Comparing bioactivity profiles of diverse nanomaterials based on high-throughput screening (HTS) in ToxCast™

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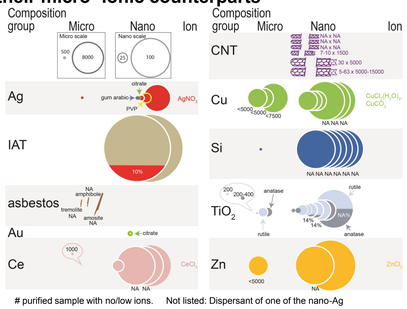
Introduction and objectives

- Over 2800 nanomaterials (NMs) and numerous nanoproducts are in commerce and few have toxicity data.
- To prioritize NMs for toxicity testing, high-throughput screening (HTS) of biological activity may be the only practical and timely approach to provide the information necessary.
- Goals: Screen 62 NMs as a case study, and use the HTS results to prioritize NMs for further research/hazard identification

Approach

Screened 62 samples of nanomaterials and their micro- ionic counterparts



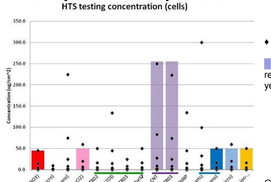
purified sample with no/low ions. Not listed: Dispersant of one of the nano-Ag

NM physicochemical property characterization

Endpoints	Method	Samples	As received: Dry material	As received: Suspension	(Re)suspended: In stock (H_2O + serum)	(Re)suspended: In 4 testing media
Size distribution and shape	TEM, SEM, DLS	Nano and micro	√	√	√	X
Surface area	BET	Nano and micro	√		√	√
Chemical composition	XRD, TOC	All samples	√	√		
Crystal form	XRD	Applicable	√	√		
Impurity	XPS	CNT	√			
Total metal concentration		Metallic			√	√
Total non-metal concentration		Non-metallic			√	
Ion concentration	ICP-MS and others	Applicable			√	√
Zeta potential, surface charge	Zetasizer	Nano and micro			√	

Characterized by CEINT, except BET, which will be measured by NIST and NIOSH.
√ included in our project. Dark green – competed. Light green – partially completed.

Testing concentration ranges vary by NM class to reflect potential exposure



♦ Testing concentration

MPPD predicted lung retention of NM after 45 year exposure

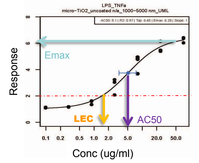
Gangwal et al. 2011 Environ Health Perspect 119(11):1539-46.

HTS assays cover a broad range of bioactivities

DNA
RNA
Protein
Function/
Phenotype

Main type of result by assay platform	Primary / cell line	Species	Cell type	#of endpoint	# of direction (time points)	# of results per NM per conc
Transcription factor activation	Cell line	Human	Hepatocytes (HepG2)	48	NA (1)	48 LEC
Protein expression profile	Primary	Human	•Umbilical vein endothelial cells (HUVEC) •HUVEC+Peripheral blood mononuclear cells •Bronchial epithelial cells •Coronary arterial smooth muscle cells •Dermal fibroblasts-neonatal (HDFn) •Epidermal keratinocytes + HDFn	87	2 (1)	174 LEC
Cell growth kinetics	Cell line	Human	Lung (A549)	1	2 (numerous)	2 AC50 (at 80 hr)
Toxicity phenotype	Primary Cell line	Rat Human	Hepatocytes Hepatocytes (HepG2)	16	NA or 2 (4)	96 AC50
Developmental malformation	NA	Zebrafish	embryos	Aggregated as 4	NA (NA)	2 BMD

Data processing to calculate LEC, AC50, or BMD



- AC50 = the concentration that generate 50% of maximal effect (Emax)
- LEC = Lowest effective concentration, the lowest testing conc. that induces a significant change
- BMD = bench mark dose, we used estimated concentration that has 10% population with significant change (figure not shown)

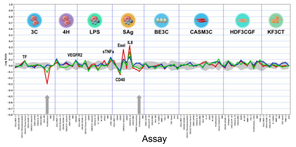
Profiles of immune responses

3 asbestos samples had similar immune response profiles

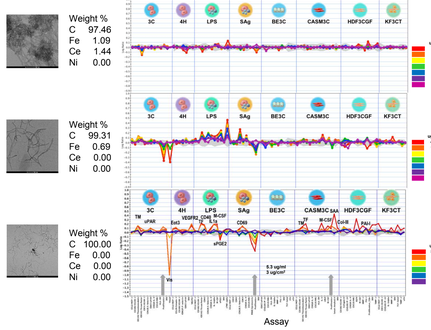
R009 RTI amosite, high *in vivo* toxicity

