

# Potential Ecotoxicity of Nanoparticles Released to the Environment



**Patricia McClellan-Green, Ph. D.**

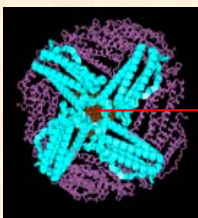
# Definition: nanotechnology

- research and technology development at the atomic, molecular and macromolecular levels using a length scale of approximately one to one hundred nanometers in any dimension
- the creation and use of structures, devices and systems that have novel properties and functions because of their small size
- the ability to control and manipulate matter on an atomic scale”

(Nanotechnology Workgroup, Science Policy Council, USEPA, December 2005

## NANO-SIZED PARTICLES (<100 nm): NATURAL AND ANTHROPOGENIC SOURCES

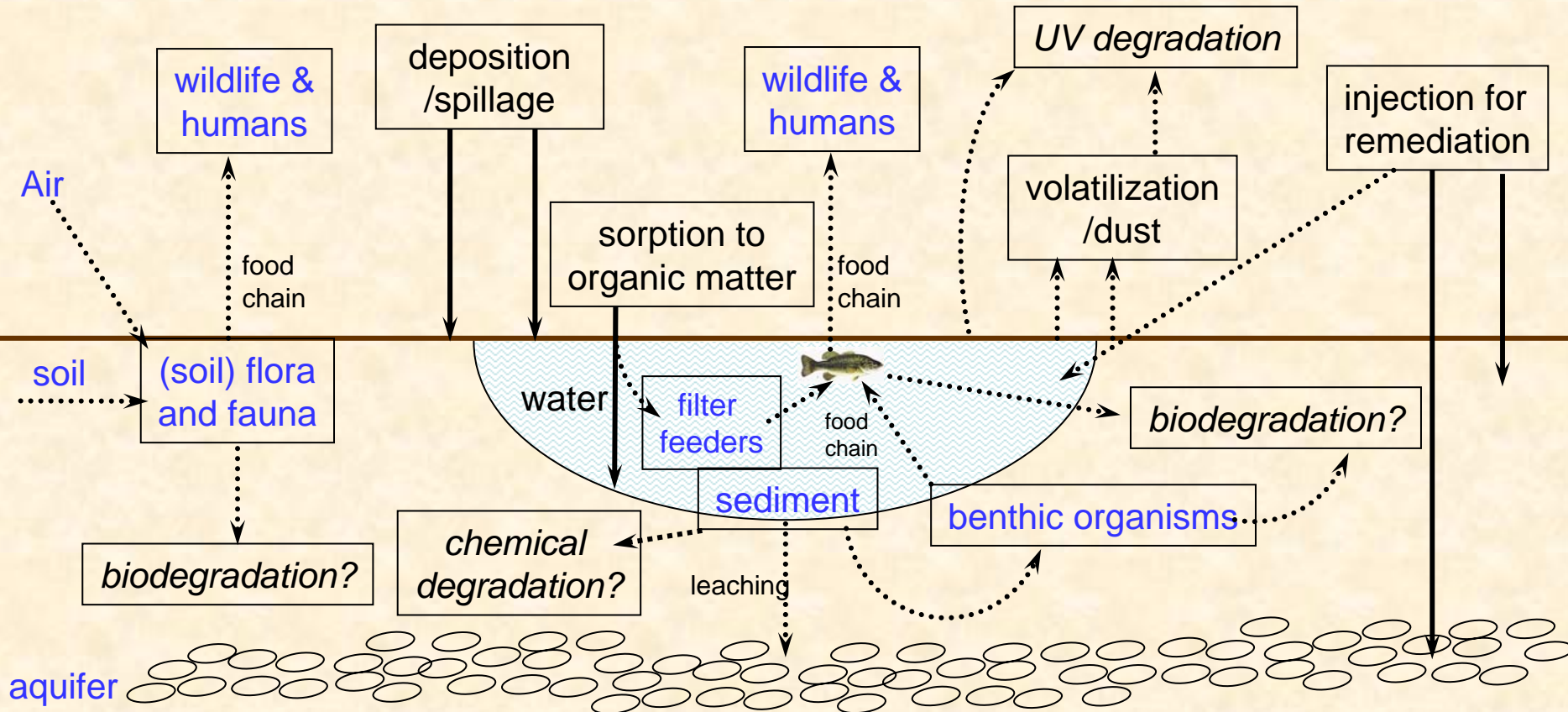
Natural	Anthropogenic	
	<i>Unintentional</i>	<i>Intentional</i>
<p>gas to particle conver. forest fires volcanoes (<i>hot lava</i>) viruses</p> <p>biogenic magnetite: <i>magnetotactic bacteria;</i> <i>Protoctists, Mollusks,</i> <i>Arthropods, fish, birds,</i> <i>human brain, [meteorite]</i></p> <p>ferritin (12.5 nm) lipoprotein particles <i>fabrics,</i> <i>(1-75 nm, plasma)</i></p>	<p>internal combustion eng. power plants incinerators airplane jets</p> <p>metal fumes <i>(smelting, welding, etc.)</i> polymer fumes other fumes heated surfaces frying, broiling, grilling electric motors</p>	<p>engineered nanoparticles: <i>(controlled size and shape, designed for functionality)</i> <i>metals, semiconductors, metal oxides</i> <i>quantum dots/rods</i> <i>fullerenes, nanotubes</i> <i>nanowires</i> <i>nanoshells</i> <i>nano ....</i> <i>(nanotechnology applied to many products: cosmetics, medical, electronics, optics, displays, etc.)</i></p>
<p><u>Potential Exposure Routes:</u></p> <p style="font-size: 1.2em; color: blue;"><b>Respiration      Ingestion      Dermal      Injection</b></p>		



