NIEHS
National Institute of Environmental Health Sciences

RISK e Learning

Nanotechnology – Applications and Implications for Superfund

October 18, 2007
Session 8:
"Nanoparticles: Ecotoxicology"
Stephen Klaine, Clemson University
Patrick Larkin, Santa Fe Community College

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Nanomaterials in the Environment: Carbon in Aquatic Ecosystems

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Outline

- Challenges of working in aquatic ecosystems
- Carbon nanoparticles
- Surface modification to stabilize suspension
- Natural Organic Matter: nature's way of stabilizing nanoparticles

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Nanoparticles in Aquatic Ecosystems

Nanoparticle behavior

Nanoparticle-organism interactions

Mode of Action

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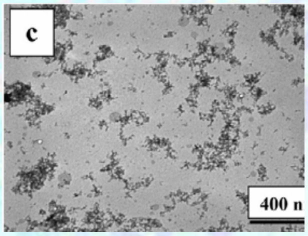
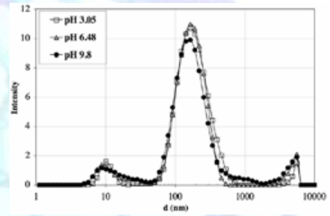
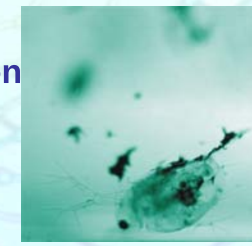
Nanoparticles in Aquatic Ecosystems





Particle Size Distribution
(Fullerols) Brant et al 2007

Particle Aggregation
(Fullerols) Brant et al 2007



Particle Stability
(SWNT) Roberts et al 2007

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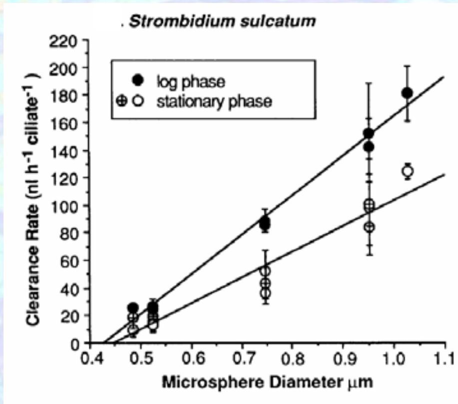
Nanoparticles in Aquatic Ecosystems



Nanoparticle size matters to filter-feeders

(Marine ciliate) Christaki et al 1998

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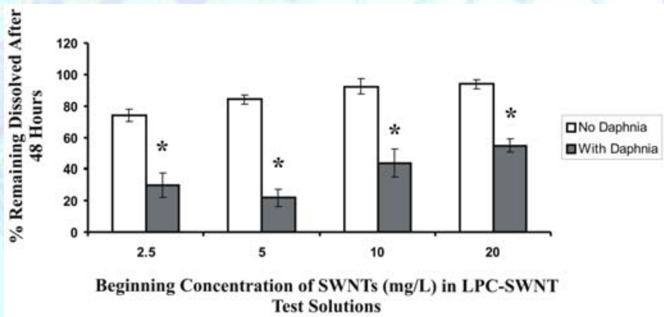
Nanoparticles in Aquatic Ecosystems

Filter-feeders modify nanoparticle suspensions



(SWNT) Roberts et al 2007

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Carbon Nanoparticles

- **Carbon quantum dots**

(Sun et al 2006)

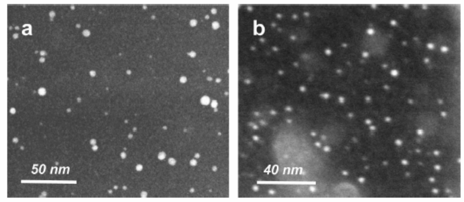
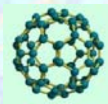
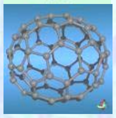
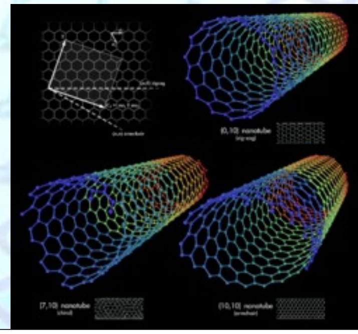


Figure 2. Representative STEM images of carbon dots surface-passivated with (a) PEG_{1500N} and (b) PPEI-EI.