R015 Libby amphibole, moderate *in vivo* toxicity

R011 El Dorado tremolite , low *in vivo* toxicity



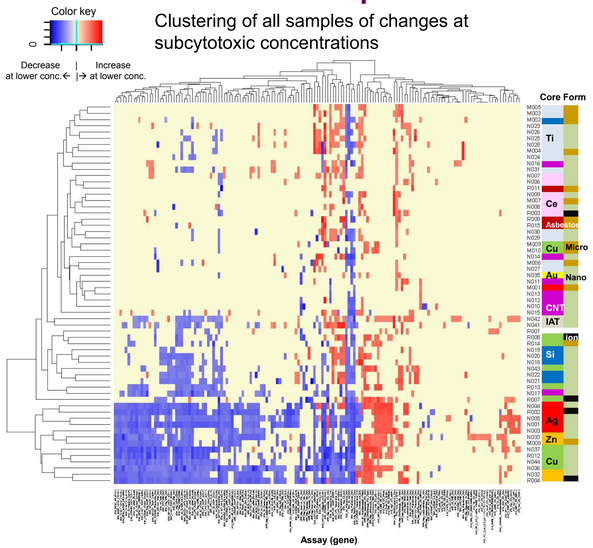
CNTs had different immune response profiles from each other and from asbestos at non-cytotoxic concentrations



•Given the many CNT physicochemical properties (length, wall number, rigidness, etc) can contribute to their bioactivities, further physicochemical characterization is needed to associate CNT physicochemical properties to bioactivities.

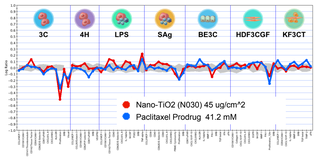
Profiles of all response

Clustering of all samples of changes at subcytotoxic concentrations

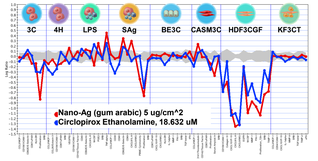


- Activities shown as log transformed LEC/AC50/BMD at mass/surface area. Red for increase; blue for decrease. Deeper colors indicate LEC/AC50/BMD at lower concentrations
- Ag, Cu and Zn were active in more assays than other core materials.
- Nano and ion had similar profiles.
- Most assays were changed in one direction (either up or down), and only few have changes in both directions.

Profile matching suggests possible targets that were not directly measured

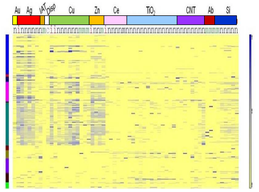


- Nano-TiO_2 has a similar profile as Paclitaxel, a microtubule stabilizer interfering with mitosis
- Pearson's coefficient > 0.7
- Gheshlaghi, ZN *et al.* (2008) Toxicity and interaction of titanium dioxide nanoparticles with microtubule protein. *Acta Biochim Biophys Sin (Shanghai)* 40(9): p. 777-82

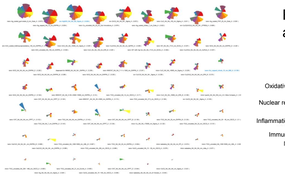


- Nano-Ag had a similar profile as ciclopirox, a N+ K+ ATPase inhibitor
- Pearson's coefficient > 0.7
- Nechay BR, Saunders JP. (1984) Inhibition of adenosine triphosphatase in vitro by silver nitrate and silver sulfadiazine *J Environ Pathol Toxicol Oncol* 5(4-5):119-26

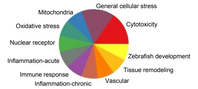
Ranking of *in vitro* bioactivity



Assays were divided into groups (slices of ToxPi) by the function/ pathway the assay target is associated.



