Exposure in Biological Systems

Review of the State of the Science

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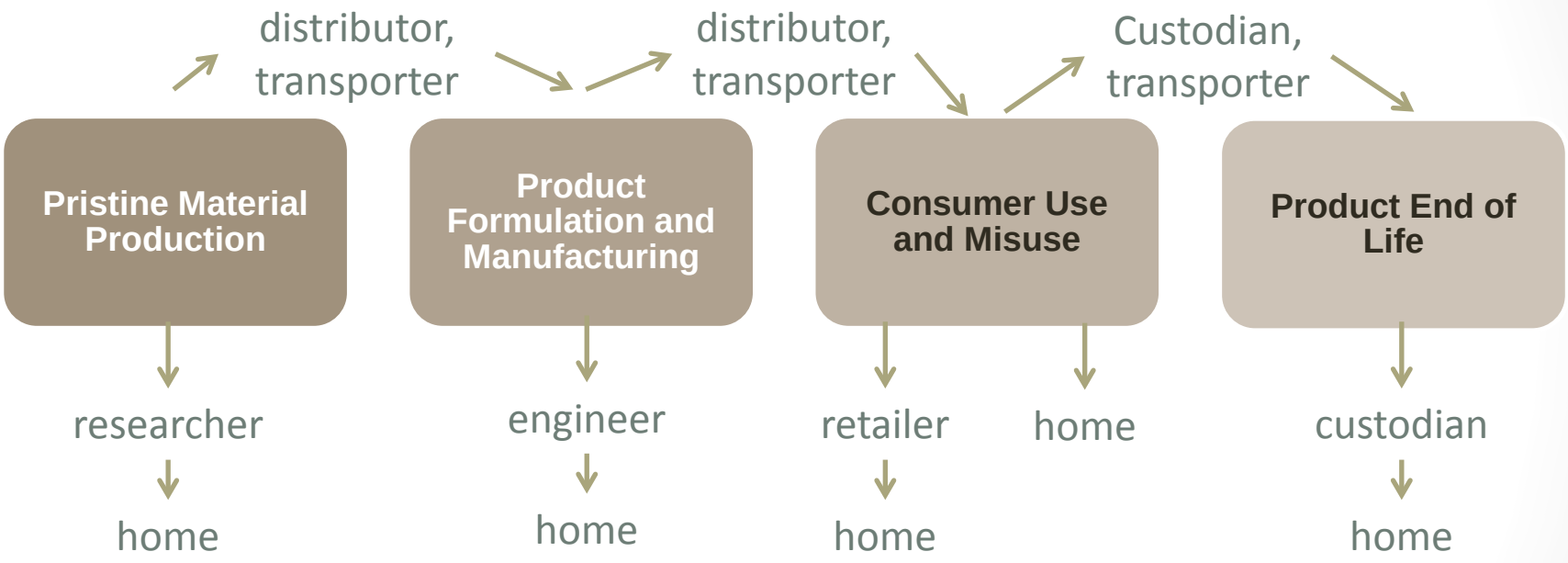
Outline of Talk

- Exposure across the product life
  - Biological intake
  - Hazard continuum
  - Mitigating exposures
- Nanomaterial monitoring
  - Detection and measurement
  - Biological monitoring measurands
  - Quantifying exposure
- Biological (toxicological) responses
  - Methods
  - Relevance to exposure science

