Integrative Nanotoxicology: Linking Rapid Assays and Informatics to Understand Nanomaterial –Biological Interactions

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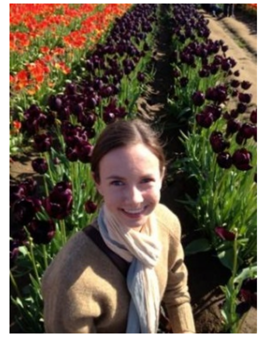

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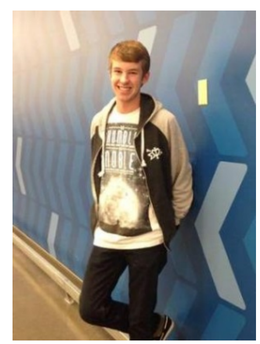


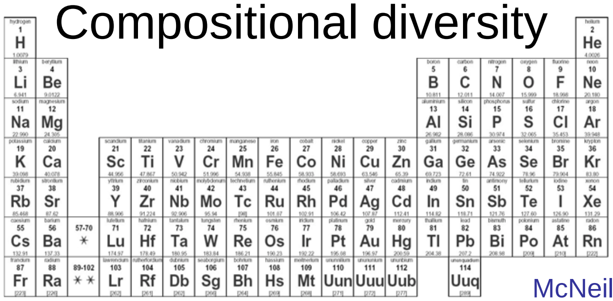
Arek Engstrom

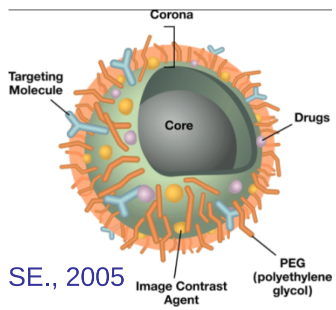
Macklin Turnquist

Need for Rapid Assays

Nanomaterial Diversity

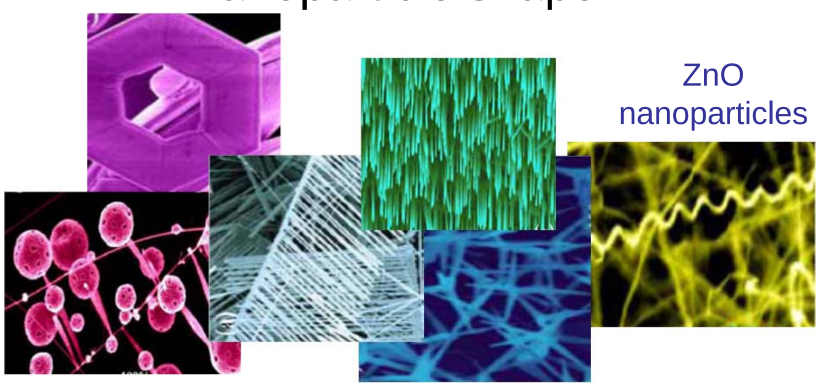
Compositional diversity





McNeil SE., 2005

Synthesis process influences nanoparticle shape

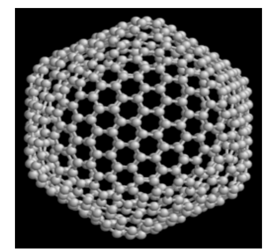


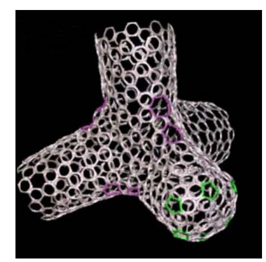
ZnO nanoparticles

Nanomaterial Complexity

Relative importance of nanomaterial characteristics are unknown

Physicochemical properties
Chemical Structure
Core Particle Composition
Size
Shape
Charge
Surface Chemistry
Surface Area
Agglomeration State
Zeta Potential





http://www.vincentherr.com/cf/nanomain.html

Small changes in nanomaterial can alter conditional behaviors of nanomaterials (performance, exposure, hazard)

Biological Impacts Evaluations

Embryonic Zebrafish Assay

In vivo system to rapidly screen for biological impacts

General Attributes

Share molecular, cellular and physiological characteristics with other vertebrates

Develop rapidly

Easy to maintain

Toxicity Evaluation

Large sample sizes

Many routes of exposure

Transparent - non-invasive evaluations

Amenable to mechanistic evaluations

Investigate genomic → whole animal responses in same organism

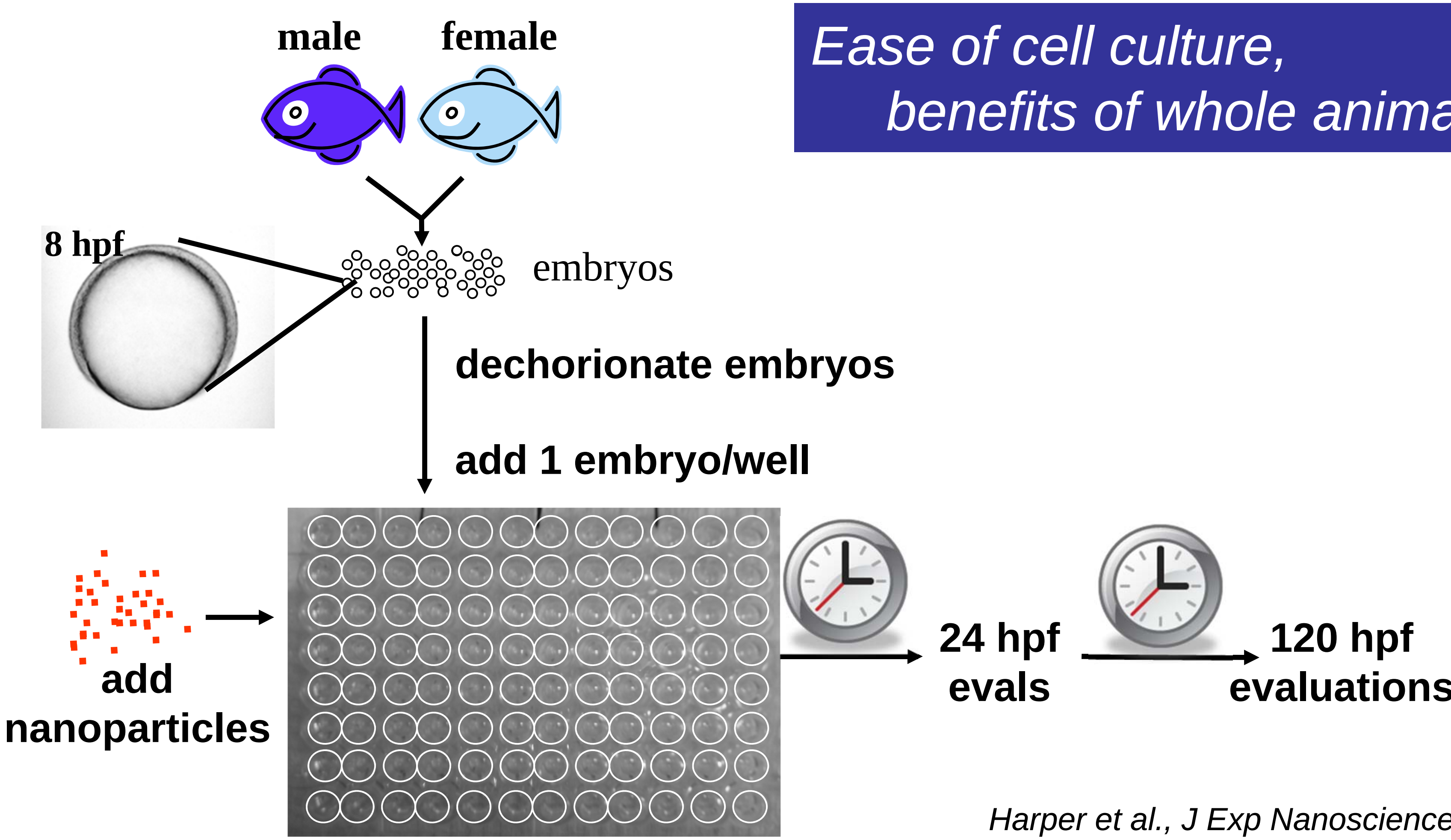
Full suite of molecular signaling necessary and active early in development