Ranking results shown as ToxPi



Ranking with concentration as total mass/surface area

All samples are nanomaterials, except marked (* for ion, M for micromaterials, d for "deioned" samples, in which most ions were removed)

Chemical Name (form-core_coat/cap_coat/cap material_size_source_letter)	Score	Ag	Cu	Zn	Si	CNT	Ti	asbestos
nano-Ag_coated_gum arabic_6_nm_Duke_A	0.87							
nano-Ag_capped_NA_15_nm_ENPRA_A	0.83	*						
ion-AgNO3_NA_NA_NA_Sigma_A	0.82							
nano-Cu_uncoated_NA_25_nm_Sun Innovations_A	0.68							
ion-ZnCl2_NA_NA_NA _Sigma_A	0.67			*				
nano-Ag_coated_citrate_7_nm_Duke_A	0.62							
nano-CuO_NA_NA_<50_nm_Sigma_A	0.61							
ion-CuCl2(H2O)2_NA_NA_NA _STREM_A	0.61			*				
nano-Ag_coated_PVP_25_nm_Duke_A	0.59							
nano-ZnO_coated_triethoxycaprylylsilane_130_nm_ENPRA_A	0.57							
nano-ZnO_uncoated_NA_100_nm_ENPRA_A	0.55							
micro-ZnO_NA_NA_<5000_nm_Sigma_A	0.46							
nano-CuO_NA_NA_<50_nm_EPA_B	0.46		d	M				
nano-Cu(OH)2_NA_NA_NA_nm_SePRO_A	0.40							
nano-IAT-with-Ag-ion_NA_NA_172.5_nm_NCSU_A	0.39							
nano-IAT_NA_NA_172.5_nm_NCSU_A	0.34							
nano-Cu(OH)2_NA_NA_NA_nm_DuPont_A	0.34							
nano-SiO2_NA_NA_NA_nm_ENPRA_D	0.29							
nano-SiO2_NA_NA_NA_nm_ENPRA_E	0.28							
nano-SiO2_NA_NA_NA_nm_ENPRA_B	0.27							
dispersant for NM300_NA_NA_NA _ENPRA_A	0.26							
nano-SiO2_NA_NA_nm_ENPRA_C	0.25							
nano-MWCNT_NA_NA_7-11 x 1500_nm_ENPRA_A	0.25							
ion-CuCO3_NA_NA_NA _Sigma_A	0.24		*					
micro-CuO_NA_NA_<5000_nm_Sigma_A	0.23							
nano-Cu(OH)2_NA_NA_NA_nm_EPA_B	0.21		d					
nano-Au_capped_citrate_10_nm_BBI_A	0.20	Au	Au	Au	Au	Au	Au	Au	Au
nano-SiO2_NA_NA_NA_nm_ENPRA_A	0.19							
nano-CNT_NA_NA_NA_nm_OPPT_E	0.19							
nano-MWCNT_NA_NA_5-63 x 5000-15000_nm_ENPRA_A	0.18							
nano-MWCNT_NA_NA_30 x 5000_nm_ENPRA_A	0.17							
nano-TiO2_uncoated_NA_10_nm_OECD_A	0.17							
nano-TiO2_uncoated_NA_27.5_nm_OECD_A	0.17							
micro-CuO_NA_NA_<5000_nm_EPA_B	0.17		M, d					
ion-CeCl3_NA_NA_NA _Sigma_A	0.16							
micro-quartz_NA_NA_534_nm_U.S. Silica Company_A	0.16			M				
nano-CNT_NA_NA_NA_nm_OPPT_F	0.15							
nano-TiO2_NA_NA_7_nm_ENPRA_A	0.14							
nano-TiO2_NA_NA_NA_nm_ENPRA_A	0.14							
nano-CNT_NA_NA_NA_nm_OPPT_C	0.14							
nano-CNT_NA_NA_NA_nm_OPPT_B	0.14							
micro-Cu_NA_NA_<75000_nm_Sigma_A	0.13		M					
nano-TiO2_NA_NA_20_nm_ENPRA_B	0.13							
nano-TiO2_uncoated_NA_32_nm_OECD_A	0.10							
nano-TiO2_uncoated_NA_30 - 40_nm_OECD_A	0.10							
nano-Ce(VI)O_NA_NA_NA _nm_ENPRA_B	0.10							
nano-Ce(VI)O_NA_NA_NA_nm_ENPRA_A	0.10							
micro-TiO2_uncoated_NA_1000-5000_nm_UML_B	0.09					M		
micro-TiO2_uncoated_NA_200_nm_OECD_A	0.09					M		
nano-TiO2_NA_NA_20_nm_ENPRA_A	0.08							
nano-CeO2_uncoated_NA_15 - 30_nm_OECD_A	0.08							
micro-asbestos_NA_NA_NA_nm_RTI_A	0.07							M
micro-asbestos_NA_NA_NA_nm_Libby_A	0.07							M
micro-TiO2_uncoated_NA_1000-5000_nm_UML_A	0.07					M		
micro-TiO2_uncoated_NA_200 - 400_nm_OECD_A	0.06					M		
micro-Ag_NA_NA_NA_nm_Sigma_A	0.06	M						
nano-TiO2_uncoated_NA_21_nm_Evonik_A	0.05							
nano-CeO2_uncoated_NA_70 -105_nm_OECD_A	0.05							
nano-CNT_NA_NA_NA_nm_OPPT_D	0.04							
micro-asbestos_NA_NA_NA_nm_El-Dorado_A	0.04							M
micro-CeO2_NA_NA_NA_nm_Sigma_A	0.02							