(Nanomaterial) Exposure across the product life

Biological exposure per individual

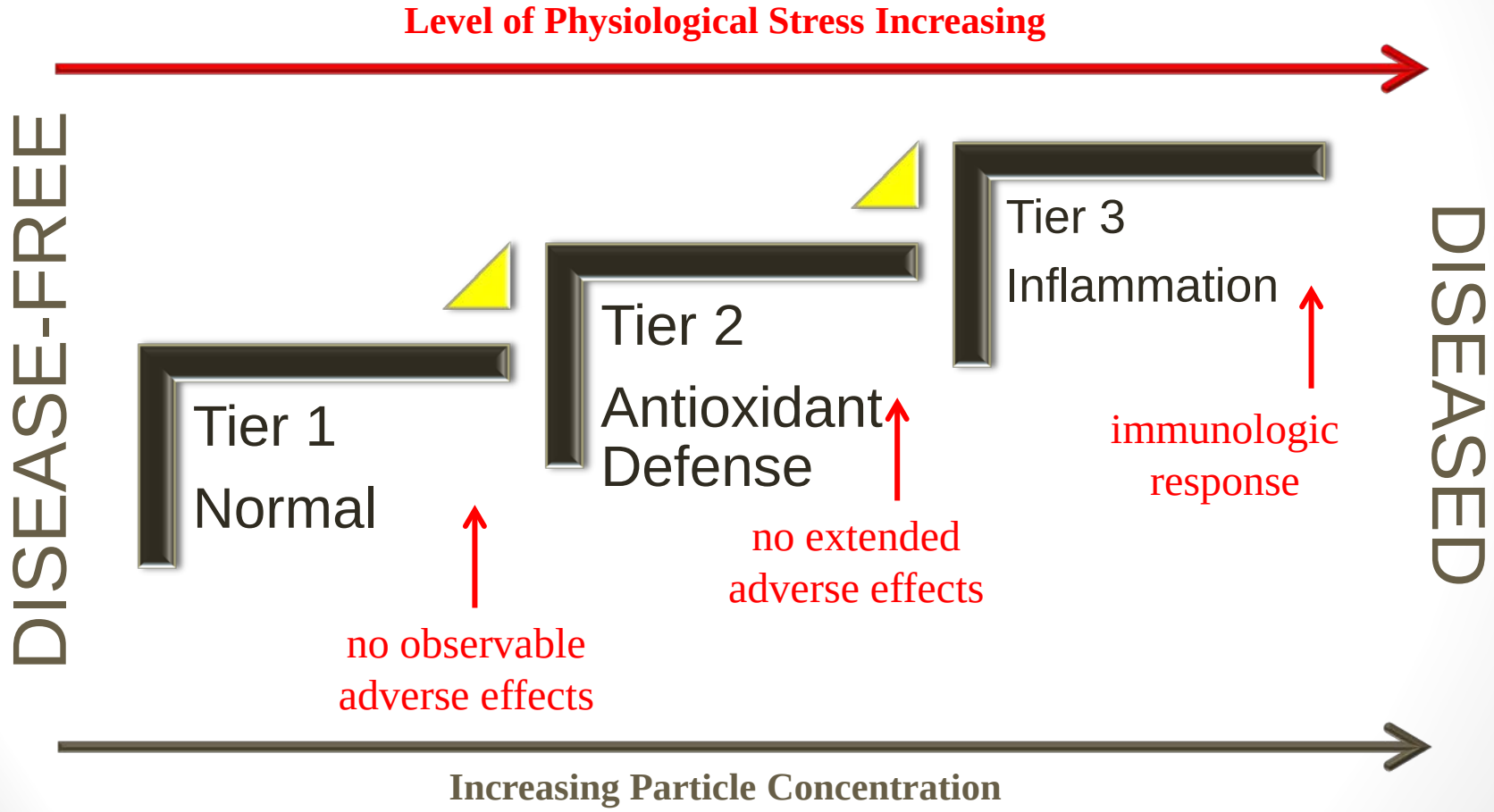


Biological intake has been shown though inhalation, ingestion, and dermal exposures

"There is a need to define the product intake fraction to quantify and compare exposures to consumer products"

- Jolliet, O. EST ahead of print (2015)
- Powers, C., et al. *Environment Systems and Decisions 35(1):76 (2015)*

Exposure is inevitable; Hazard exists on a continuum; Dose makes the poison



- Sayes C. et al. *Pharm Res* 31(9):2256 (2014)
- Li, N. et al. *Free Radic Biol Med* 44(9): 1689 (2008)

Hazard Continuum for Nanomaterials

More examples of the onset of disease:

Physical or Chemical Property	Transient Response	Sustained Response	Literature Evidence
High aspect ratio in shape	Frustrated macrophage, congestion	Fibrosis	Poland, C., et al. *Nature Nanotech* 3(7):423 (2008)
Small particle size (<10 nm)	Local penetration & inflammation	Abnormal ADME	Lim, G., et al. *J. Neurosci.* 20(15):5709 (2000)
High metal content	Dermatitis, allergies, hypersensitivity	Cancer, metal fume fever, infertile	Carter, J., et al. *TAAP* 146(2):180 (1997)
ROS	Oxidative stress	Cancers	Diehn, M., et al. N*ature* 458(7239):780 (2009)
Burnt carbon (smoke)	Asthma	Lung cancer, heart disease	Bruce, N. et al *Bul. WHO* 78(9) : 1078 (2000)
Airborne crystals	Granulomas	Silicosis	Mossman, B. et al. AJRCCM 157(5):1666 (1998)

Biological intake

1. Aerosol inhalation
   - Breathing vapors, small particulates
2. Ingestion
   - Swallowing aerosols, not washing hands
3. Dermal
   - Skin contact through abrasions, not washing hands
4. Puncture wounds
   - Used syringe needles or contaminated glassware
5. Eyes, nose, mouth
   - Splashes

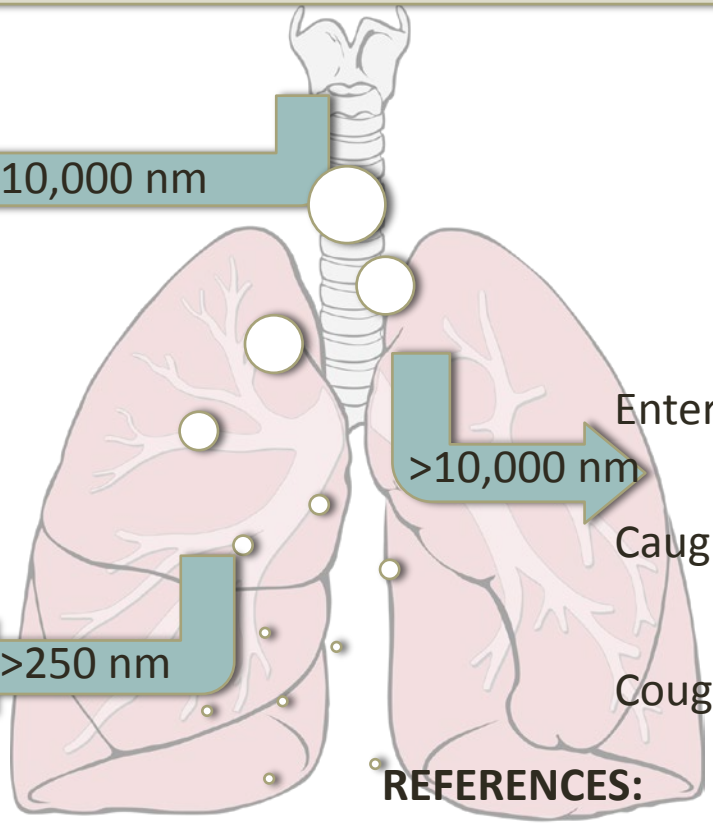
Common STOP-WORK Procedure

- Wash exposed area with warm soapy water for 15 minutes
- Flush eyes at eye wash station
- Call or visit the infirmary
- If injury is severe, call 9-1-1
- Report the incident to your supervisor
- File an Injury Report