Biological Impacts Evaluations

Embryonic Zebrafish Assay

Experimental Design

Ease of cell culture, benefits of whole animal!



Harper et al., J Exp Nanoscience, 2008

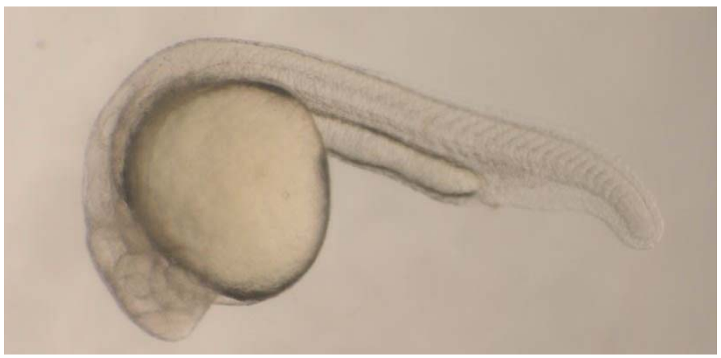
Biological Impacts Evaluations

Embryonic Zebrafish Assay

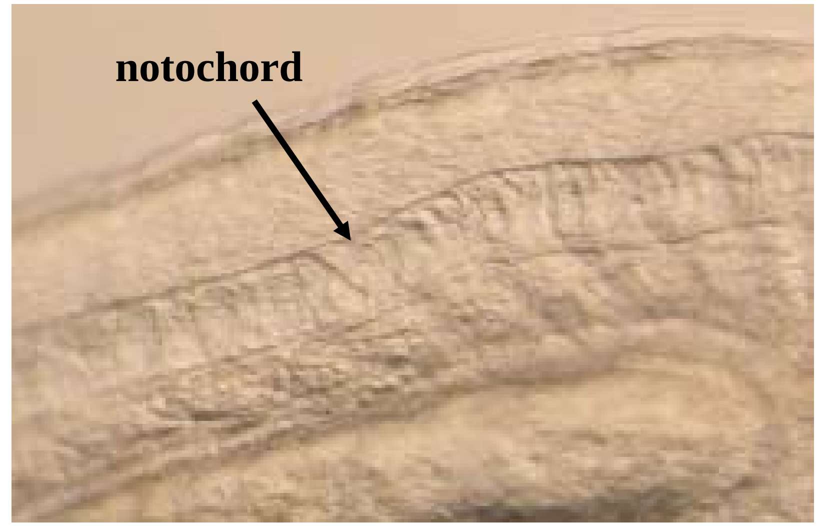
24 hpf evaluations

Mortality (**mort**)
Developmental progression (**dp**)
Spontaneous movement (**sm**)
Notochord (**nc**)

