Traditional Toxicity Studies: are they applicable and how do they reflect what will happen as particles enter and move in the environment.

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Traditional Toxicity Studies

Standard organisms: rat, Daphnia, fathead minnow, honeybee, fly, worm

- §Dose them and Kill them
- §Reproductive and long-term studies if lucky

Traditional Toxicity Studies

How well do these predict toxicity in environment for other chemicals?

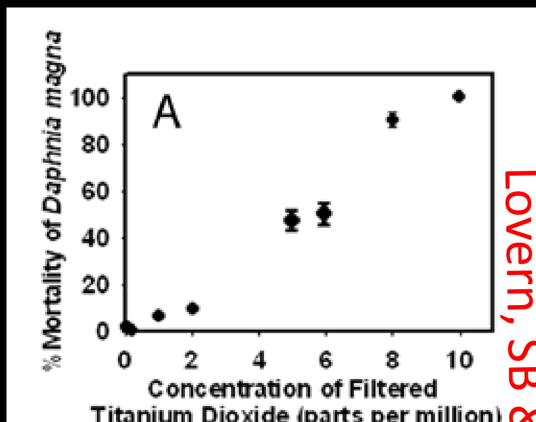
- §Does not cover transformation in environment
- §Does not cover fate and compartmentalization
- §Does not provide information on every organism or effect

Traditional Toxicity Studies

Can we use them for nanos?

I. We can use current testing strategies for nanos with adjustments

I. Nanoparticles can be toxic



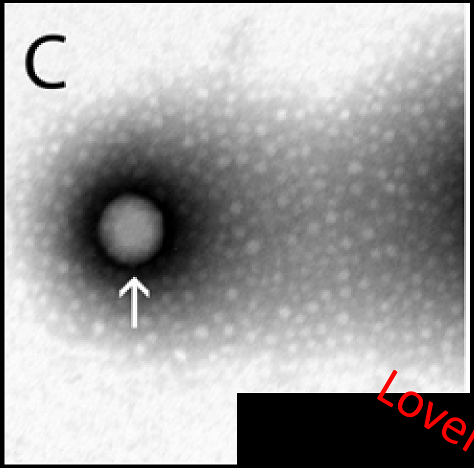
Lovern, SB & R Klaper. 2006.

LC50 = 5.456ppm

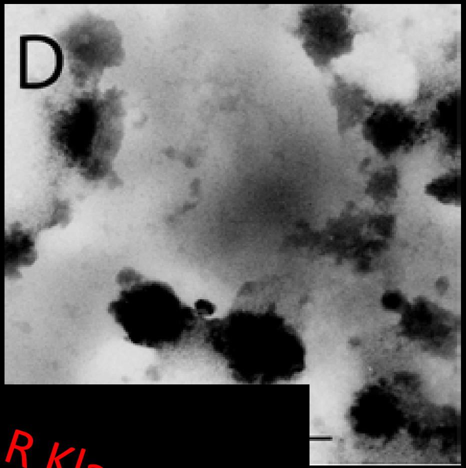
Mortality after Exposure to Titanium Dioxide or Fullerenes

LC50 = 1.970ppb

III. The cause of this difference is debatable



Aggregation Size Matters



Lovern, SB & R Klaper. 2006.

Lovern, SB & R Klaper. 2006.

III. The cause of this difference is debatable

- Surface chemistry?
- Solubility?
- Surface area?
- Charge?

- For science and nanomaterial development this is important---Is it important for assessing the risk?

Traditional Toxicity Studies

Can we use our toxicity studies to examine mechanism of action?

II. Nanoparticle toxicity depends on particle type (core, surface chemistry)

Klaper et al. 2009. *Environmental Pollution*

Klaper et al. 2009. *Environmental Pollution.*

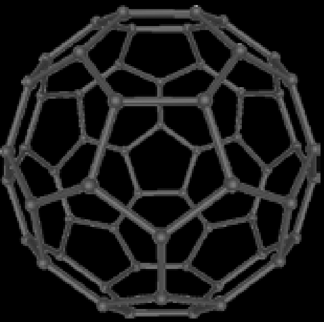
IV. Toxicity May Be Partly Due to Oxidative Stress