Image: Alex Matus, http://sun.aos.wisc.edu

Inhalation Exposure

Many studies and guidance documents have focused on inhalation as the primary route of exposure to nanoparticles



Trapped by the mouth and nos

<10,000 nm

Coughed, sneezed, or ingested

>10,000 nm

Enter the conductive airways

Caught in mucociliary escalator

Coughed, sneezed, or ingested

Inhaled into the alveolar region of lung

>250 nm

Indication that NPs may enter the bloodstream and translocate

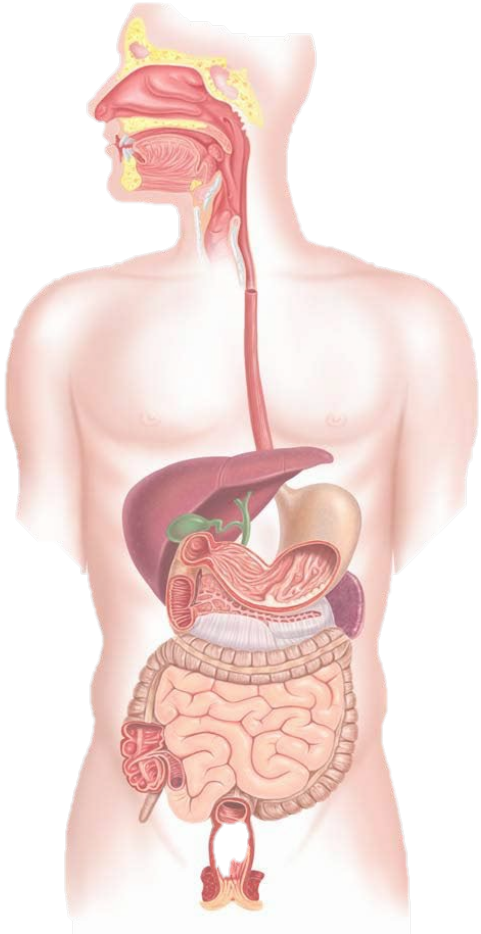
REFERENCES:

- Choi, H., et al. *Nat biotech* 28(12):1300 (2010)
- Oberdörster, G., et al. *JNN* 9(8):4996 (2009)
- Elder, A., et al. *EHP* (2006):1172

Ingestion Exposure

Exposure via ingestion is perhaps the least well researched biological exposure pathway

- Some nanomaterials are proposed for use in food packaging industry
- Some nanomedicines are meant to be ingested and translocate
- Nano-agents transform significantly during the digestion process



Digestion consists of 3 steps:

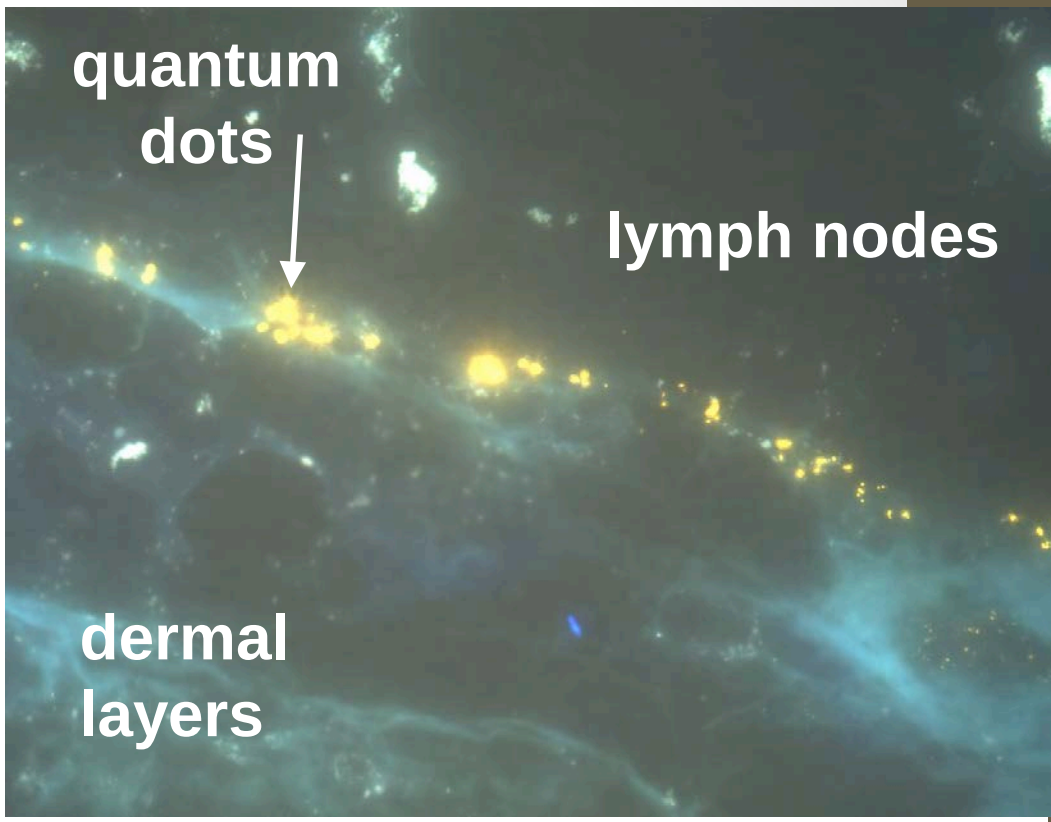
- **Step 1** – Saliva, pH ~6.5-7.0, residence time of 5 min
- **Step 2** – Gastric juice, pH ~2.0 - 3.0, residence time of 2 hours
- **Step 3** – Duodenal juice + bile juice, pH ~ 7.0 – 8.0, residence time of 2 hours