CONTROL



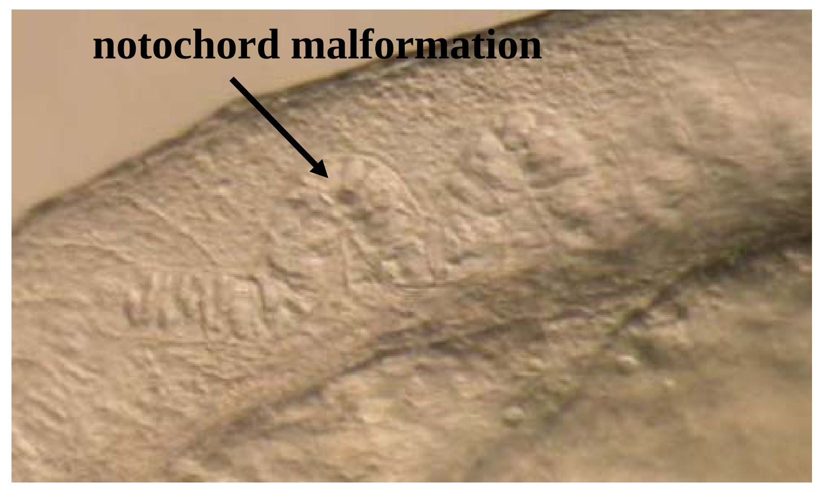
CONTROL



varying degrees of delayed development

EXPOSED

EXPOSED



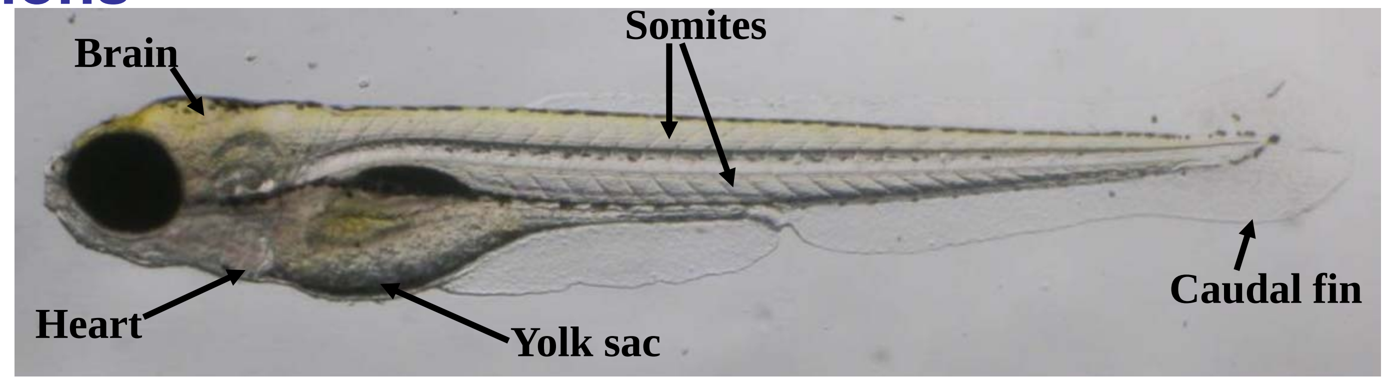
Biological Impacts Evaluations

Embryonic Zebrafish Assay

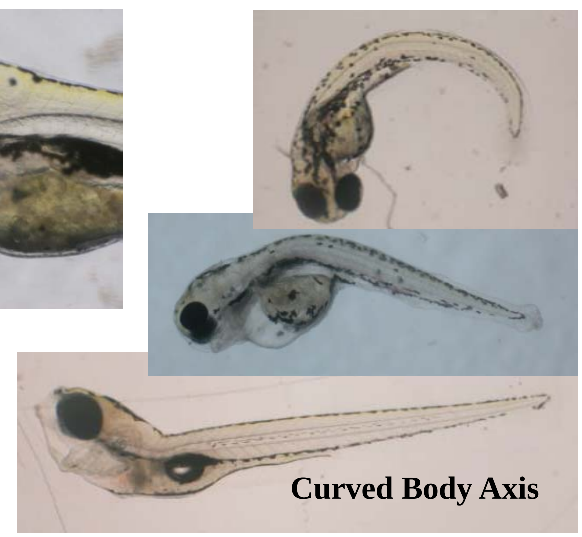
120 hpf evaluations

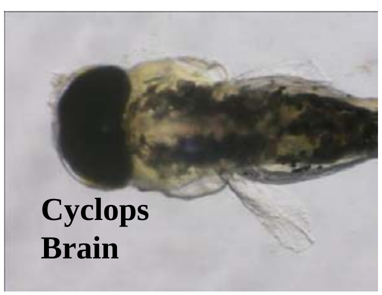
Mortality (**mort**)
Yolk sac edema (**YSE**)
Body axis (**axis**)
Eye
Snout
Jaw
Otic vessicle (**otic**)
Pericardial edema (**PE**)
Brain
Somites
Pectoral fin (**pfin**)
Caudal fin (**cfin**)
Pigmentation (**pig**)
Circulation (**circ**)
Trunk
Swim bladder (**swim**)
Motility (touch response, **tr**)