- **C60** 
- **C70** 



- **Single-walled nanotubes**

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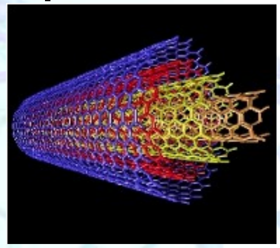
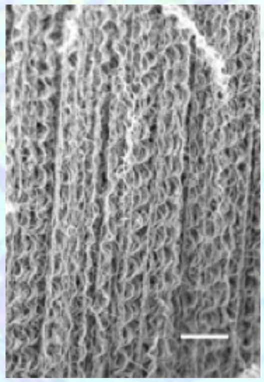
Carbon Nanoparticles

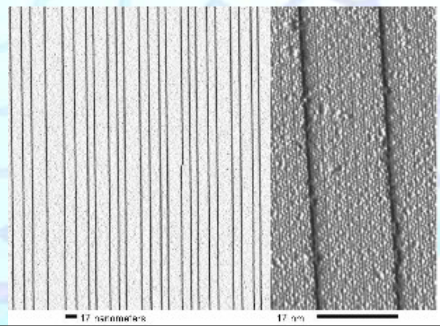
- **Multi-walled nanotubes**





- **Nanocoils**

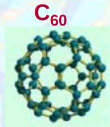
- **Nanowires**



Surface Modification

- **Micelle wrapping**

C_{60}



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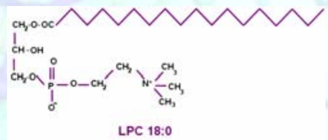
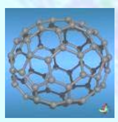


Excitation: 530 nm,
Emission: 585 nm
TRITC Equivalence

- **Pi stacking**

C_{70}



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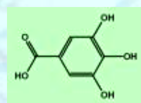

Gallic acid

Excitation: 488 nm, Emission: 535 nm
Calcein-AM Equivalence

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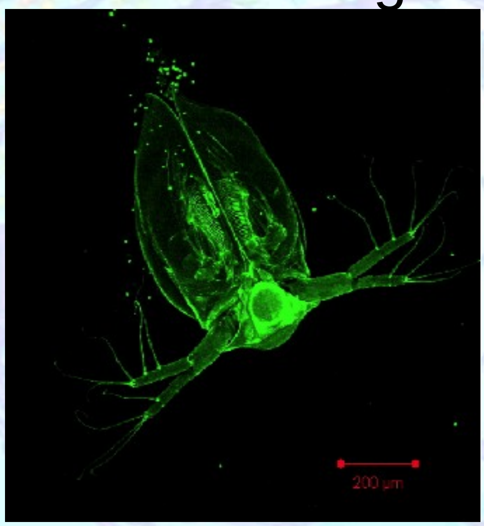
Daphnia magna (water flea) exposed to C70 + gallic acid



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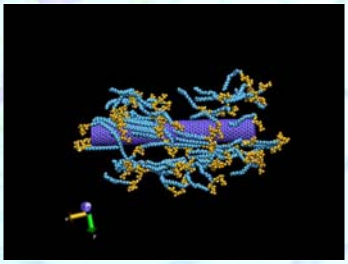
(Seda et al, in prep)

Surface Modification

SWNT and Lysophospholipids self assemble in water



(Qiao and Ke, 2006)

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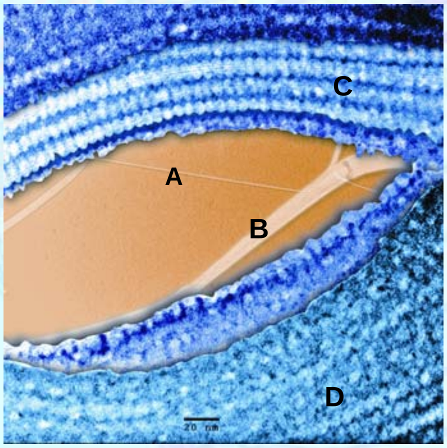
Surface Modification



"Curious Eye"

(Wu, et al 2006)

Lysophospholipid@SWNT

Binding

Left is an EM image of SWNTs.
A: SWNT;
B: SWNT bundle;
C: Phospholipid coated SWNTs;
D: Excess phospholipids.