REFERENCES

- Rogers, K., et al. *STE* 420:334 (2012)
- Quadros, M., et al. *EST* 47(15):8894 (2013)

Other exposures

- **Ocular, nasal, dermal and puncture wound exposure through various barriers are also dependent on the size of the nanomaterial**
- **Methods have been developed to measure concentration of material/chemical at these exposure site**
  - Dermal exposure assessment method (DREAM) (SA_{skin} & $SA_{particles}$)
  - Pseudo-skin method
  - Setting threshold limit value (TLV) based on toxicity data



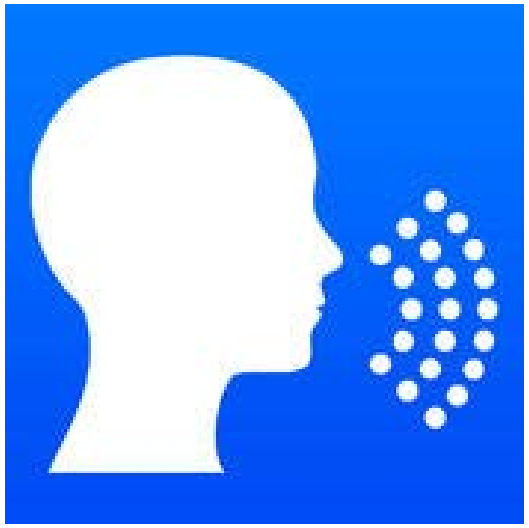
REFERENCES:

- **Nanoparticle (quantum dots) penetrate the dermal layers of the skin.** *Image courtesy of the FDA-NCTR*
- Johnson, D., et al. *EHP* 49 (2010).
- Bergamaschi, E. et al. *Nanotoxicology* 3(3):194 (2009)
- Warheit, D., et al. *Pharm. Ther.* 120(1):35 (2008)
- Dahm, M., et al. *Ann Occup Hyg* 56(5): 542 (2012)

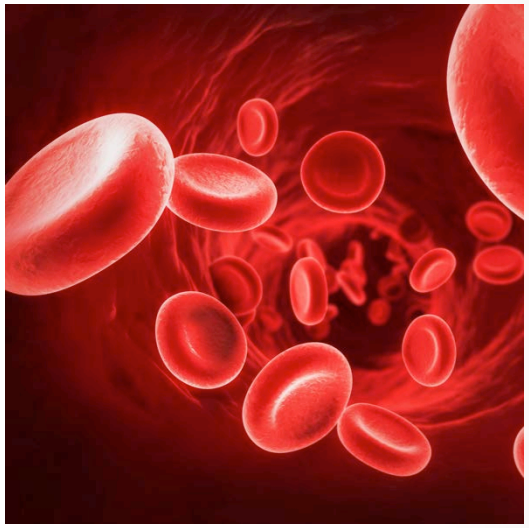
Nanomaterial monitoring

Monitoring is classified as Personal, Area, or Biological

Area



Personal



Biological

Monitoring is defined as observe and check the quality of (something) over a period of time; keep under systematic review
The most useful monitoring data is when personal, area, and biological samples are collected within the same system

A graded approach to measurements

The most useful monitoring data is when personal, area, and biological samples are collected within the same system

1 **Screen areas and processes**
Consider the particular characteristics of a facility

2 **Collect samples at source and personal space**
Including chemical and physical properties of the nanomaterial

3 **Analyze biological fluids**
Probing for changes in biomarker levels
Attention to immediate biological response

Area **Personal** **Biological**

REFERENCES:
UC Santa Barbara (http://www.cns.ucsb.edu)
SafeNano (http://www.safenano.org/knowledgebase/guidance/safehandling/)
NanoSafe, Inc. (http://www.nanosafeinc.com)
NIOSH (http://www.cdc.gov/niosh/topics/nanotech/)

Detection and Measurement of Nanoparticles - AREA

Current Methods

- Condensation nucleus or particle counters (CPC or CNC); particles are activated to droplets detected/quantified optically
- Ion-charged trapping electrometry: gives a sensitive proxy of surface area
- Measuring the size dependent Brownian motion over time (particles)
- Raman and Rayleigh scattering (photons)
- Scanning Electron Microscopy (SEM) with Energy Dispersive X-Ray Spectroscopy (EDS)
- Scanning Transmission Electron Microscopy (STEM)
- High Resolution Transmission Electron Microscopy (HRTEM)

Aerosol

Liquid

Both

Coupling to Size Selecting Instruments

- Differential Mobility Analyzer (DMA)
- APS and Scanning Mobility Particle Sizer (SMPS)
- Impactors: separate and count nanoparticles from larger particles
- Aerosol Mass Spectrometry: particles are vaporized, ionized, and analyzed