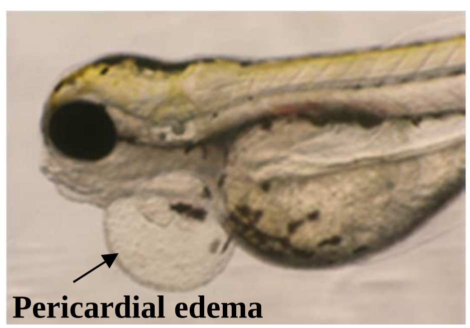
CONTROL



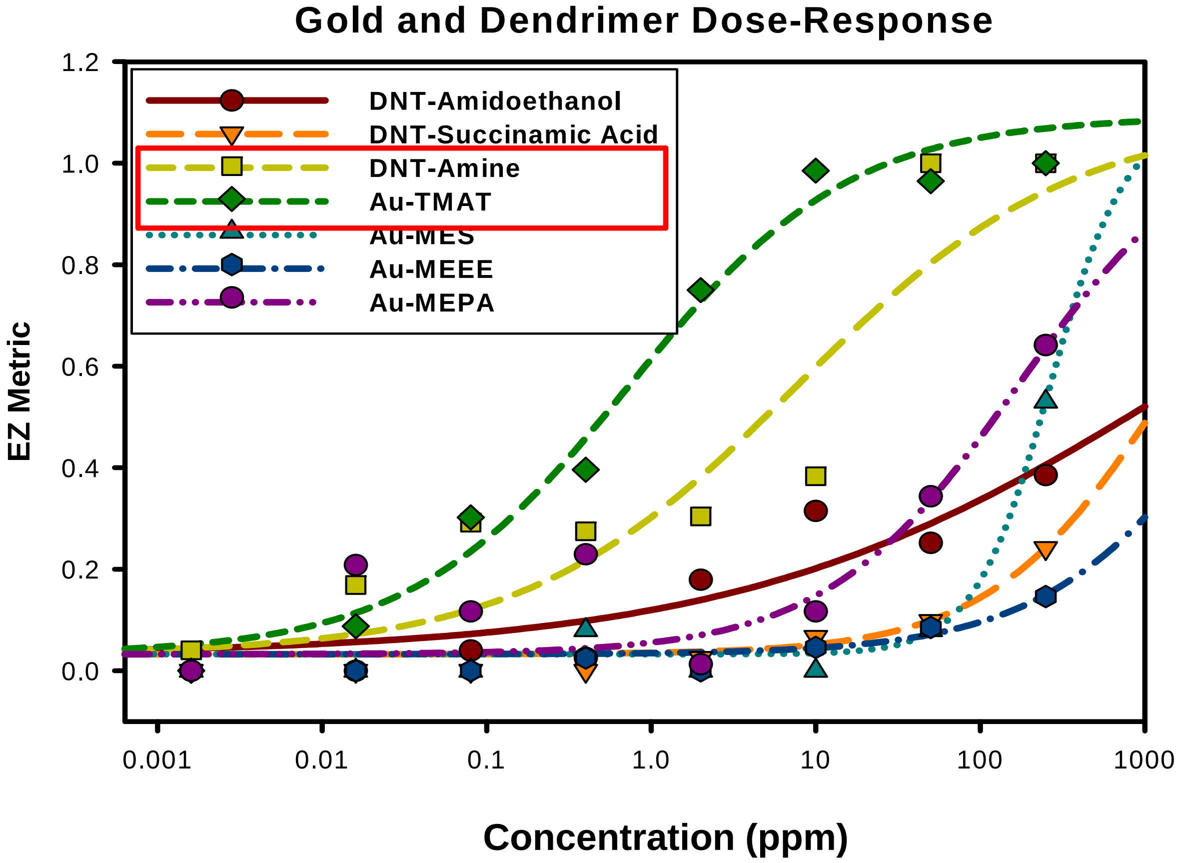
EXPOSED







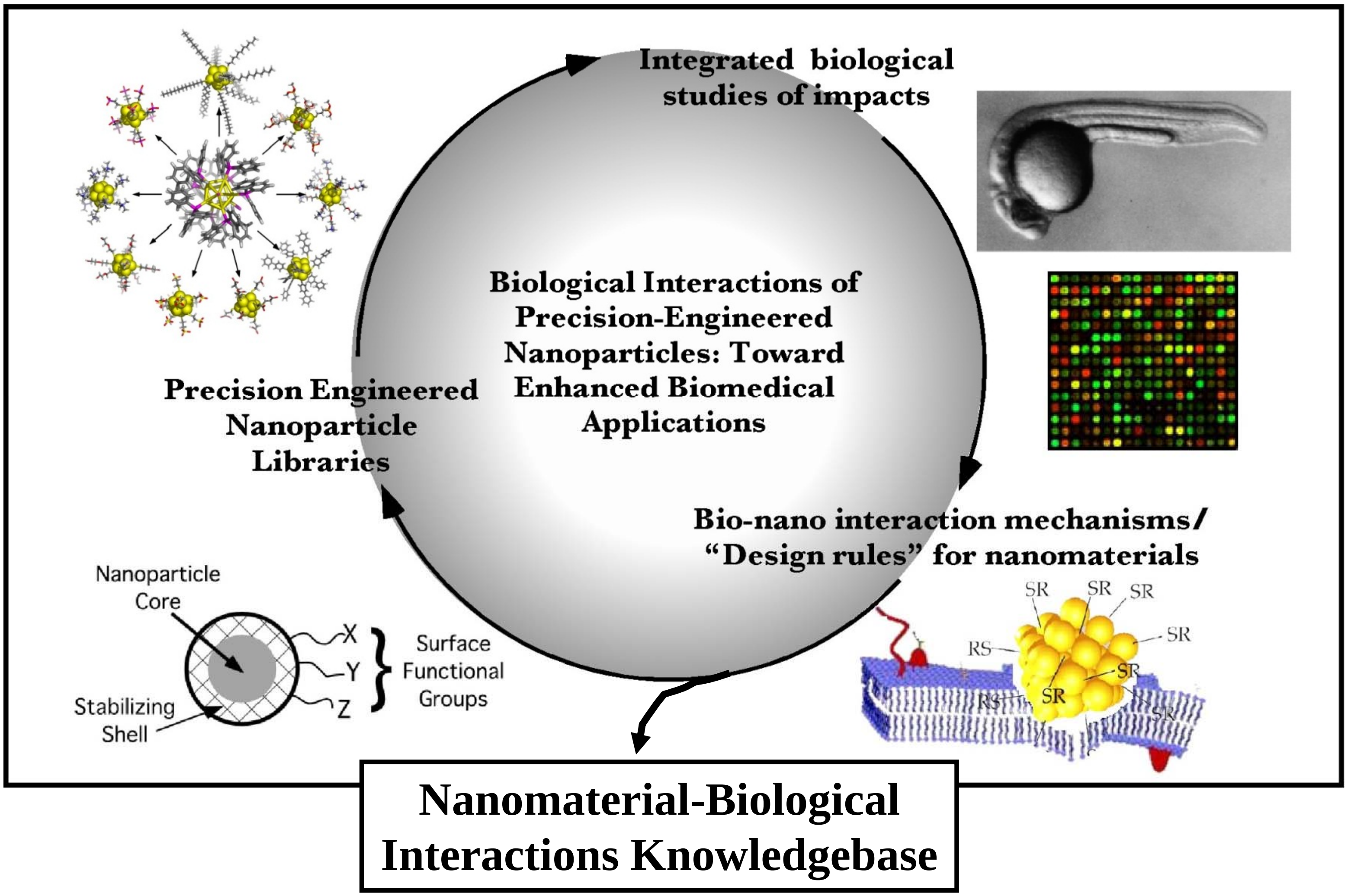
Concentration-Response to Identifiy Concerning Surface Chemistry



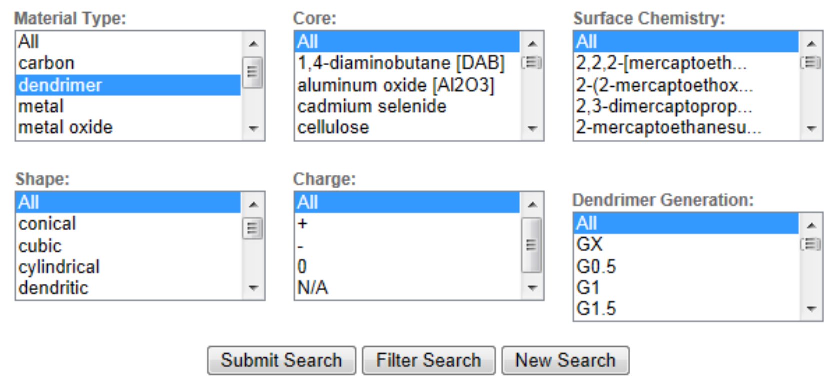
Nanomaterial	Charge
DNT-amidoethanol	neutral
DNT-succinamic acid	negative (-)
DNT-amine	positive (+)
Au-MEEE	neutral
Au-MEPA	negative (-)
Au-MES	negative (-)
Au-TMAT	positive (+)

nanomaterial-biological interactions

Embryonic Zebrafish Assay: Iterative Testing to Gain Knowledge



Nanomaterial-Biological Interactions Knowledgebase



Link to material record

Link to experimental data

Data repository

Nanomaterial								EZ Metric								
ID	Family	Core	Surface Chemistry	Shape	Size	Charge		Concentration								
nbi_0010	metal	gold [Au]	2-mercaptoethanesu...	spherical	1.5	-		control	16 ppb	80 ppb	400 ppb	2 ppm	10 ppm	50 ppm	250 ppm	Data
Average Values								0.00	0.00	0.00	0.07	0.08	0.05	0.00	0.08	View
nbi_0004	metal	gold [Au]	2-(2-mercaptoethox...	spherical	0.8	0		control	16 ppb	80 ppb	400 ppb	2 ppm	10 ppm	50 ppm	250 ppm	Data
Average Values								0.00	0.00	0.04	0.02	0.04	0.04	0.09	0.15	View
nbi_0007	metal	gold [Au]	N,N,N-trimethylamm...	spherical	1.5	+		control	16 ppb	80 ppb	400 ppb	2 ppm	10 ppm	50 ppm	250 ppm	Data
Average Values								0.03	0.13	0.36	0.74	0.77	0.98	1.03	1.00	View
nbi_0013	metal	gold [Au]	6-mercaptohexanoic...	spherical	10	-		control	16 ppb	80 ppb	400 ppb	2 ppm	10 ppm	50 ppm	250 ppm	Data
Average Values								0.00	0.08	0.02	0.10	0.04	0.13	0.57	0.99	View
nbi_0012	metal	gold [Au]	N,N,N-trimethylamm...	spherical	10	+		control	16 ppb	80 ppb	400 ppb	2 ppm	10 ppm	50 ppm	250 ppm	Data
Average Values								0.00	0.00	0.00	0.00	0.04	0.08	0.65	0.93	View