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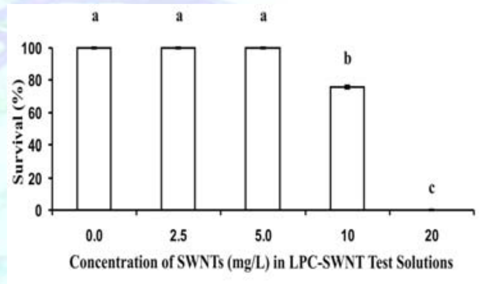
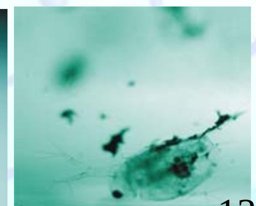
Surface-Modified SWNT-Biota Interaction

Daphnia magna
(water flea)





Control	45 minutes	1 hour	20 hours

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Roberts et al 2007

Natural Organic Matter: Nature's way of stabilizing nanoparticles

Natural organic matter (NOM) is used to describe the complex mixture of organic material, such as humic acids, hydrophilic acids, proteins, lipids, amino acids and hydrocarbons, present in surface waters and resulting from the decay of biota within the watershed.

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Natural Organic Matter: Nature's way of stabilizing nanoparticles

NOM is composed of a mixture of complex molecules varying from low to high molecular weights, including diagenetically altered biopolymers and black carbons.

NOM can vary greatly, depending on its origin, transformation mode, age, and existing environment, thus its biophysico-chemical functions and properties vary with different environments.

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Natural Organic Matter: nature's way of stabilizing nanoparticles

NOM stabilizes fullerene suspensions

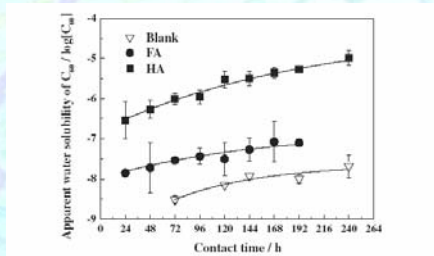

Figure 1. Relationships between contact time and apparent water solubility of C_{60} as a log $[C_{60}]$ in the presence of HSs. Concentration of HSs 100 mg L^{-1}, pH 6.0, ionic strength 0.1, and blank 0.1 M NaCl aq.

Terashiuma and Nagao, 2007

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Natural Organic Matter: nature's way of stabilizing nanoparticles

NOM stabilizes MWNT suspensions

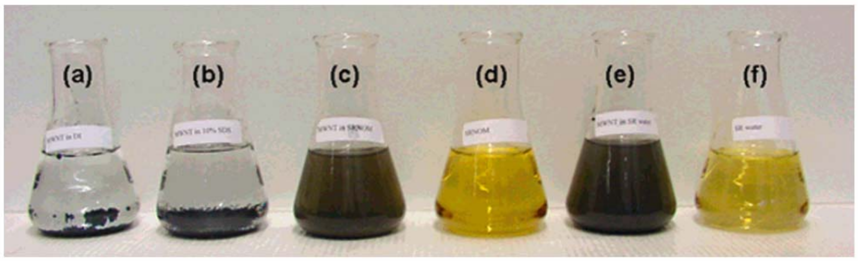

FIGURE 1. Visual examination of (a) organic-free water, (b) 1% SDS solution, (c) 100 mg C/L SR-NOM solution, and (e) Suwannee River water after adding 500 mg/L MWNTs, agitating for 1 h, and quiescent settling for 4 days. The 100 mg C/L SR-NOM solution and Suwannee River water without MWNT addition are also shown in panels d and f.