Detection and Measurement of Nanoparticles - PERSONAL

- Protective Equipment
  - Dermal exposure reduction
    - Gloves
    - Lab coats
    - Based on conventional IH
  - Inhalation exposure reduction
    - Respirators, dust masks
    - HEPA filtration
  - Ocular exposure reduction
    - No contact lens
    - Safety glasses or goggles
- Monitoring
  - Personal samplers
  - Gravimetric measuring (filter-based)
  - Photometric measuring

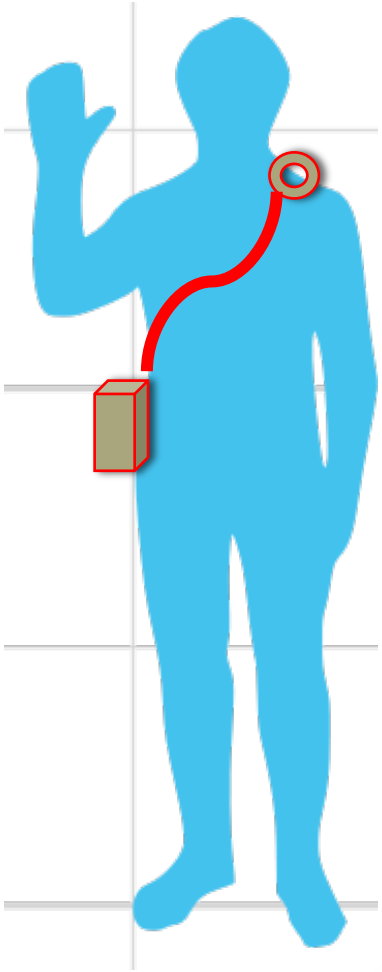


Image courtesy Wikimedia

Detection and Measurement of Nanoparticles - BIOLOGICAL

- Quantify exposure by measuring nanomaterials
  - Collection of tissue or body fluid for examination of contaminant concentration (parent material OR metabolite)
  - Biological exposure indices (BEI)
    - Intended for use in biological monitoring where the goal is the determination of the worker's internal dose of a chemical
- Quantify exposure by measuring biological markers
  - Relating the biomarker concentration to the nanomaterial internal dose
  - Measured in individual's blood, urine, or exhaled breath
  - Development of new methods for markers of biological effects
    - DNA and protein adducts
    - Chromosomal Aberrations
    - Genetic Markers

- *Morgan, M. The Biological Exposure Indices… EHP 105(1):105-115 (1997).*
- *Hemminki, K. DNA adducts in biomonitoring. J Occup Environ Med 37(1):44-51 (1995).*

Challenges in quantifying exposure by measuring biological markers

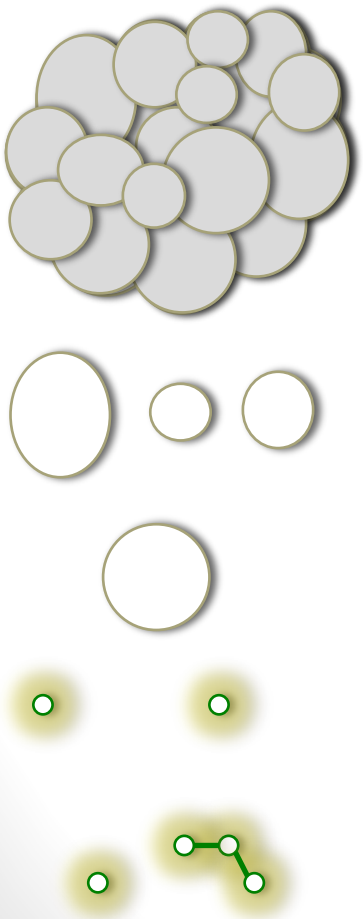
- No specific biomarker (gene, protein, enzyme, other) exists
- Type of exposure could change the biological response (single vs. multiple; direct vs. indirect)
- Environmental factors are still be assessed (efficacy of clothing, PPE, and even skin as barriers)

Potential Solutions

- Understand and catalog/categorize metabolites of nanomaterials
- Continue pathway-specific toxicity research over dose and time study designs

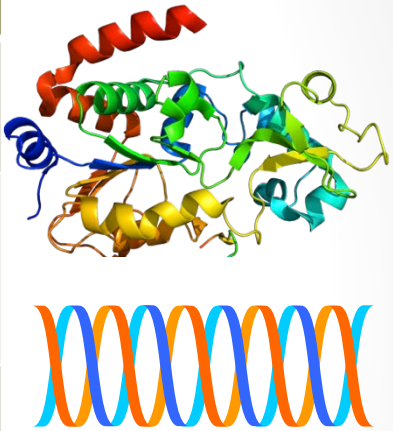
As the analyte size decreases, so does the methodology

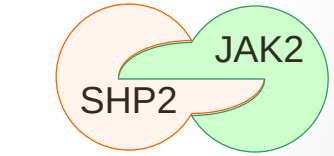
MEASURING NANOMATERIALS



Detection Method	Target	Detection Method
DLS, SEM, optical scope	Micro 10^{-6}	Colorimetric/ enzymatic
DLS, TEM	Nano 10^{-9}	ELISA, fluorescence, luminescence
ICP-MS, Raman, FTIR	Pico 10^{-12}	LC/MS, MALDI-TOF MS, GC, electrospray

MEASURING BIOLOGICAL MARKERS





The same understanding is needed in regard to sample concentration

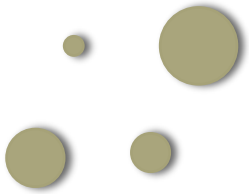
References:

- Cheng, M. et al. Curr Op Chem Bio 10:11 (2006)
- Cheng, F., et al. *Biomat* 26(7):729 (2005)
- Lynch, I., et al. *Adv Coll Interfac Sci* 134:167 (2007)

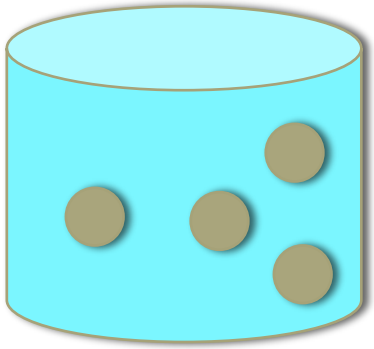
Detection and Measurement of Nanoparticles

- What do we need?
  - **Reliable methods** that detect and measure NPs in the media in which humans are exposed
  - **Identified properties** that are relevant to RISK and can be measured at low sensitivity

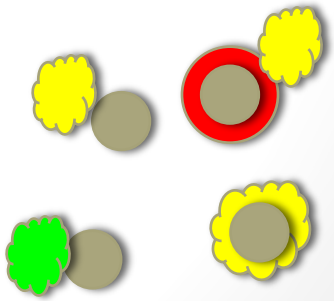
Size: NP dimensions are below diffraction limit of visible light



