NCCT ToxCast Program for Nanomaterial Prioritization: High-Throughput Screening, Consideration of Exposure, and Bioactivity Profiling/Modeling

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ORD Nanomaterials Research Program Symposium

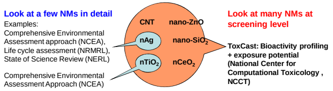
Research Issue

So many nanomaterials (NMs), so little time and resource

• Currently assessment of NM is case-by-case, and the research of NM toxicity and exposure cannot keep up with the development of new materials and applications. Prioritization of NM assessment is needed.

How to prioritize nanomaterial research for risk assessment?

Prioritization by considering both hazard and exposure



Hypothesis: NMs affect same toxicity pathways as conventional chemicals, and ToxCast program can be applied to prioritize NM research.

Existing *in vitro* studies are not sufficient for modeling need

• Diverse nanomaterials
• Various preparation methods
• Often lack detailed characterization
• One nanomaterial at a time or only very few concentrations at a time
→ Extremely difficult, if not impossible, to find correlations between nanomaterial characteristics and effects with confidence

Timetable

Classes of NM of interest:

nano-Ag, CNT, nano-TiO_2, nano-CeO_2, nano-ZnO, $nSiO_2$

Major steps	Feb 2010 - Jan 2011	Feb 2011- Jan 2012	Feb 2012- Jan 2013
1. Develop handling protocols	Some NM classes	All NM classes	
2. Determine testing concentration ranges	Some	All	
3. Characterize NMs	Some	All	
4. Perform High-throughput screening (HTS)	Some	Some	All
5. Analyze HTS data		Some	All
6. Apply ToxCast methodology		Some	All

Research Approach and Initial Findings

Applying ToxCast chemical prioritization tools to NM

- ToxCast program uses statistical computer models with bioactivity profiling and exposure information to prioritize research
- Extensive *in vitro* and *in vivo* high-throughput screening (HTS) of NM biological activity + Computational profiling
  → Identify key NM physico-chemical characteristics influencing its activities (can be a base for developing structure-activity relationships in computer models for NM)
  → Characterize biological pathway activities
  → Classify and prioritize NM hazard

+ Exposure information
  → Prioritize research

Initial NMs for testing

- Nano-Ag (4 types) + $AgNO_3$ + dispersant only
- Nano-TiO_2 (9) + micro-TiO_2 (4)
- CNTs (~8)
- Nano-CeO_2/CeO (4) + Ce ion
- Nano-ZnO (2) + Zn ion
- Nano-SiO_2 (5)
- reference materials: nano-Au (1) and micro-quartz (micro-SiO_2) (1)

Sources: Engineered NanoParticle Risk Assessment (ENPRA) in EU, Organisation for Economic Co-operation and Development (OECD) in EU, Center for the Environmental Implications of NanoTechnology (CEINT) at Duke, National Institute of Standards and Technology (NIST) in USA, and commercial sources in USA.

Collaborators

- Center for the Environmental Implications of NanoTechnology (CEINT) at Duke
- National Health and Environmental Effects Research Laboratory (NHEERL) at EPA
- National Toxicology Program (NTP)
- National Institutes of Health (NIH) Chemical Genomics Center (NCGC)

Major Steps

1. Develop and test handling protocols

Criteria:
• Suitable for all classes of nanomaterials across assays
• Allows comparison with others' results
• Mimic realistic exposure scenarios:
No post-manufacturer wash or purification
No surfactants

Approach:
• Compared protocols used in CEINT, ENPRA, and Japan NIST
• Tested ENPRA dispersion protocol on selected NM
• Tested dispersant on zebrafish embryos

Decision: ENRPA protocol
• Sonication of NM in pure water with 2% serum for 16 min

Major Steps (con't)

2. Identify potential real world exposure concentrations to determine concentration ranges tested in HTS

- *In vitro* testing concentrations:

  The highest reported NM concentration in the air
  + PMMD (Multiple Path Particle Dosimetry) model
  → Estimated NM concentration in the lung alveolar region
  → Use to set high end of in vitro testing concentration

- Zebrafish embryo testing concentrations:

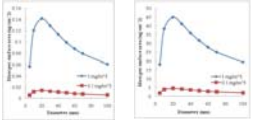
  Consider estimated environmental concentrations (water, sediments etc.) + reported toxic concentrations in fish embryos
  → Use to set high end testing concentration

- Initial findings:

Table 1. Examples of reported NM concentrations in air

NM	Highest particle count (#/cm^3)	Mnfg. / Lab	Particle size (nm)	Mass conc. (mg/m^3)	Reference
Nano-Ag	85,900	Mnfg.	35	0.02	(Methner et al. 2010)
	31,000	Mnfg.	100	0.16	(Park et al. 2009)
	7,000	Lab	150	0.12	(Tsai et al. 2009)
		Mnfg.	-	0.094	(Miller et al. 2010)
MWCNTs	35,800	R&D Lab	20 x 500	0.01	(Methner et al. 2010)
		Mnfg.		0.3208	(Lee et al. 2010)
CNTs	10,000	Lab	8 x ?	0	(Bello et al. 2008)
Nano-TiO_2	144,800	Mnfg.	40	0.02	(Methner et al. 2010)
	140,000	-	16	0.001	(Hameri et al. 2009)

MPPD estimate human retained alveolar mass concentrations (nano-Ag and nano-TiO_2)



24 hr exposure — Full working life (45 yr, 8h/d, 5d/wk)

Proposed testing concentrations

	In vitro HTS	Zebrafish embryos
CNT	0.01 3– 250 ug/cm^2	0.11 – 250 mg/L
Nano-Ag	0.002 – 45 ug/cm^2	0.04 – 87.48 ng/L
Micro-quartz	0.02 – 300 ug/cm^2	0.11 – 250 mg/L
Nano-Au	0.01 – 189 ug/cm^2	0.11 – 250 mg/L

3. Characterize NMs in various conditions

Aim for extensive characterization

- as received from supplier (powder, dispersion)
- as exposed (in stock solution; in cell culture media without and with cells; in embryo-rearing solution for zebrafish embryos without embryos or fish, with embryos, and with fish)
- in exposed cells (in cells, in zebrafish embryos and zebrafish)

4. Perform ToxCast HTS Assays for NM

A. Model organism assay: Developmental effects in zebrafish embryos (Stephanie Padilla)

- Zebrafish embryos will be dosed with NM from fertilization until day 5 post fertilization; this covers formation of all major organ systems.
- On day 6, the larvae will be assessed for malformations.

- Tested 5 rearing solutions for both embryo development and nano-Ag aggregation.
  - Full strength EPA moderately hard reconstituted water (1X MHRW) produced healthy embryos and least NM aggregation

B. Cellular assays:

- **Cells:**

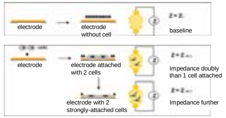
  Primary human cells
  Endothelial cells, monocytes, keratinocytes, fibroblasts, proximal tubule kidney cells, small airway epithelial cells

  Biotransformation-competent cells
  Primary rat hepatocytes

  Cell lines
  HepG2 human hepatoblastoma, A549 human lung carcinoma, HEK 293 human embryonic kidney

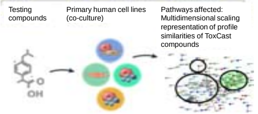
- **Assay formats:**

a. Human cell growth kinetics (ACEA Biosciences)



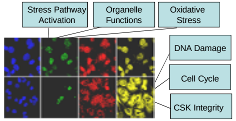
b. Cytotoxicity profiling in 9 primary human cell systems (BioSeek, Inc.) (Houck et al., 2009)

Use protein expression profiles to characterize NM effects relevant to human tissues and disease conditions.



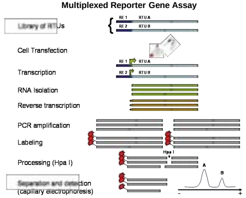
c. High-content imaging of cellular phenotype in human and rat liver cells (Cellumen)

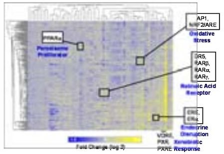
Use automated fluorescent microscopy to determine effects of chemicals on toxicity biomarkers in HepG2 cells and primary rat hepatocytes.



d. Multiplexed Transcription Factor Activity in human liver cells (Attagene, Inc.) (Martin et al., 2010)

Use signatures for prediction of toxicological outcomes of compounds in HepG2 cells.





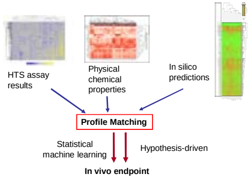
5. Analyze HTS Data

- Identify molecular targets, pathways and phenotypic endpoints affected.
- Determine assay endpoints significantly affected by nanomaterials.
- Calculate potency and efficacy for positive findings.
- Evaluate alternative methods of expressing potency (e.g. concentration by weight/volume, number of particles, surface area, etc.).

6. Apply ToxCast methodology

Toxicity Signature Matching

A toxicity signature is in essence a function that takes as input the results of a set of assays run on a chemical, and produces a prediction of the toxicity of that chemical for a specific *in vivo* endpoint.



Expected Impact

- **Find relationships between bioactivities and NM characteristics or testing conditions.**
- **Recommend a dose metric for NMs *in vitro* studies.**
- **Establish associations to *in vivo* toxicity or pathways identified from testing of conventional chemicals with ToxCast HTS methods**
- **May be able to identify structure-activity relationships using physico-chemical properties of NMs.**

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