Hyung et al, 2007

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Natural Organic Matter: nature's way of stabilizing nanoparticles

NOM stabilizes <u>most</u> carbon nanoparticle suspensions

***400 mg/L nanoparticles**

- **A: Water**
- **B: 100 mg/L NOM**
- **C: 100 mg/L NOM + C_{60}**
- **D: 100 mg/L NOM + C_{70}**
- **E: 100 mg/L NOM + SWNT**
- **F: 100 mg/L NOM + MWNT**
- **G: 100 mg/L NOM + Nanocoil**
- **H: 100 mg/L NOM + Nanowire**

**Sonicated in small quantities for 30 min

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(Edgington et al, in prep)

Acute Toxicity of NOM Stabilized Carbon Nanoparticle Suspensions (96 hr)

- **C60 - no mortality** **(Lovern & Klaper,2006, 70% mortality at 9 mg/L)**
- **C70 - no mortality**
- **MWNT - 10% mortality**
- **Nanowire - no mortality**
- **Nanocoil - no mortality**

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*25 mg/L (nominal) nanoparticles

Creating Reproducible Nanoparticle Suspensions - SOP

- 25 mg/l carbon nanoparticles were suspended via sonication in a solution containing 15 mg/l dissolved organic carbon.
- After 24 hours, an average of 7 mg/l had fallen out of suspension to the bottom of the tube. Concentration at 24 h was 18 ± 0.5 mg/l. (n=12; cv = 5.9%)

(Edgington et al, in prep)

Acute Toxicity of NOM Stabilized Carbon Nanoparticle Suspensions to *D. magna* (96 hr)

NOM SOURCE (USA)	LC50 Value (95% C.I.)
Black River (SC)	1.91 (1.40-2.62)
Suwannee River (GA)	2.99 (2.36-3.81)
Edisto River (SC)	4.09 (3.41-4.91)

[NOM] = 15 mg/l Carbon

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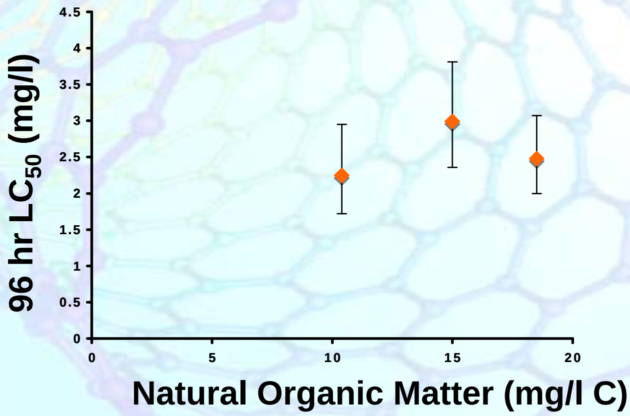
(Edgington et al, in prep)

Influence of Suwannee River NOM on the Toxicity of MWNT to *D. magna*



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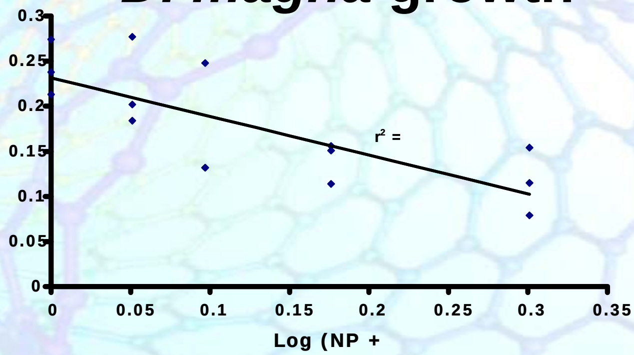
(Edgington et al, in prep)

Influence of MWNT suspended in 10 mg/l Suwannee River NOM on *D. magna* growth



D. magna **exposed to MWNTs in NOM for 96 hours**
(MWNT concentrations range from 0-1 mg/L)

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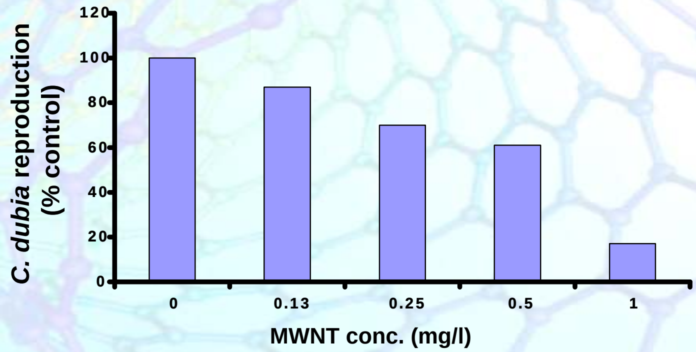
Taylor and Roberts (*In prep.*)

Influence of MWNT suspended in 10 mg/l Suwannee River NOM on *Ceriodaphnia dubia* reproduction



Reproduction over a 7 day period in *C. dubia* exposed to MWNT-NOM is decreased by as much as 80%.