5-7 nm Iron-containing cavity

Tires, sunscreen, toothpaste, sanitary ware coatings, food products.

# Model of nanoparticle movement through the Environment



# Exposure, Uptake, Translocation, and Excretion of NM

—→ Confirmed routes  
- - - -> Potential routes

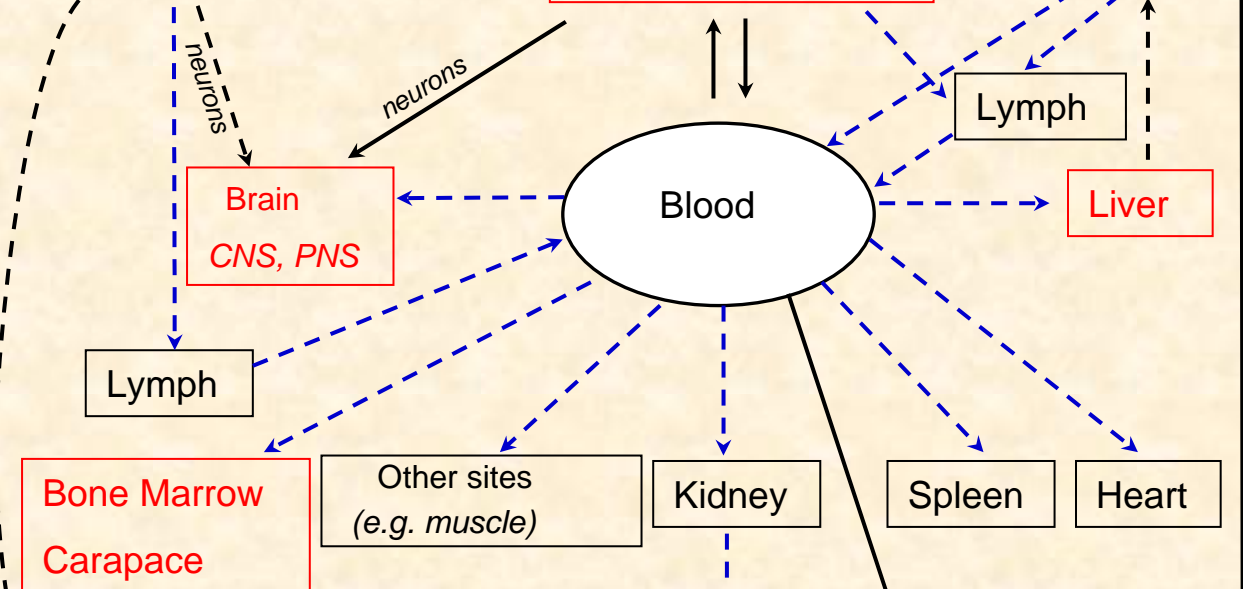
Exposure Media



Uptake Pathways



Translocation and Distribution

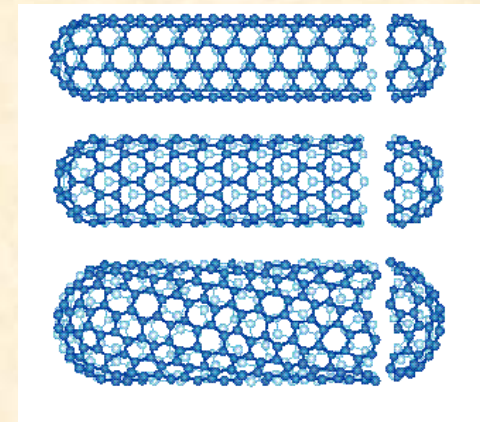
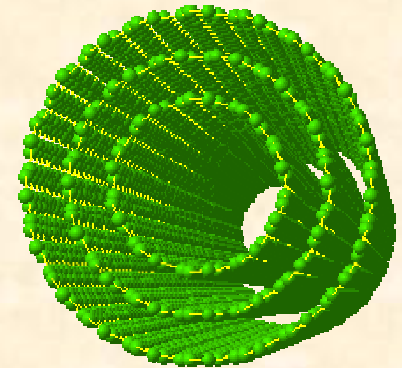
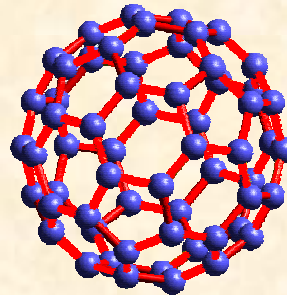


Excretory Pathways



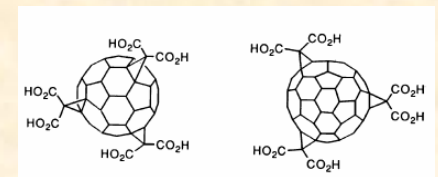
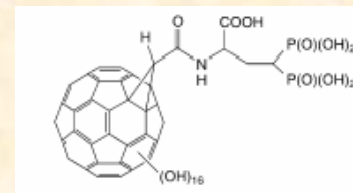
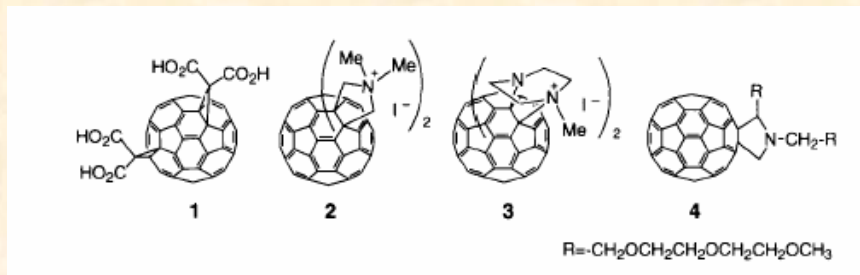