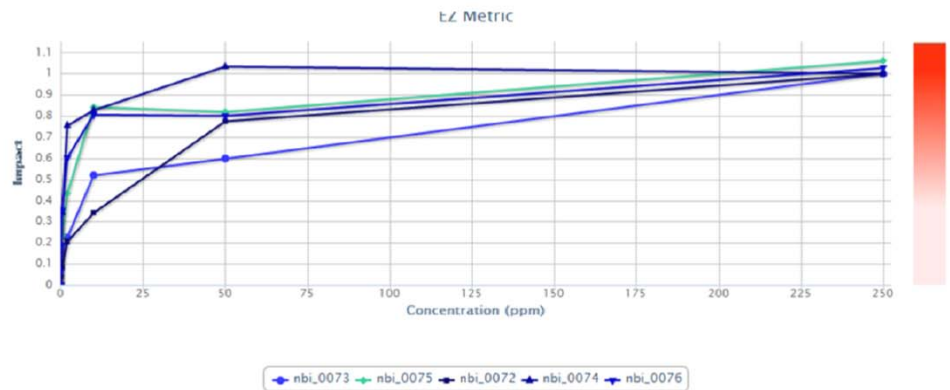
http://nbi.oregonstate.edu/

Material Record

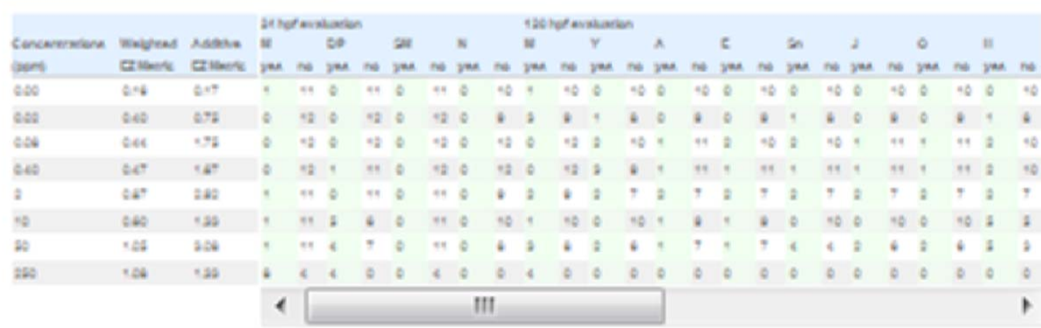
NBI Knowledgebase

Field	Value
NBI Material Identifier	44
Particle Descriptor	alumina-doped silicon diox
Investigator / Material Data Contributor: Name	Stacey Harper
Investigator / Material Data Contributor: Affiliation	Oregon State University
Investigator / Material Data Contributor: Email	stacey.harper@oregonstat
Material Type	metal oxide
Manufacture Date	
Manufacturer	Sigma-Aldrich
Synthesis Process	
Synthesis Precursors	
Purity	pure
Types of Impurities	
Primary Particle / Material Core Data:	
Primary Particle Size: Avg. (nm)	24.5
Primary Particle Size: Min. (nm)	0
Primary Particle Size: Max (nm)	49
Method of Size Measurement	BET
Instrument Used for Size Measurement	
Core Shape	irregular-angular
Core Structure	
Crystal Structure	
Core Atomic Composition	silicon dioxide [SiO2];aluminum oxide [Al2O3]
Number of Core Atoms	
Mass Core Atoms (ng)	
Core Shell / Coating (if present):	
Shell Composition	
Shell Surface Shape	
Shell Linkage	
Surface Linkages / Ligands (if present):	
Outermost Surface Functional Groups	
Surface Chemistry Linkage Group / Type	
Density of Surface Covered with Ligands (%)	
Minimum Number of Ligands	
Maximum Number of Ligands	
Complete Material:	
Mass of Core + Shell + Linkages and Ligands (ng)	