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Gevertz and Roberts (*In prep.*)

Summary and Conclusions

- Particle size, shape and surface chemistry may play critical roles in environmental fate and effects of carbon nanoparticles.
- Surface-modified carbon nanoparticles may have longer residence times in the water column
- Carbon nanoparticle suspensions are more stable in NOM
- Source of NOM appears to influence MWNT bioavailability and toxicity
- NOM concentration does not influence MWNT bioavailability and toxicity at > 10 mg/l carbon

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Collaborators and Funding

- Clemson University:
  - Brandon Seda and Aaron Edgington, ENTOX Ph.D. students
  - P.C. Ke, Department of Physics
  - R. Qiao, Department of Mechanical Engineering
  - A. Mount, Department of Biological Science
  - Y.P. Sun, Department of Chemistry
- University of North Texas
  - A. Roberts, Institute of Applied Sciences
- Georgia Institute of Technology
  - E. M. Perdue, School of Earth and Atmospheric Sciences

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Literature Cited

- Brant, J.A., J. Labille, C.O. Robichaud, and M. Wiesner. 2007. Fullerol cluster formation in aqueous solutions: Implications for Environmental Release. *J. Colloid and Interface Science* 314:281-288.
- Christaki, U., J.R. Dolan, S. Pelegri, and F. Rassoulzadegan. 1998. Consumption of picoplankton-size particles by marine ciliates: Effects of physiological state of the ciliate and particle quality. *Limnol. Oceanogr.* 43(3):458-464.
- Hyung, H., J.D. Fortner, J.B. Hughes, and J.H. Kim. 2007. Natural Organic Matter Stabilizes Carbon Nanotubes in the Aqueous Phase. *Environ. Sci. Technol.* 41:179-184.
- Lovern, S. B.; Klaper, R., Daphnia magna mortality when exposed to titanium dioxide and fullerene (C-60) nanoparticles. *Environ. Toxicol. Chem.* 2006, 25, (4), 1132-1137.
- Qiao, R. and P.C. Ke. 2006. Lipid-Carbon Nanotube Self Assembly in Aqueous Solution. *J. Am. Chem. Soc.* 128 (2006), 13656.
- Roberts, A.P., A.S. Mount, B. Seda, J. Souther, R. Qiao, S. Lin, P.C. Ke, A.M. Rao and S.J. Klaine *Environ. Sci. Technol.* 41:3025-3029, 2007
- Terashiuma, M. and S. Nagao. 2007. Solubilization of [60] Fullerene in Water by Aquatic Humic Substances. *Chemistry Letters* 36(2):302-303.
- Wu, Y., Q. Lu, J.S. Hudson, A.S. Mount, J.M. Moore, A.M. Rao, E. Alexov, and P.C. Ke. 2006. Coating Single-Walled Carbon Nanotubes with Phospholipids, *J. Phys. Chem. B* 110, 2475.
- Sun, Y.P, B.Zhou, Y. Lin, W. Wang, K.A. Shiral Fernando, P.Pathak, M.J. Meziani, B.A. Harruff, X. Wang, H. Wang, P.G. Luo, H. Yang, M.E. Kose, B. Chen, L.M. Veca, and S.Y. Xie. 2006. Quantum-sized Carbon Dots for Bright and Colorful Photoluminescence. *J. Am. Chem. Soc.* 128:7756-7757.

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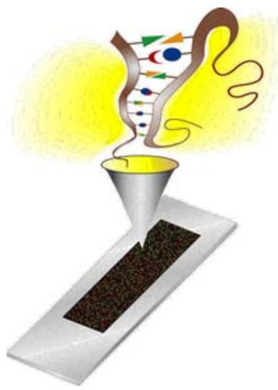
Screening of a nanoparticle using *in vivo* and microarray studies



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-Independent Ecotoxicology Consultant
-Santa Fe Community College

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Andrew Maynard, Ph.D.

Woodrow Wilson International Center for Scholars

Project on Emerging Nanotechnologies at the Woodrow Wilson International Center for Scholars

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Reference for nano study

● Oberdorster et al., (2006) Rapid environmental impact screening for engineered nanomaterials: A case study using microarray technology. Project on emerging Nanotechnologies at the Woodrow Wilson International Center for Scholars, Washington D.C. USA.