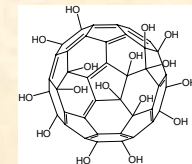
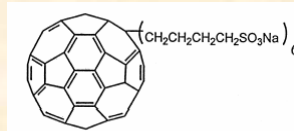
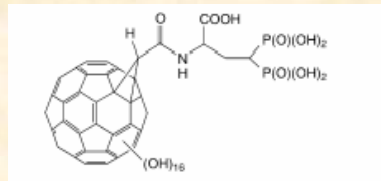
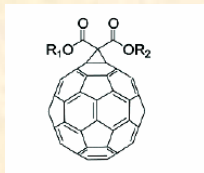
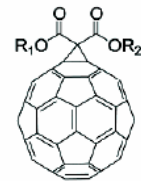
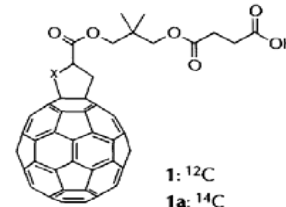
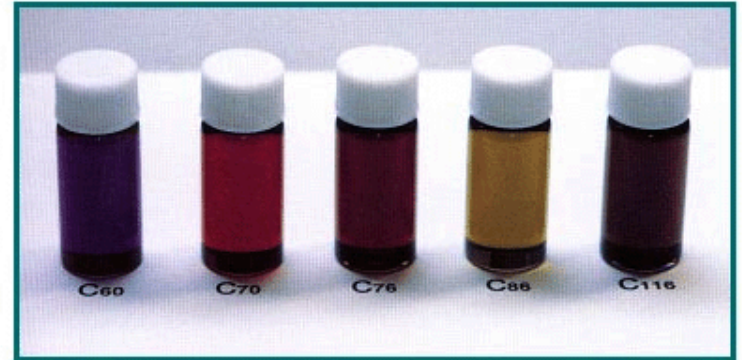
# Carbon-based nanomaterials

- Fullerenes
  - “Buckyballs”
- Nanotubes
  - Single walled (SWNT)
  - Multi walled (MWNT)
- Nanofibres/nanofibrils
- Uses
  - Coatings to minimize static electricity
  - Fuel lines, hard disk drive handling trays
  - Electrostatically paintable car components
  - Flame retardant fillers for plastics
  - Field emitter sources in flat panel displays



