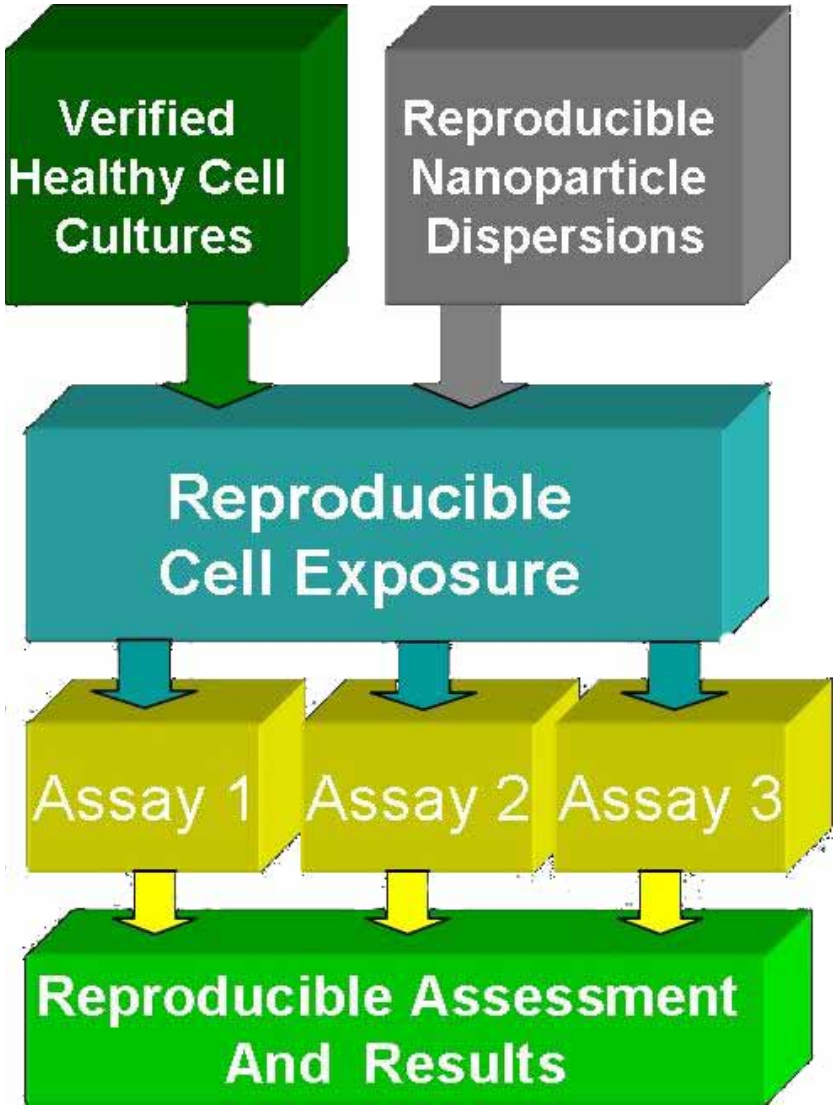
Headed by Prof. Kenneth Dawson, University College, Dublin



http://nanoehsalliance.org

Why Do Round-Robin Testing?

Goal: *Not* to learn something about what nanoparticles do to cells per se, but to learn if those outcomes have predictive value for the in vivo situation.



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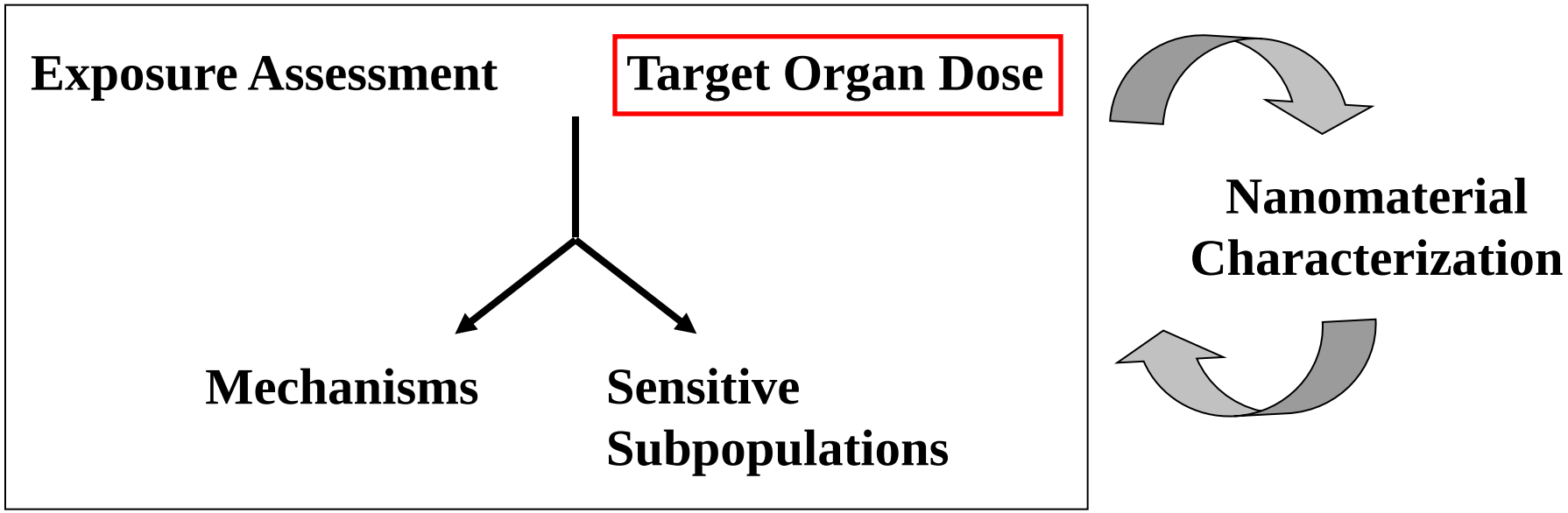
- Can we really move forward if we don't?
- Too much at stake to proceed without validated methodology or methodology that is not fully understood.

Why Do Round-Robin Testing?

- Results from round-robin testing tend to be trusted:
  - Robust and transparent
  - Impact of bias is lower

Framework for Defining Human Health Risks and Benefits of Nanomaterials



Adapted from Elder et al., 2009

Critical Decisions

- What cell types to use and why?
  - RAW264.7
- What particles to use and why?
  - Positively-charged polystyrene, cerium oxide
- What assays should be used?
  - MTS ‘viability’ assay, intracellular fluorescein oxidation, propidium iodide uptake
- What is an appropriate positive control?
- Do the positive and negative controls have to be particles?

What Are the Sources of Variability?
e.g. Cell Culture

- Where were the cells obtained?
- How many passages have the cells undergone?
- What is the process by which cells are removed from their growth substrate?
- What is the type/source of serum?
- How confluent do the cells get and at what rate?
- How often is media changed and what are the criteria for doing so?

Variability in Cultured Cell Growth Characteristics *(RAW264.7 Cells)*

- Five groups participated in a cell growth and health validation study
- The inter-laboratory variability in growth rates within the first 24-48 hrs after plating the cells was low
- Within 96 hrs after plating, though, the variability increased
- This demonstrates the care that needs to be taken with respect to the timing between cell plating, exposure, and assessments of cytotoxicity or oxidative stress

What Are the Sources of Variability? *Endpoints*

- What are the sources of interference with a given assay?
- Do we all have the same degree of nanoparticle agglomeration?
- How are the particles dispersed, in what, and how often?
- To what degree do we need to use the same instrumentation?
  - e.g. plate readers