Klaper et al. 2009. *Environmental Pollution.*

Klaper et al. 2009. *Environmental Pollution.*

Traditional Toxicity Studies

Adding other measures to inform our knowledge regarding nanomaterial effects

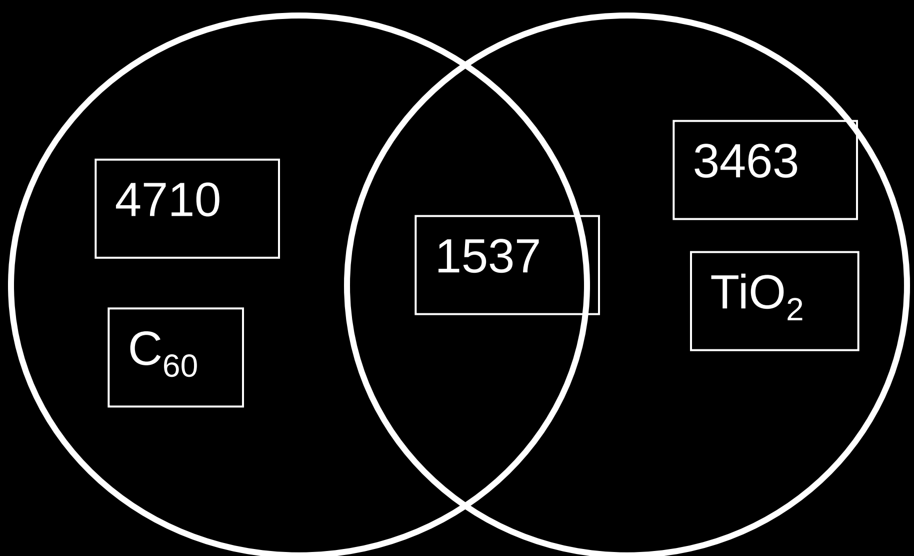


Genomic studies

- Normalized libraries
  - Fullerene, Titanium dioxide
  - Collaboration with DGC and JGI
  - 5000 sequences each library
  - Annotation project

Differential Gene Expression Depending on Type of Nanoparticles

TiO2 versus Fullerenes



Genes and Functional Categories of C_{60} Exposures

- Apoptosis or Anti-apoptosis
  - Caspase 8 precurser
  - Oxidoreductase
  - Serine kinases
- Chitin processes
  - chitinases
- Metabolism
  - Glutathione S-transferase
- Electron transport
  - Cytochrome P450's
  - Cytochrome C oxidase
  - NADH dehydrogenase
- Translation
  - 40,60S Ribosomal
- Protein phosphorylation

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Nanoparticle specific testing?: The cellular and gene expression effects of manufactured nanoparticles on primary cell cultures of rainbow trout macrophages