Concentration: low concentration require single chromophore detection technology

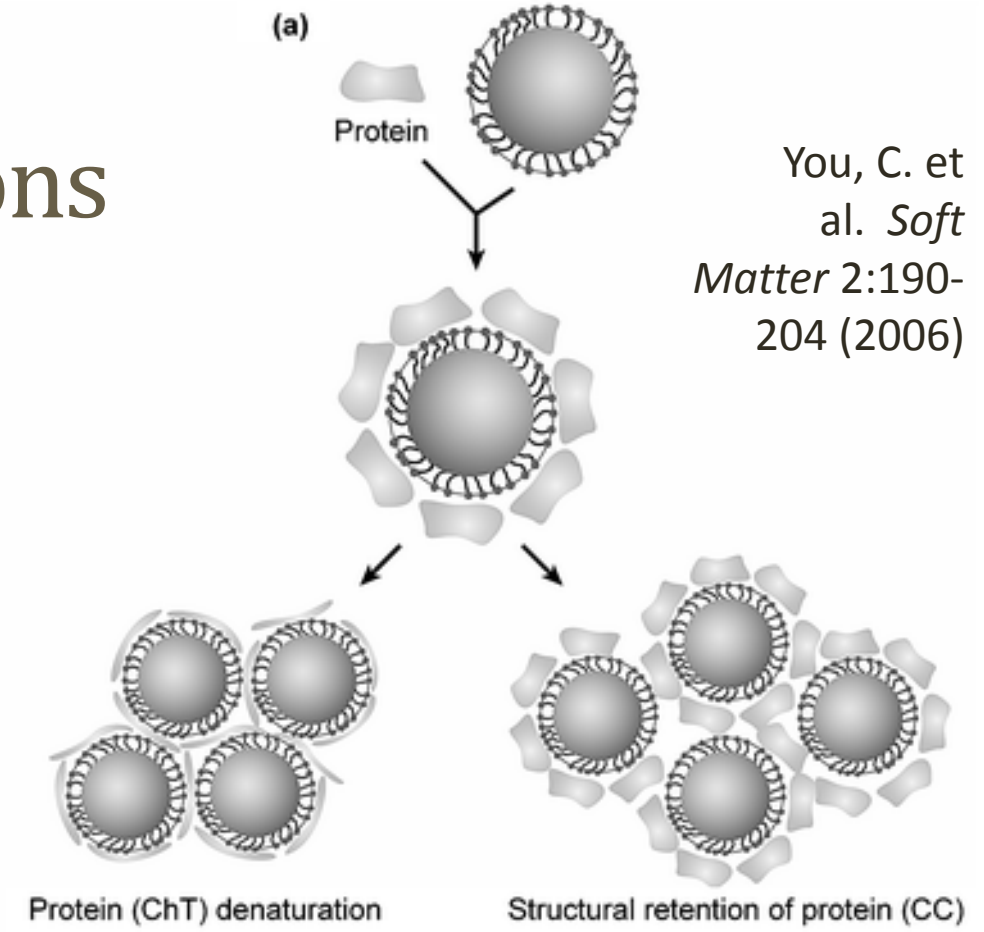


Composition: differentiate between core and surface

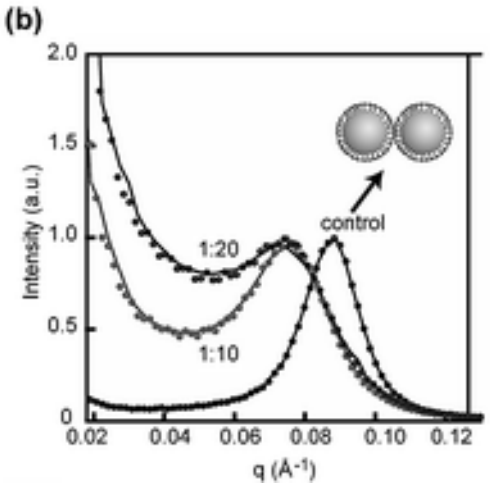


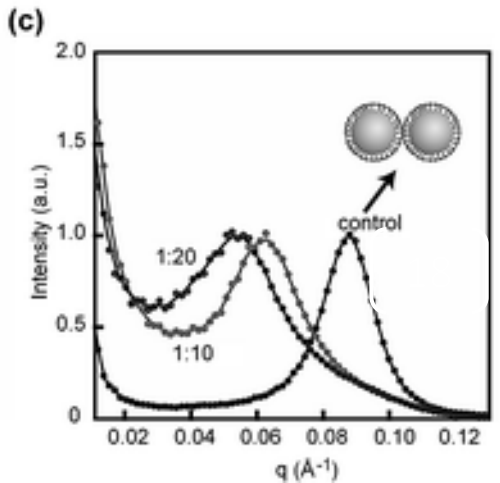
Bio-nano interactions

- Same dimensions
- Biomolecules are folded and shaped by weak bonds (side groups, H-bridges, and salt bridges)
- NPs disrupt their structure
  - Immediately adsorb onto the surface of the molecule at biological exposure site
- Adsorption is dependent on particle surface characteristics
- This phenomenon compromises detection method & risk evaluation



You, C. et al. *Soft Matter* 2:190-204 (2006)





Bio-nano interactions

- It is important to consider the “dose rate”
  - Spread within the body
  - Decay in number concentration
  - Metabolites of individual particles
  - Solubility – use of surfactants pose new questions

One of the major emerging issues to be discussed with the “bio-nano interface” field is the particle grouping with little or no solubility (or those particles that do not biodegrade at the bioaccumulation site

Potential Path Forward

- **Learn from the polyaromatic hydrocarbon community**
  - "Determination of the DNA and protein adducts of PAHs is the most suitable way of estimating this risk"
  - Angerer, J. International Archives of Occupational and Environmental Health. 70(6):365 (1997).
- **Use mass spectroscopy in toxicity studies to better understand biomarkers in fluids**
  - "We propose that LC-MS/MS be used to characterize proteins found in both synthetic and natural NPs"
  - Martel, J. Anal Biochem. 418(1):111 (2011).
- **Apply mechanistic biochemistry principles**
  - "The MALDI-TOF signature changed significantly when the characteristics of the nanoporous silica were altered"
  - Terracciano, R. et al. PROTEOMICS 6(11):3243 (2006)

Can the already-published nanotoxicology data tell us anything about exposure?

Exposure routes

- Inhalation
- Ingestion
- Dermal
- Muscous

Triggered pathways

- Sensitization/irritation
- Inflammation
- DNA damage and repair

Cell and tissue damage

- Lung, cardiovascular, liver

Form

- Metabolites
- Cradle to grave
- E-fate
- Particle kinetics

Accumulation, translocation

- Mucous membrane
- Skin penetration
- Body burden
- Lymph system
- Macrophages

Pathway	Major Finding	Citation
NFκB	Quantum dot nanoparticles induce the NFκB pathway even at low concentrations	A. Romoser, et al. Molecular Immunology 48 (2011) 1349-1359
NF-κB and AP-1	MWCNT induce oxidative stress which can trigger AP-1 and NfκB pathways even at low doses	P. Ravichandran, et al. Apoptosis 15 (2010) 1507–1516
NF-κB and JNK/P53	Silica nanoparticles induce apoptosis through the JNK/p53 pathway and pro-inflammatory response through the NFκB pathway	X. Liu, et al. Biomaterials 31 (2010) 8198-8209
Caspase 8/t-Bid independent apoptosis	Titanium dioxide nanoparticle exposure induces a mitochondrial apoptosis pathway independent of the caspase 8/t-Bid pathway	Y. Shi, et al. Toxicology Letters 196 (2010) 21-27
MAPK	MAPK proteins induce the NFκB pathway which is responsible for controlling much of the inflammatory response	A. Romoser, et al. Toxicology Letters 210 (2012) 293-301
NRF2	NRF2 pathway is induced by nanoparticle exposure and different cell lines have differential susceptibility	J. Berg, et al. Toxicology in Vitro 27 (2013) 24-33
ATF-2	Silica nanoparticle exposure activates ATF-2 pathway even at subtoxic doses	B. Mohamed, et al. Journal of Nanobiotechnology 9 (2011) 1-14
DDR	Silica nanoparticles induce DDR via Chk1-dependent G2/M checkpoint signaling pathways	J. Duan, et al. PLoS One 8 (2013) 1-13
Apoptosis	Gold nanoparticles induce multiple modes of cell death simultaneously, including apoptosis and necrosis	M. Lin, et al. J Nanopart Res 15 (2013) 1745-1759
DDR	Zinc oxide nanoparticles induce DNA damage and p53 is a major component of thi DDR	K. Ng, et al. Biomaterials 32 (2011) 8218-8225
DDR	Nanoparticle physiochemical characteristics dictate DNA damage and response	S. Barillet, et al. J Nanopart Res 12 (2010) 61–73
DDR and Inflammation	Silver nanoparticles can modulate gene expression and protein function leading to defective DDR and inflammatory response	P. AshaRani, et al. Genome Integrity 3 (2012) 1-14
Inflammation	Al2O3, Au, Ag, SiO2 nanoparticle exposure showed sublethal pro-inflammatory responses related to ROS generation, and ZnO and Pt nanoparticle exposure showed lethal genotoxic responses	R. Rallo, et al. Environ. Sci. Technol 45 (2011) 1695–1702
Apoptosis	Carbon black nanoparticle exposure induces apoptosis through ROS dependent mitochondrial pathway whereas titanium dioxide nanoparticles induce cell death through lysosomal membrane destabilization and lipid peroxidation	S. Hussain, et al. Particle and Fibre Toxicology 7 (2010) 1-17
Apoptosis	Silver nanoparticle exposure induces oxidative cell damage through inhibition of reduced glutathione and induction of mitochondria-involved apoptosis	M. Piao, et al. Toxicology Letters 201 (2011) 92-100
Apoptosis	Silica nanoparticle exposure induces ROS mediated apoptosis which is regulated through p53, bax/bcl-2 and caspase pathways	J. Ahmad, et al. Toxicology and Applied Pharmacology 259 (2012) 160-168
Autophagy	Gold nanoparticle exposure induces autophagy and oxidative stress	J. Li, et al. Biomaterials 31 (2010) 5996-6003