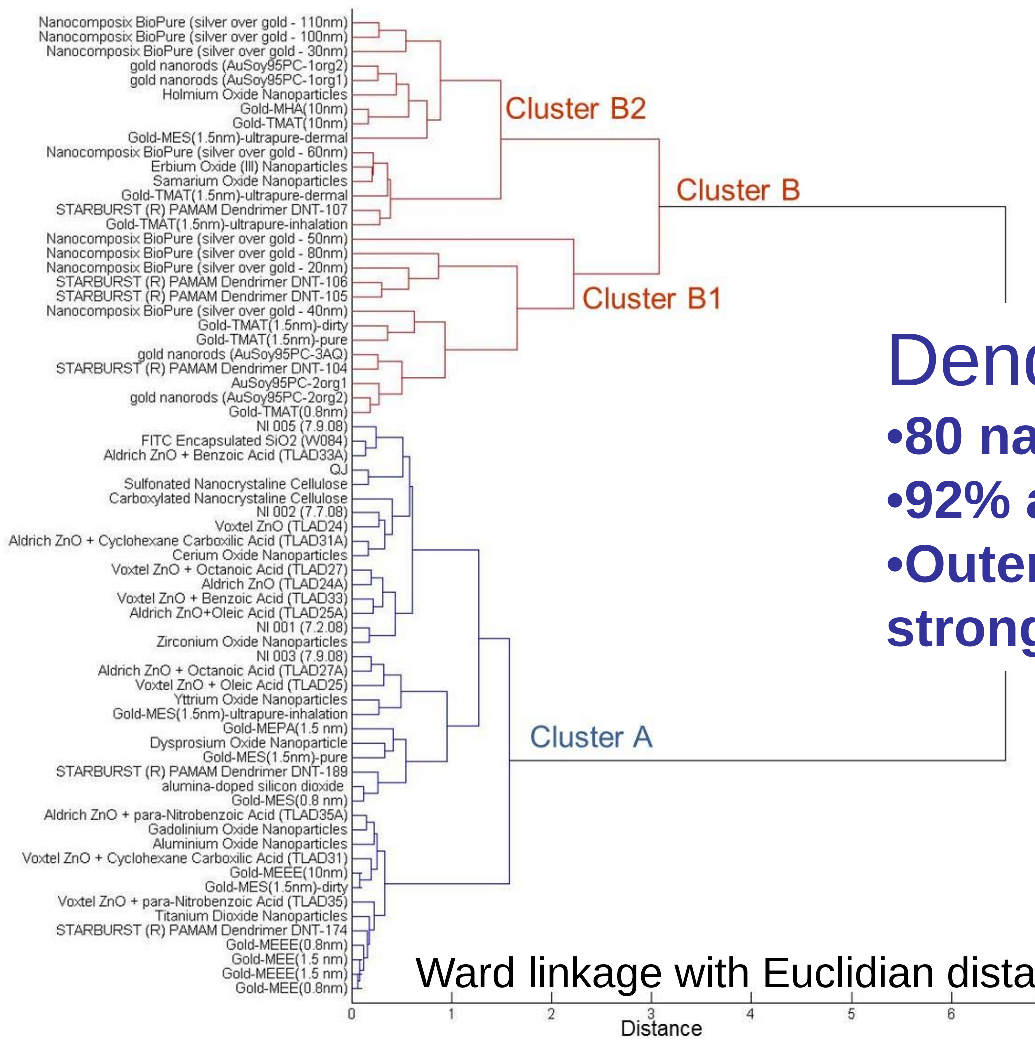
Experimental Data



Nanomaterial							EZ Metric								
ID	Family	Core	Surface Chemistry	Shape	Size	Charge	Concentration								
nbi_0073	metal	gold [Au]	phosphatidylcholine	rod	7	0	control	16 ppb	80 ppb	400 ppb	2 ppm	10 ppm	50 ppm	250 ppm	Data
Average Values							0.00	0.00	0.00	0.15	0.23	0.52	0.60	1.00	View
nbi_0075	metal	gold [Au]	phosphatidylcholine	rod	14	0	control	16 ppb	80 ppb	400 ppb	2 ppm	10 ppm	50 ppm	250 ppm	Data
Average Values							0.00	0.00	0.09	0.24	0.43	0.84	0.82	1.06	View
nbi_0072	metal	gold [Au]	phosphatidylcholine	rod	7	0	control	16 ppb	80 ppb	400 ppb	2 ppm	10 ppm	50 ppm	250 ppm	Data
Average Values							0.04	0.00	0.00	0.08	0.21	0.34	0.78	1.00	View
nbi_0074	metal	gold [Au]	phosphatidylcholine	rod	22	0	control	16 ppb	80 ppb	400 ppb	2 ppm	10 ppm	50 ppm	250 ppm	Data
Average Values							0.00	0.00	0.00	0.34	0.75	0.83	1.03	1.00	View
nbi_0076	metal	gold [Au]	phosphatidylcholine	rod	14	0	control	16 ppb	80 ppb	400 ppb	2 ppm	10 ppm	50 ppm	250 ppm	Data
Average Values							0.00	0.06	0.19	0.35	0.60	0.81	0.80	1.03	View



Clustering Analysis of EZ Metrics



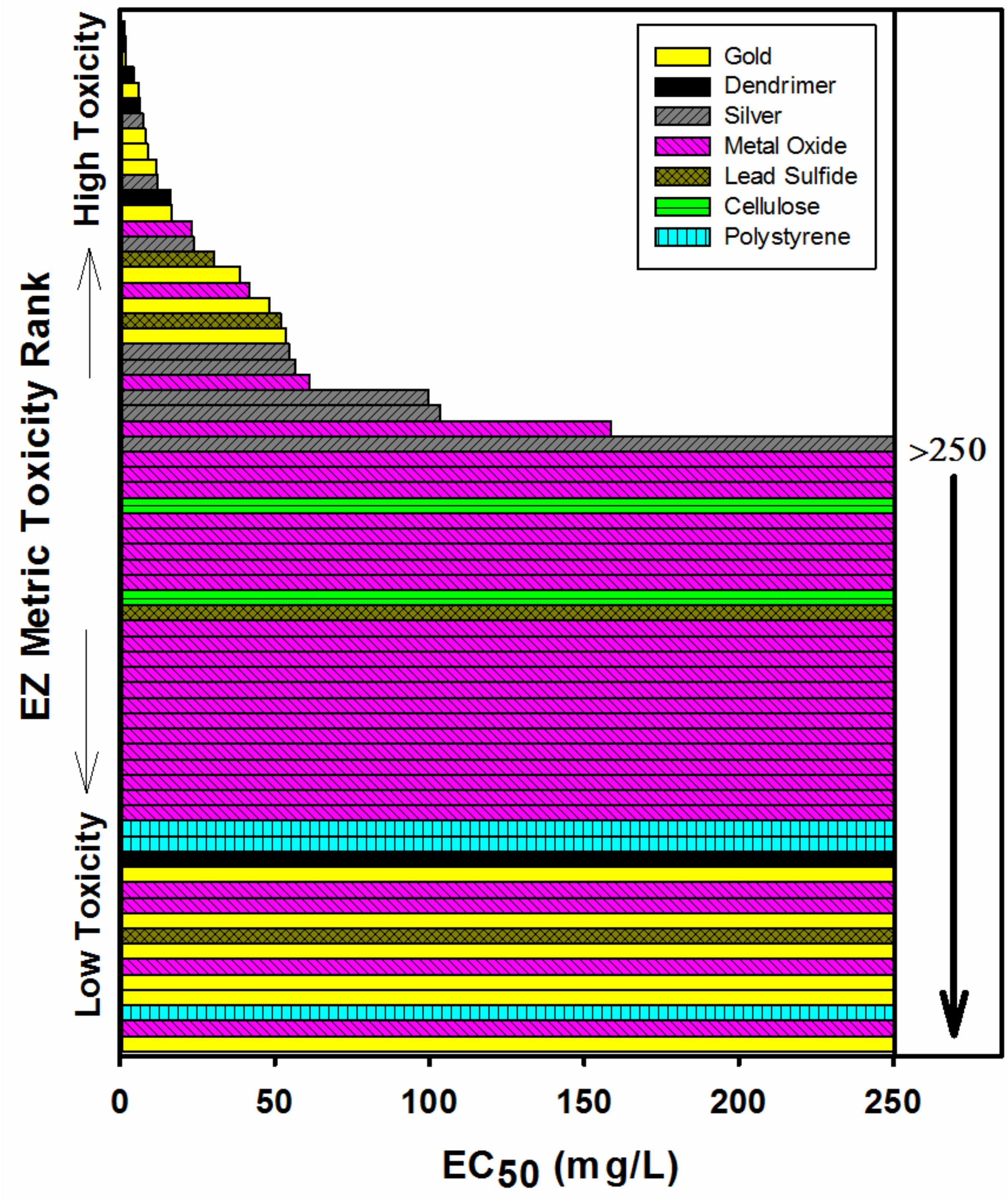
Ward linkage with Euclidian distance measure