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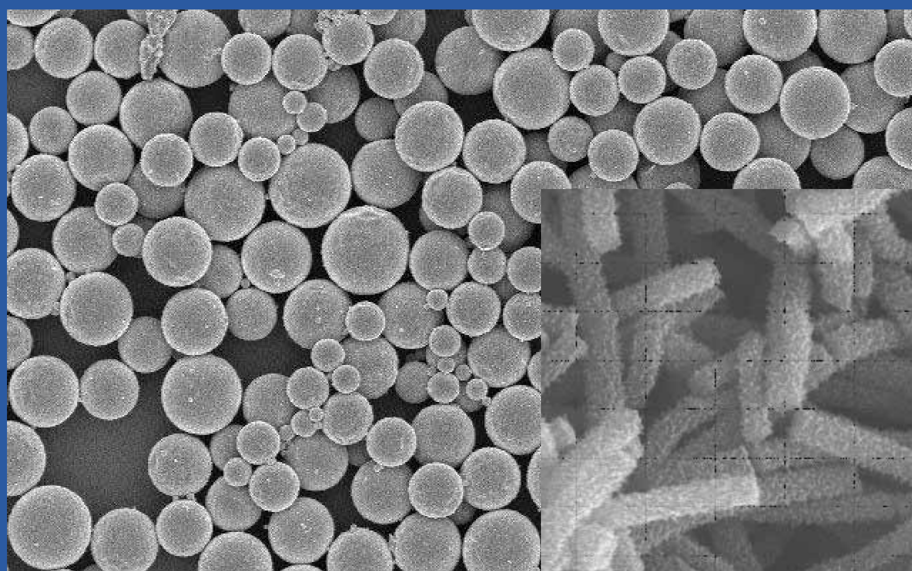
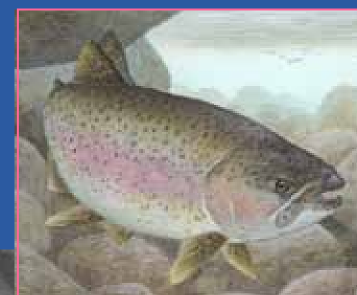
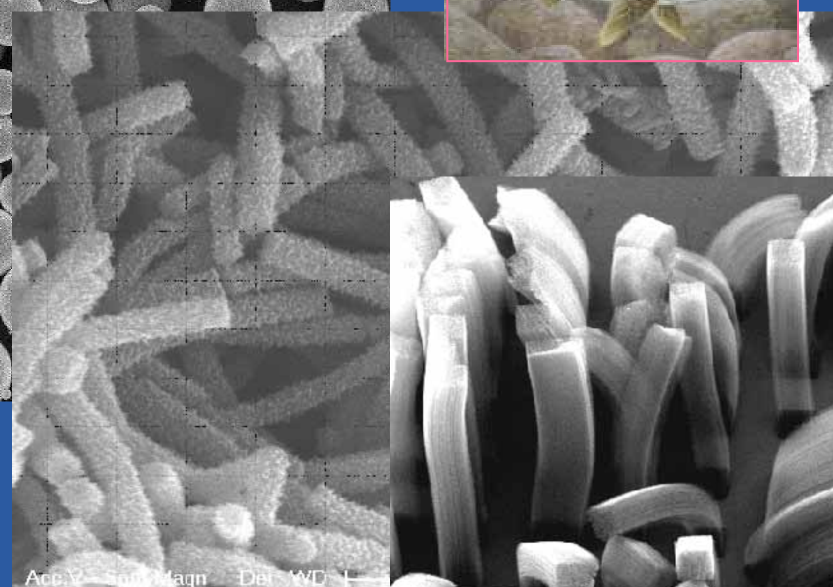
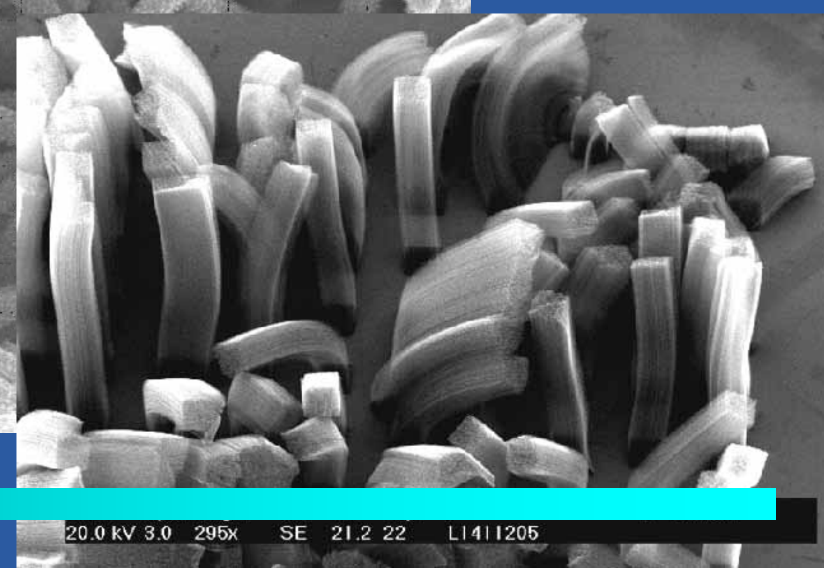
Nanoparticle specific testing?:

The cellular and gene expression effects on immune response
Nanoparticles should be considered foreign