Web site: www.nanoproject.com

Outline of talk

(1) Background of project

(2) Daphnia studies
- -Exposures
- -Results

(3) Fathead minnow studies
- -Exposures and results
- -Background on arrays
- -Array results

(4) Conclusions

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Background

The increasingly rapid introduction of nano-based substances into the marketplace requires new methods to assess both short and long-term potential environmental impacts of these compounds.

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Background

To test the nanoparticles we used a standard EPA-approved ecotoxicology test using daphnia with assays using a newly developed, 2000-gene DNA array for the fathead minnow.

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Background

We collaborated directly with a company, Toda America, that manufactures Reactive Nano-Iron Particles (RNIP).

These particles are currently being used to remediate toxic waste sites.

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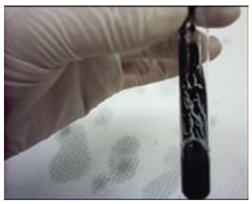
Background

Toda America graciously donated 1 kg (250 g RNIP in 750 mL water, as a slurry) for toxicity testing.



Surface Stabilized iron slurry

Ingredients:

Fe:	16.5 %
Fe_3O_4:	8.5%
H_2O:	75%

specific gravity: 1.25

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Daphnia exposures

Water fleas (*Daphnia magna*) were used to examine the toxicity of RNIP.

Daphnia are small crustaceans that live in fresh water such as ponds and lakes.

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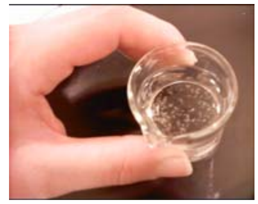
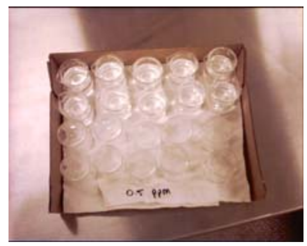
Daphnia exposures



This species is easily grown and maintained in a laboratory setting.



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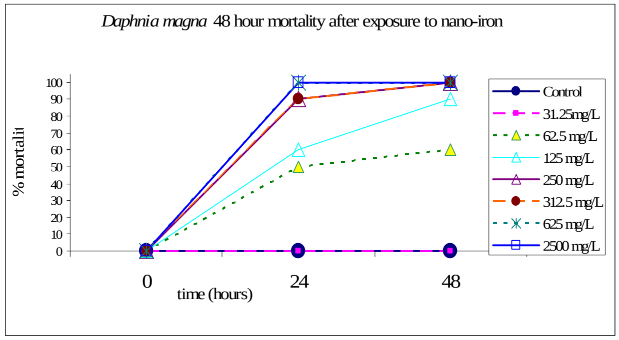
Daphnia range finding studies

The 48-hour LC_{50} of RNIP was found to be ~55 parts per million (ppm).



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RNIP toxicity

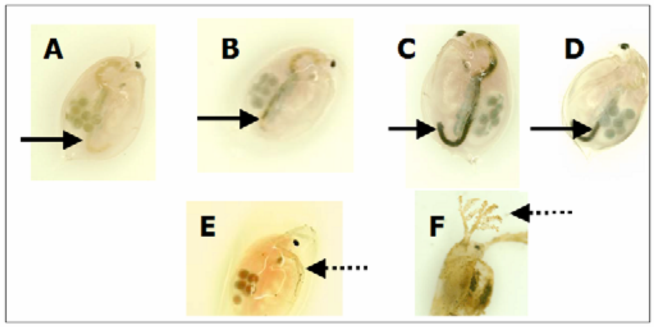
Based on a toxicity rating scale, RNIP would be considered slightly toxic.

Category	LD50 oral mg/kg (ppm)
I (Highly toxic)	Less than 50
II (Moderately toxic)	51-500
III (Slightly toxic)	Over 500
IV (Practically non-toxic)	-

Toxicity scales as defined in: M. A. Kamrin, *Pesticide Profiles: Toxicity, Environmental Impact, and Fate,* Lewis Publishers (Boca Raton, FL, 1997), p. 8

Coating of daphnia

Daphnia ingested RNIP and this NP also coated their carapace, including filtering apparatus and appendages



A = control; B = 3 mg/L; C = 7.5 mg/L; D = 15 mg/L; E = 30 mg/L; F = 125 mg/L (dead daphnid). All daphnids shown are 21 days old and eggs are visible in their brood pouches (small green circles).