Dendrogram plot

- **80 nanomaterial files**
- **92% accuracy**
- **Outermost surface chemistry strongest predictor**

Nanomaterial Hazard Ranking Based on EZ Metric Scores

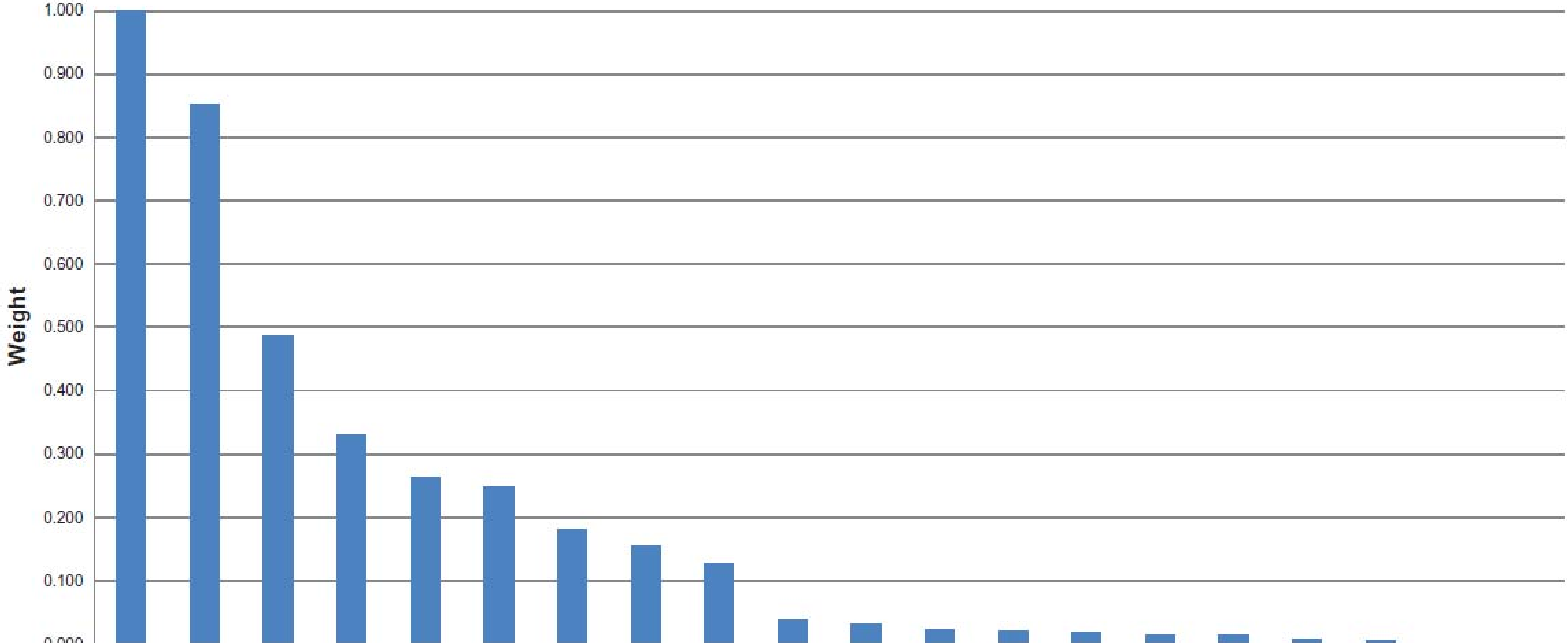
Color-coded to core composition



NEI Miner Analysis

82 nanomaterials at 8 concentrations

RELEIF algorithm attribute weights with respect to the 24 hpf mortality.