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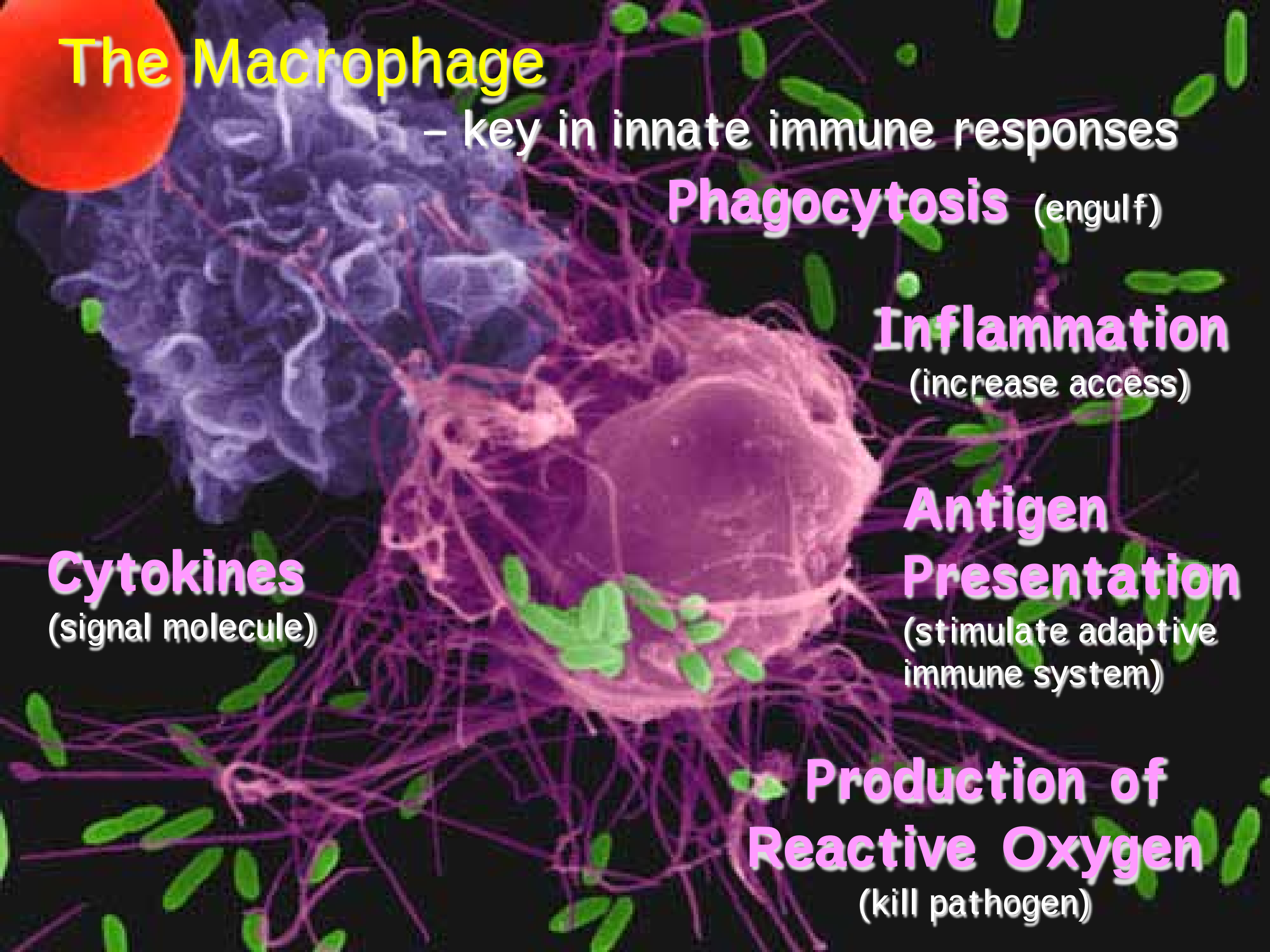