8 h					24 h			
Silver	Fullerol	QD	TiO_2		Silver	Fullerol	QD	TiO_2
0.42	1.03	0.63	0.81	ADORA2A	0.67	-	0.74	0.41
0.79	1.21	1.01	1.03	C5	0.82	1.77	1.12	1.45
0.68	0.80	0.87	0.94	CASP1	1.20	1.71	1.40	1.06
1.18	0.85	0.88	1.05	CASP4	1.28	1.27	1.00	1.03
0.81	0.62	0.77	0.88	CCL2	0.96	0.82	1.07	1.36
1.12	1.01	1.01	1.05	CD55	1.94	2.26	1.50	0.95
0.95	0.63	0.77	0.84	CHUK	1.76	1.59	1.28	0.77
0.65	1.13	0.93	0.96	COLEC12	0.88	1.44	0.97	0.81
0.55	1.11	0.92	1.14	FN1	1.69	2.24	1.76	1.73
27.63	1.13	0.74	0.80	HMOX1	11.16	1.79	1.48	0.63
0.48	0.96	0.86	1.01	IFNA1	1.13	1.34	1.46	2.16
0.95	1.02	0.69	0.92	IFNGR1	1.31	1.62	1.49	1.33
0.91	0.76	0.87	0.82	IFNGR2	0.90	1.70	1.73	1.42
0.92	1.20	1.00	1.14	IKBKB	1.12	2.26	1.15	1.68
0.79	1.23	0.50	1.34	IL10	1.46	1.08	0.68	1.90
0.60	1.26	1.36	0.79	IL1A	5.13	2.26	2.37	2.18
0.78	0.80	0.55	1.09	IL1B	4.24	1.90	1.26	2.26
0.28	1.32	0.97	0.52	IL1F7	29.50	14.73	13.96	36.11
0.65	0.83	0.86	0.93	IL1R1	0.63	1.56	1.17	0.96
0.92	1.66	1.03	1.36	IL1RAP	1.99	2.39	1.33	2.13
1.11	0.65	0.99	0.56	IL1RL2	0.40	1.64	1.09	1.20
1.52	0.81	0.88	0.77	IL6	2.64	2.42	1.58	1.08
0.89	1.20	1.18	1.88	IRAK1	2.71	2.29	3.19	3.20
0.77	0.58	0.70	0.64	IRAK2	1.39	0.63	0.74	0.82
0.76	1.30	0.82	1.21	IRF1	0.77	1.54	1.45	1.81
0.84	1.05	0.85	1.04	LY96	1.82	1.24	1.09	0.90
0.73	1.05	0.92	1.15	MAPK14	1.26	1.58	1.48	1.51
0.78	0.85	0.94	0.94	MAPK8	1.56	1.81	1.58	1.63
0.94	1.20	1.00	1.16	MIF	1.67	1.35	1.09	0.90
0.77	1.18	1.18	1.34	MYD88	1.25	1.74	1.45	1.78
0.90	0.90	0.95	0.90	NFKB1	1.47	1.30	1.16	0.80
0.72	1.29	1.14	1.29	NFKB2	1.18	1.77	2.21	1.36
1.12	1.03	0.95	1.00	NFKBIA	1.01	1.11	1.11	0.65
0.37	0.52	0.81	0.53	NLRC4	1.91	2.59	1.99	0.55
0.79	1.09	0.80	1.08	SERPINE1	0.98	1.47	0.88	1.30
0.87	1.03	1.01	1.14	TGFB1	0.94	1.55	1.62	1.77
1.17	2.59	1.80	1.92	TLR3	0.74	1.42	1.38	0.84
1.13	0.85	0.93	0.99	TLR4	1.09	1.01	0.95	0.87
1.09	1.84	1.36	0.97	TLR6	1.28	1.87	1.38	1.28
0.90	1.11	0.90	1.00	TNFRSF1A	0.84	1.13	0.86	0.37
1.16	0.71	1.02	0.75	TOLLIP	1.39	1.32	1.22	0.65
1.13	0.77	0.98	0.79	TRAF6	1.37	1.58	1.38	0.92

Immune Gene Expression Changes in Human Cells

Up- and down-regulation of family member genes after prolonged exposure shows preparation for inflammation

Fold suppressions of <0.5 are colored dark green and 0.5-0.8 light green. Fold inductions of 1.2-2.0 are pink and >2.0 red

Conclusions

- It makes sense to control exposure to those nanomaterials for which preliminary hazard data has already shown unwanted health effects or for those nanomaterials where the hazards are unknown

- When it comes to human exposure, measuring markers in biological systems is a useful tool in moving exposure science, toxicology, and nanotechnology forward

- There are some research projects discussed yesterday and today that are worth commissioning