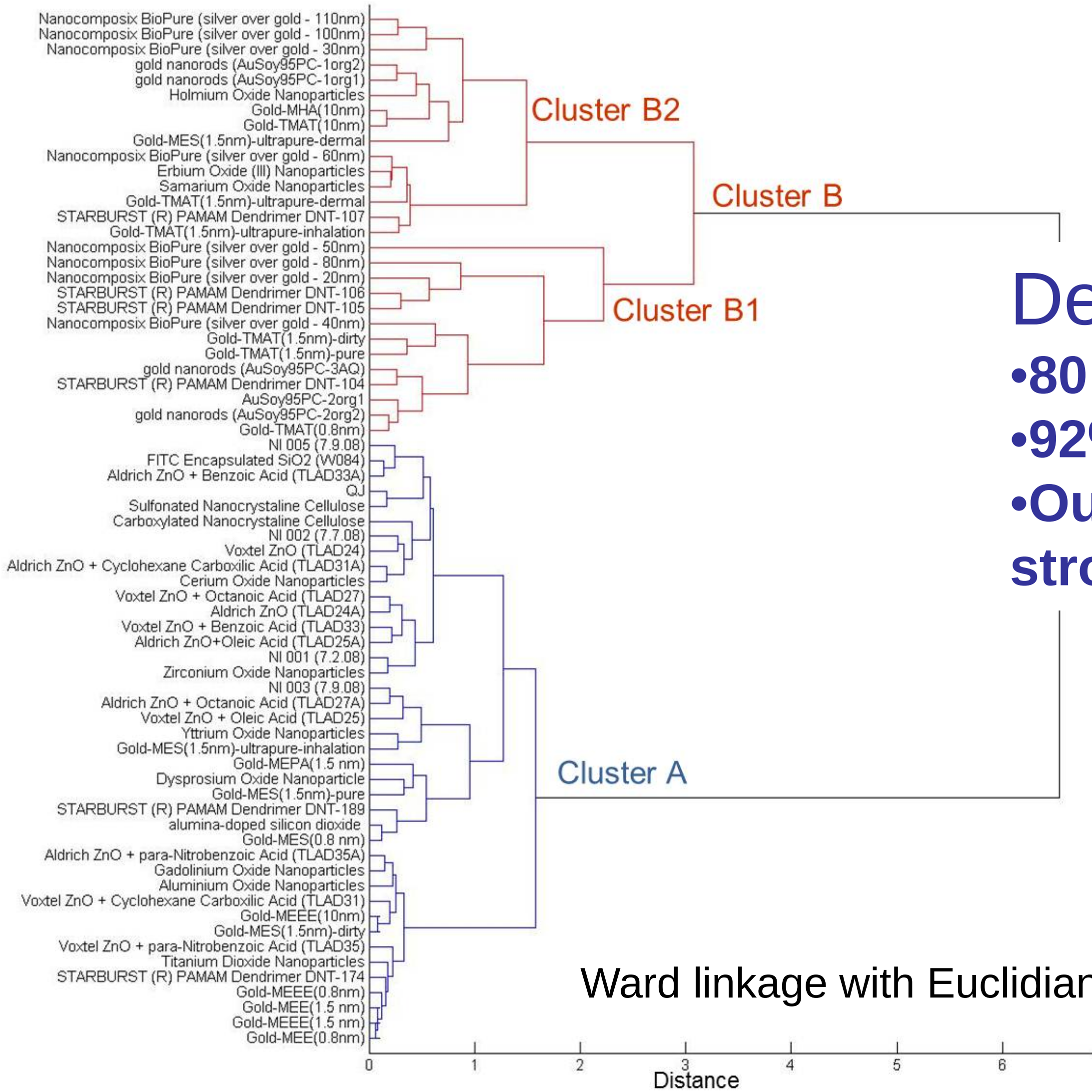


	Dosage concentrations used (ppm)	Shell composition	Outermost surface functional groups	Purity	Core structure	Surface charge	Synthesis precursors	Core atomic composition	Core shape	Primary exposure route	Material type	Minimum number of ligands	Material zeta potential in media (mV)	Solubility/ dispersity medium	Stable average agglomerate size in bedia (nm)	Primary particle size: Avg. (nm)	Primary particle size: max (nm)	Primary particle size: min. (nm)	Mass core atoms (ng)	Number of core atoms
Series 1	1.000	0.852	0.486	0.330	0.263	0.249	0.181	0.154	0.128	0.039	0.032	0.024	0.020	0.018	0.014	0.014	0.007	0.006	0.000	0.000

Tang et al., 2013

Clustering Analysis of EZ Metrics

68 nanomaterials based on summarized toxicity



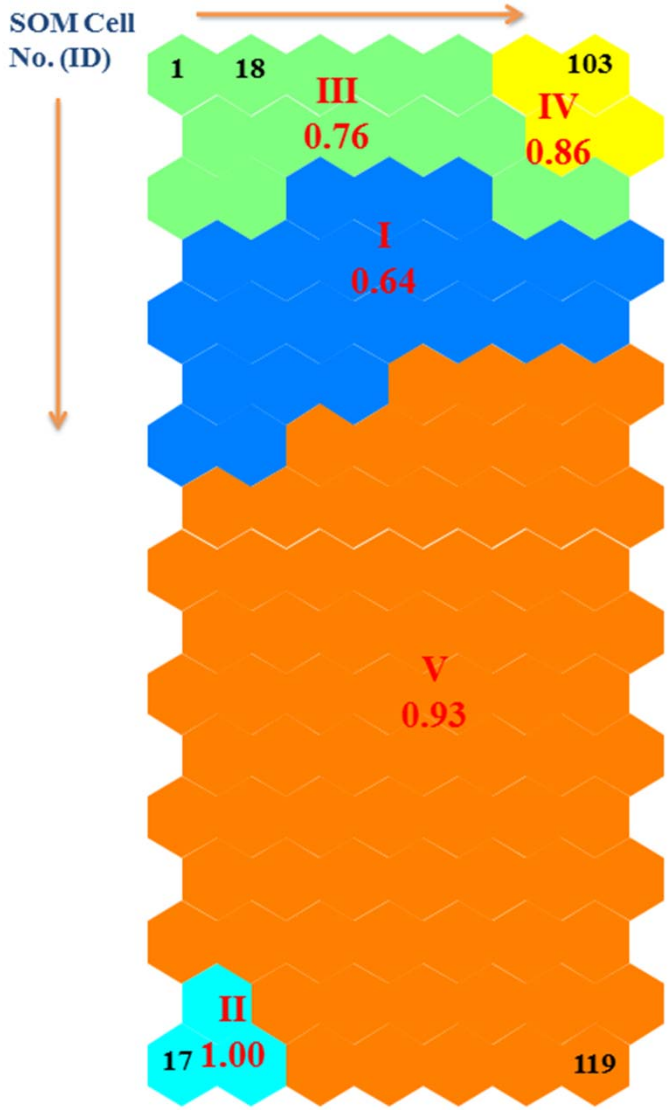
Dendrogram plot

- **80 nanomaterial files**
- **92% accuracy**
- **Outermost surface chemistry strongest predictor**

Ward linkage with Euclidian distance measure

Self-Organizing Map (SOM) Clustering

74 nanomaterials at 8 concentrations



SOM, where each cell (i.e. the hexagons) contains a certain number of similar NPs.

5 clusters of similar SOM cells were identified with a clustering index of 0.89 indicating a significant clustering pattern.

Particle concentration, surface chemistry and surface charge were related to the clustering pattern

Overall Consensus

	Exposure Concentration (ppm)	Outermost Surface Chemistry	Surface Charge	Core Composition	Partcile Size
NEI Minor	X	X	X		
MATLAB		X		X	X
Au GLM	X	X			X
SOM	X	X	X		

- Out of up to 20 input variables considered in each approach, only 5 features are shown to be predictive of nanomaterial toxicity.

- Outermost surface chemistry is the only determining feature common to all 4 modelling efforts.
  - Understanding changes in surface chemistry resulting from interactions with biological media should improve models.

- Interestingly, core composition was only found to be predictive in one case.
  - For single-composition nanoparticles like metal oxides core composition can be viewed as predictive.

Data Sharing – A case for this becoming the norm

- Widespread use of the NBI data in national and international efforts to understand nanomaterial hazards:
  - European Union's NanoSafety Modeling Cluster and NanoPUZZLES Project;
  - the US Nanomaterial Registry;
  - UCLA's Center for the Environmental Implications of Nanotechnology; and
  - Duke University's Center for Environmental Implications of NanoTechnology.
- Data mining of large experimental databases comprised of heterogeneous nanoparticles, such as the NBI, are useful for developing predictive models of nanomaterial toxicity.
- Predictive model refinement can be achieved through consensus modeling of the same large datasets.
- Data that includes thorough nanomaterial characterization <u>and</u> multiple endpoints provide the volume of information required for model development.

nanomaterial–biological interactions

Funding Acknowledgements

NIH National Institute of Environmental Health Sciences Grant #s T32ES007060 and ES017552

NIEHS National Institute of Environmental Health Sciences

USDA National Institute of Food and Agriculture Grant # 2013-67021-21181

USDA NIFA United States Department of Agriculture National Institute of Food and Agriculture

National Science Foundation Grant # 1438165

NSF

Air Force Research Laboratory Grant # FA8650-05-1-5041

AFRL THE AIR FORCE RESEARCH LABORATORY

Thank you for your attention!

"I'm on board for microbrews, but nanopizza is taking technology a step too far."

Balbus et al. (2005) Issues in Science and Technology