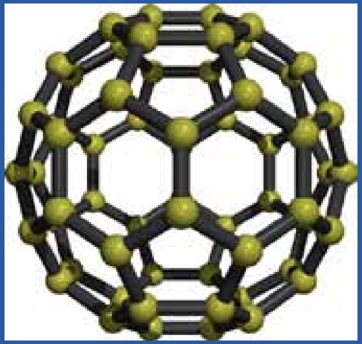
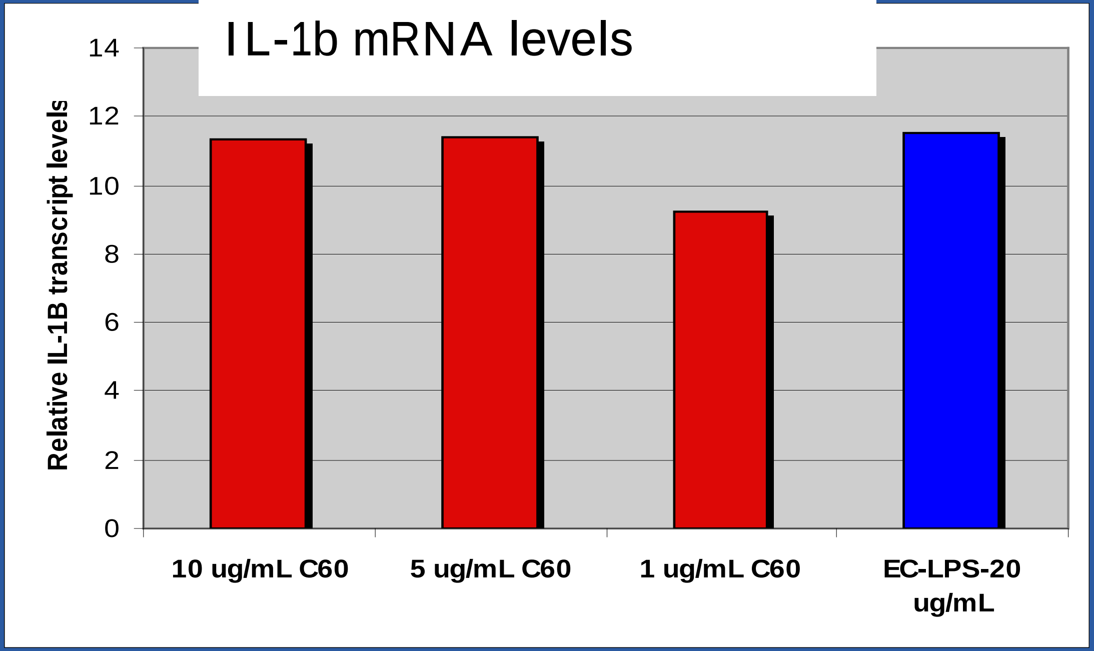
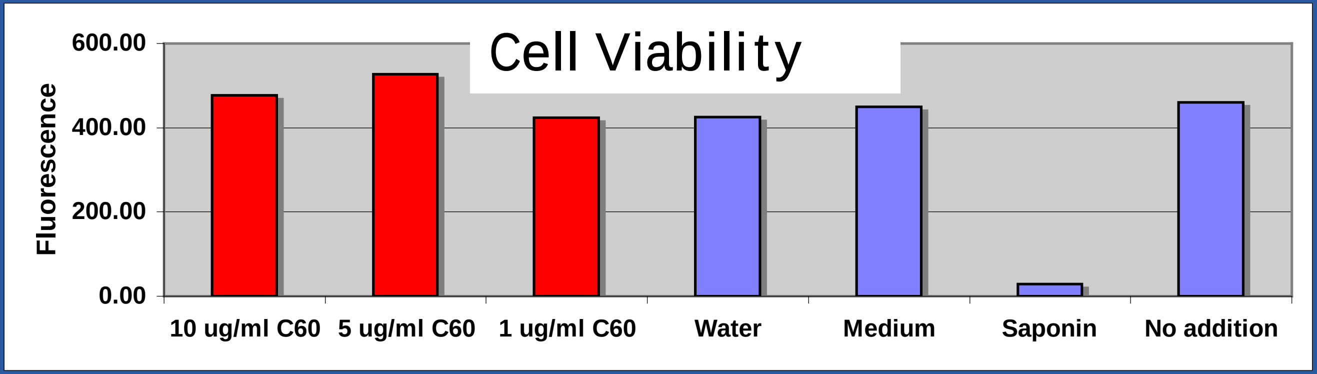