Ranking with concentration as total ion/ surface area

Ag, Zn, Cu are more active than Ce

Chemical Name	Score	Ag	Cu	Zn	Ce
nano-Ag_capped_NA_15_nm_ENPRA_A	0.86				
nano-Ag_coated_gum arabic_6_nm_Duke_A	0.78				
ion-AgNO3_NA_NA_NA_Sigma_A	0.75	*			
ion-ZnCl2_NA_NA_NA _Sigma_A	0.61			*	
nano-IAT-with-Ag-ion_NA_NA_172.5_nm_NCSU_A	0.59				
nano-Cu_uncoated_NA_25_nm_Sun Innovations_A	0.58				
ion-CuCl2(H2O)2_NA_NA_NA _STREM_A	0.58		*		
nano-CuO_NA_NA_<50_nm_Sigma_A	0.56				
nano-Ag_coated_citrate_7_nm_Duke_A	0.54				
nano-Ag_coated_PVP_25_nm_Duke_A	0.51				
nano-ZnO_coated_triethoxycaprylylsilane_130_nm_ENPRA_A	0.51			M	
nano-ZnO_uncoated_NA_100_nm_ENPRA_A	0.47				
micro-ZnO_NA_NA_<5000_nm_Sigma_A	0.43			M	
nano-Cu(OH)2_NA_NA_NA_nm_SePRO_A	0.39		*		
nano-Cu(OH)2_NA_NA_NA_nm_DuPont_A	0.32				
ion-CuCO3_NA_NA_NA _Sigma_A	0.23				
nano-Au_capped_citrate_10_nm_BBI_A	0.18	Au	Au	Au	Au
ion-CeCl3_NA_NA_NA _Sigma_A	0.15				*
micro-CuO_NA_NA_<5000_nm_Sigma_A	0.15		M		
micro-Cu_NA_NA_<75000_nm_Sigma_A	0.12		M		
nano-Ce(VI)O_NA_NA_NA _nm_ENPRA_A	0.09				
nano-Ce(VI)O_NA_NA_NA _nm_ENPRA_B	0.09				
nano-CeO2_uncoated_NA_15 - 30_nm_OECD_A	0.07				
micro-Ag_NA_NA_NA_nm_Sigma_A	0.06	M			
nano-CeO2_uncoated_NA_70 -105_nm_OECD_A	0.05				
micro-CeO2_NA_NA_NA_nm_Sigma_A	0.03				M

Key results

1. Chemical composition has more influence than size
2. Nano and corresponding ion have similar profiles
3. Most microparticles are much less active than their nano or ion counterparts, except micro-ZnO, which is almost as active as nano-ZnO.
4. LECs and AC50 rarely lower than 1 ug/ml
5. High *in vitro* activity (ToxPi ranking) were seen for Ag (nano, ion), Cu (nano, ion), Zn (nano, ion, micro) and Si (nano).
6. Medium *in vitro* activity were seen in most nano-SiO_2 and CNT, some nano-TiO_2, and 1 nano-CeO_2.
7. Low *in vitro* activity were seen in all microparticles (except micro-ZnO), some CNTs, some nano-TiO_2, most nano-CeO_2, and all 3 asbestos.
8. Assays using submerged cells may have limited sensitivity to detect inhalation effects

Conclusion

- HTS is useful for screening NM bioactivities and ranking NMs for their bioactivity.
- Profile comparison may aid predicting additional targets that were not directly measured in HTS.
- Asbestos and CNT have distinctive bioactivity profiles in our systems.
- Core composition and ion release are among key factors in influencing bioactivity profiles.

On-going analysis

- Characterize biological pathway activity
- Explore grouping and weighing options in ToxPi prioritization approach and other prioritization methods
- Identify key nanomaterial physico-chemical characteristics influencing its activities
- Compare bioactivity profiles with ToxCast chemicals (non-nano)