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FHM exposures

Fathead minnows (*Pimephales promelas*) were chosen as a model species in this study for several reasons.

- They have been used as a standard test species for aquatic toxicology since the 1960s and are widely used in eco-toxicology.
- Their reproductive physiology is well known
- They can be propagated easily in the laboratory.

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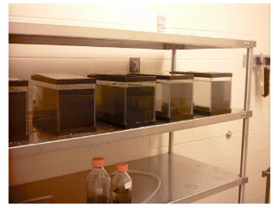
FHM exposures

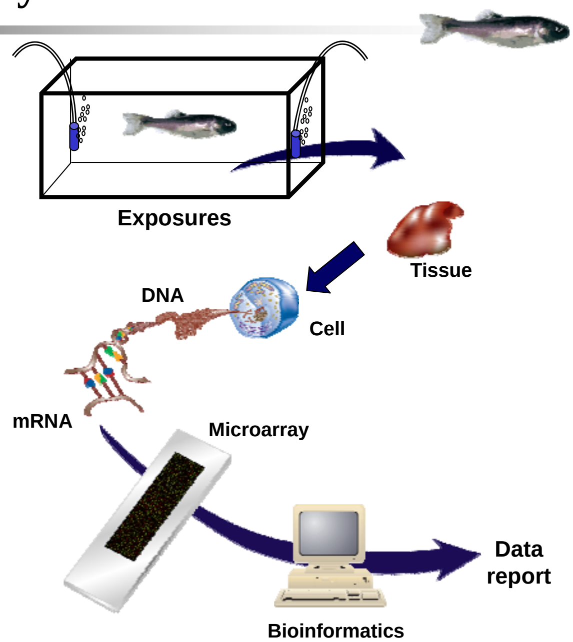


Fathead minnows were exposed for 5-days to 50 ppm of RNIP.

The concentration of RNIP used did not cause any overt physical changes (such as lesions) or mortality in the fish.

FHM array overview

FHM array experimental design



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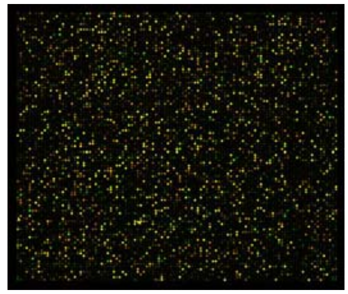
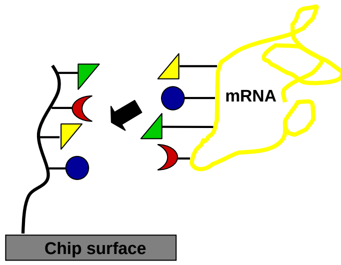
FHM arrays

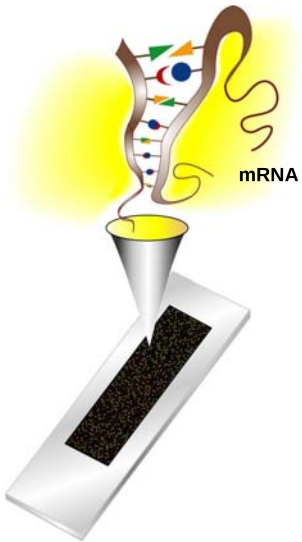
Picture of an array that was run for the experiments.

These arrays were designed using the Agilent platform.



Agilent arrays





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Custom design your on array

Agilent’s eArray

-Custom printing.

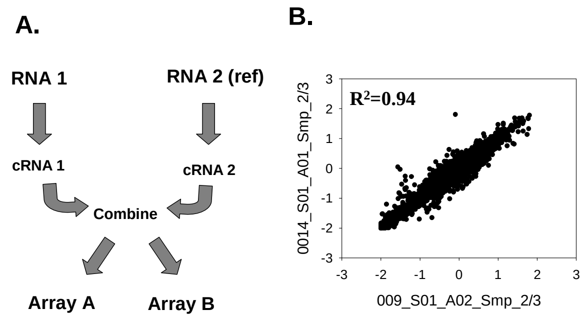
-Agilent’s manufacturing allows you to create your own microarray designs that meet your specific biological needs.