The Macrophage
– key in innate immune responses
Phagocytosis (engulf)
Inflammation
(increase access)
Antigen Presentation
(stimulate adaptive immune system)
Cytokines
(signal molecule)
Production of Reactive Oxygen
(kill pathogen)

C60 Fullerene stimulates interleukin 1b transcription



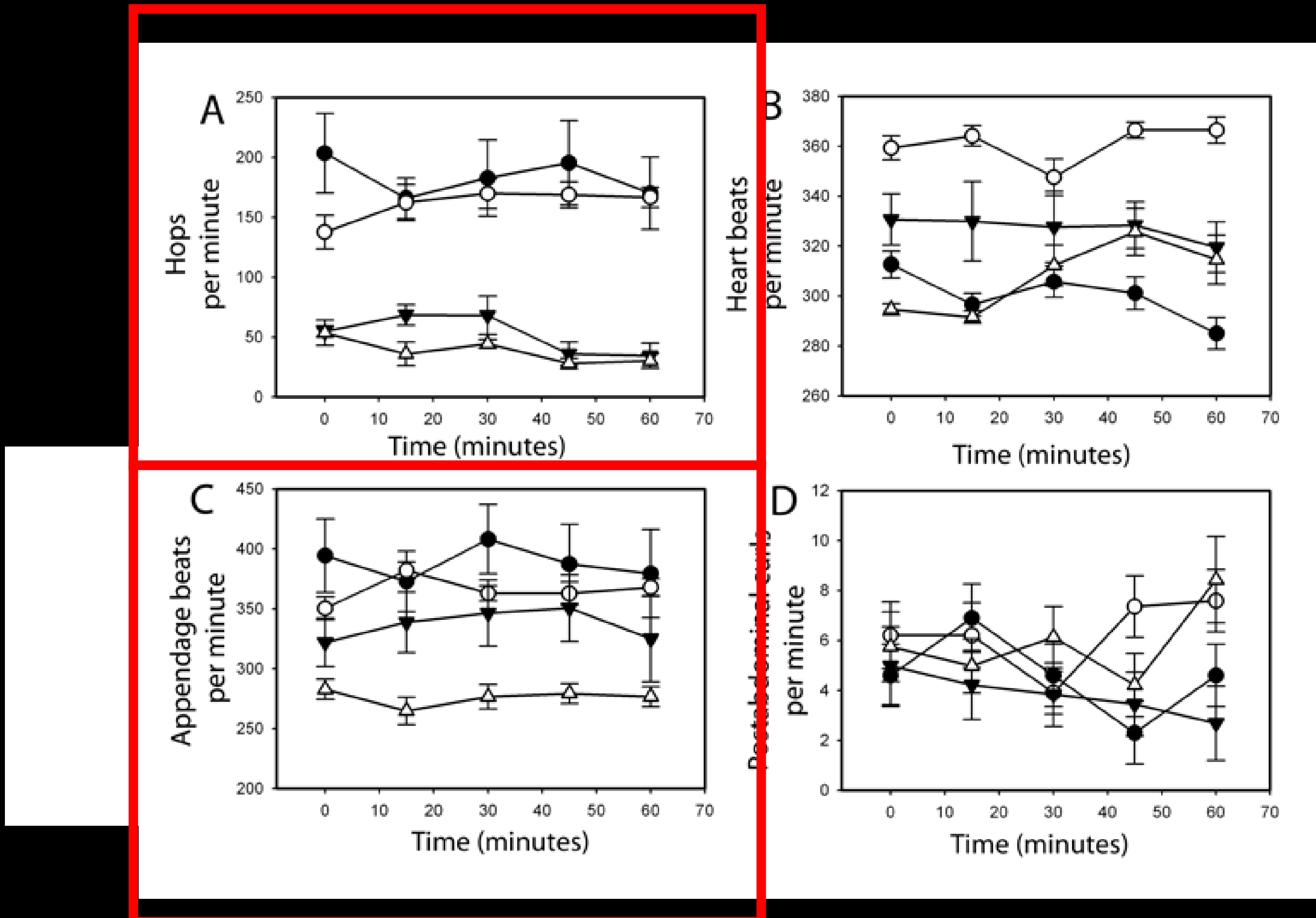




Traditional Toxicity Studies may not capture effects: Nanoparticles also interact with aquatic organisms

- **Behavior:**
- **Important for energetics**
- **More obvious to predator, problems with mating/feeding etc.**
- **Easy first indicator of response**

Behavioral changes over time with nanoparticle exposure



Control (r), TiO_2 (q), Nano-C_{60} (¡), $C_{60}HxC_{70}Hx$ (l).

From Lovern, Strickler and Klaper, 2007, Environmental Science and Technology

Laboratory Testing vs. Environmental Effects

- Our basic science research can inform us as to the characteristics of nanos that make them more or less toxic
- Primary principles as to the properties that determine toxicity and fate in environment will aid in informing testing protocols/modelling
- Keep in mind that companies will not be required to identify every form of their product in the environment for reporting requirements
- Are we going to require something different for nanos than other chemicals

What have we learned?

I. Nanoparticles can be toxic—this may be at a dose that would never occur in the environment

II. Nanoparticle toxicity depends on material

core structure, surface chemistry, aggregation state

III. The cause of this difference is debatable

IV. Mode of action may be partly due to oxidative stress

V. This is not the only way nanoparticles interact with organisms

Environmental Impact of Nanoparticles?

- Many nanomaterials---how can we make generalizations across particle types?
  - Goal: to make nanomaterials that are useful but cause the least amount of damage

- How can we take our laboratory testing to be applicable to the environmental condition of the nanomaterial?

Acknowledgements

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