-Design at your own pace and receive delivery of your arrays in weeks

200,000 sequences now publicly available for fathead minnows

Validation of arrays

Evaluation of chip reproducibility.



Larkin, P et al., (2007) Development and validation of a 2,000 gene microarray in the fathead minnow, *Pimephales promelas.* Environmental Toxicology and Chemistry.

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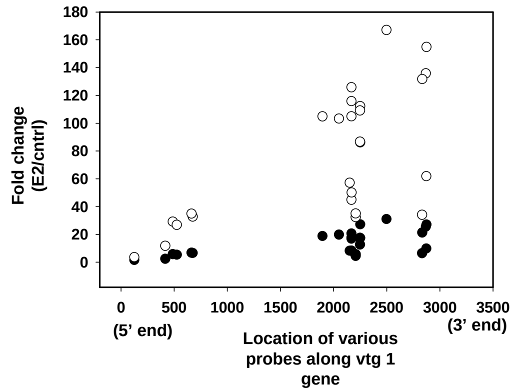
Probe validation

Relative fold change of vitellogenin 1 probes varies depending on probe location.



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Fathead minnow exposures

Differentially regulated genes in male liver

Gene Hit Definition	Fold Change	Explanation
UNDER-EXPRESSED IN LIVER – MALES EXPOSED TO NANO-IRON		
Complement component C9 precursor	**-1.3**	Involved in cell lysis, fibrinolytic, blood coagulating, and kinin systems. (Taran. Biokhimiia. 1993 May;58(5):780-7.)
OVER-EXPRESSED IN LIVER – MALES EXPOSED TO NANO-IRON		
Alpha-2 macroglobulin 2	**2.0**	Act as defense barriers – binding foreign (or host) peptides and particles. (Borth, FASEB J. 1992 Dec;6(15):3345-53.)
Alpha-2 macroglobulin 1	**1.6**	
Selenoprotein Pa precursor	**1.8**	An extracelluar glycoprotein; associates with endothelial cells; postulated to protect against oxidative injury and to transport selenium from liver to peripheral tissues. (Burk, et al., J Nutr. 2003 May: 1355 (5 Suppl 1):1517S-20S.)
Tubulin, alpha-3	**1.6**	Involved in microtubulin dynamics (growth and shortening of tubules) and possibly motor proteins used for intracellular transport. Targeted by anticancer drugs. (Pellegrini and Budman. Cancer Invest. 2005;23(3):264-73.)
Ubiquitin	**1.5**	Plays a role in the process of protein degradation. (Walters, et al. Biochim Biophys Acta. 2004 Nov 29;1695(1-3):73-87.)
Prothrombin precursor	**1.5**	Thrombin (which has multiple roles) is generated from its inactive precursor prothrombin by factor Xa as part of the prothrombinase complex. (Lane, et al. Blood. 2005 June 30; epub ahead of print.)
Antithrombin	**1.4**	Mediates the activity of heparin, a major anticoagulant. (Munoz and Linhardt, Arterioscler Thromb Vasc Biol. 2004 Sep:24(9):1549-57.)
Aldolase A fructose-biphosphate	**1.3**	Plays a role in glucose metabolism. An increase in serum aldolase is seen with muscular diseases and malignant tumors. (Taguchi and Takagi. Rinsho Byori. 2001 Nov; Suppl 116:117-24.)
Hexokinase	**1.2**	Enzyme involved in glycolosis, transcriptional regulation and regulation of apoptosis. (Kim and Dang. Trends Biochem Sci. 2005 Mar;30(3):142-50.)

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Fathead minnow exposures

UNDER-EXPRESSED IN GILL – MALES EXPOSED TO NANOIRON		
Cytosolic alanine aminotransferase (c-AAT)	**-1.2**	In striated muscles, regulates the rate of glycolosis and energy production under conditions of anaerobiosis through the formation of alanine. (Rusak and Orlicky. Physiol Bohemoslov. 1979;28(3):09-16.)

Differentially regulated
genes in male gill

Conclusions

-RNIP is considered slightly toxic based on the Daphnia exposures

-The concentration of RNIP used in the FHM studies did not cause any overt physical changes (such as lesions) or mortality in the fish.

-Very few genes were significantly changed on the FHM arrays

- Fairly good concordance was observed with the *in vivo* and array studies

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