# Spectrum of Fullerenes

- Pure fullerenes
  - $C_{60}$ ,  $C_{70}$ ,  $C_{76}$ ,  $C_{86}$ ,  $C_{116}$ .....
- Metal endohedrals
  - Metals encased by fullerene
- Surface modified fullerenes
  - Malonic acid derivatives
  - Hydroxylated fullerenes



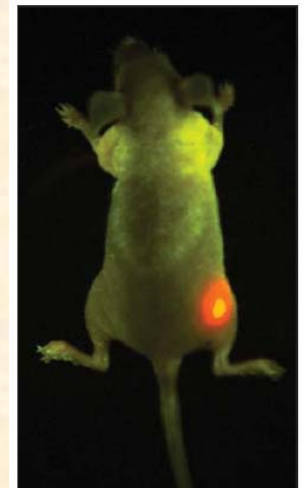
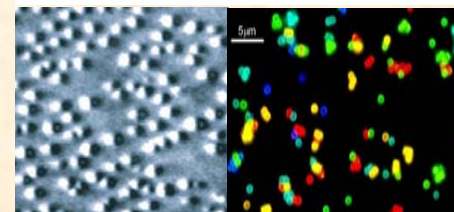
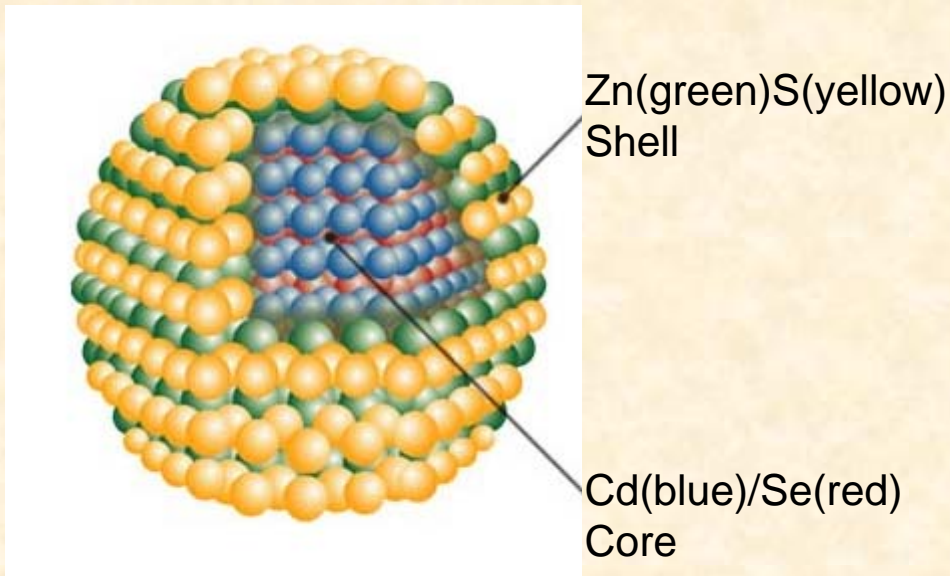
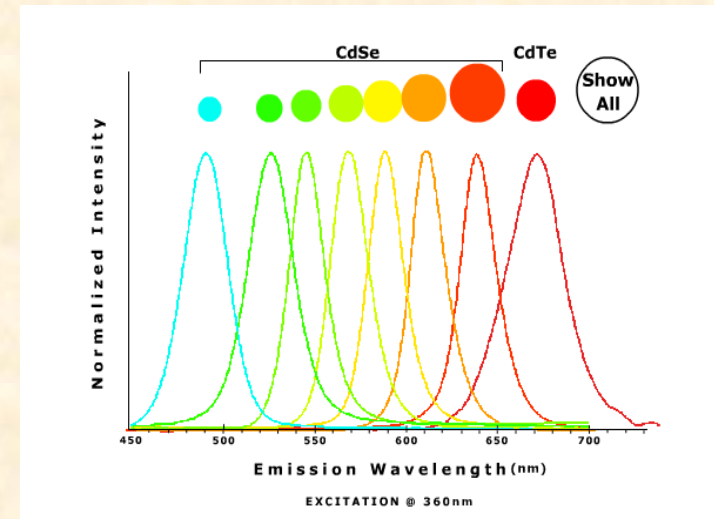
# Metal oxides

- Examples
  - Titanium, Zinc, Iron
  - Cerium, Zirconium
- Desirable properties
  - Transparent
  - UV absorbing properties
  - Photocatalytic properties
- Uses
  - Cosmetics
  - Sunscreens
  - Transparent wood protectants
  - Catalysts in remediation, self cleaning windows
  - Chemo-mechanical polishing agents for semiconductor wafers
  - Scratch resistant coatings for glass



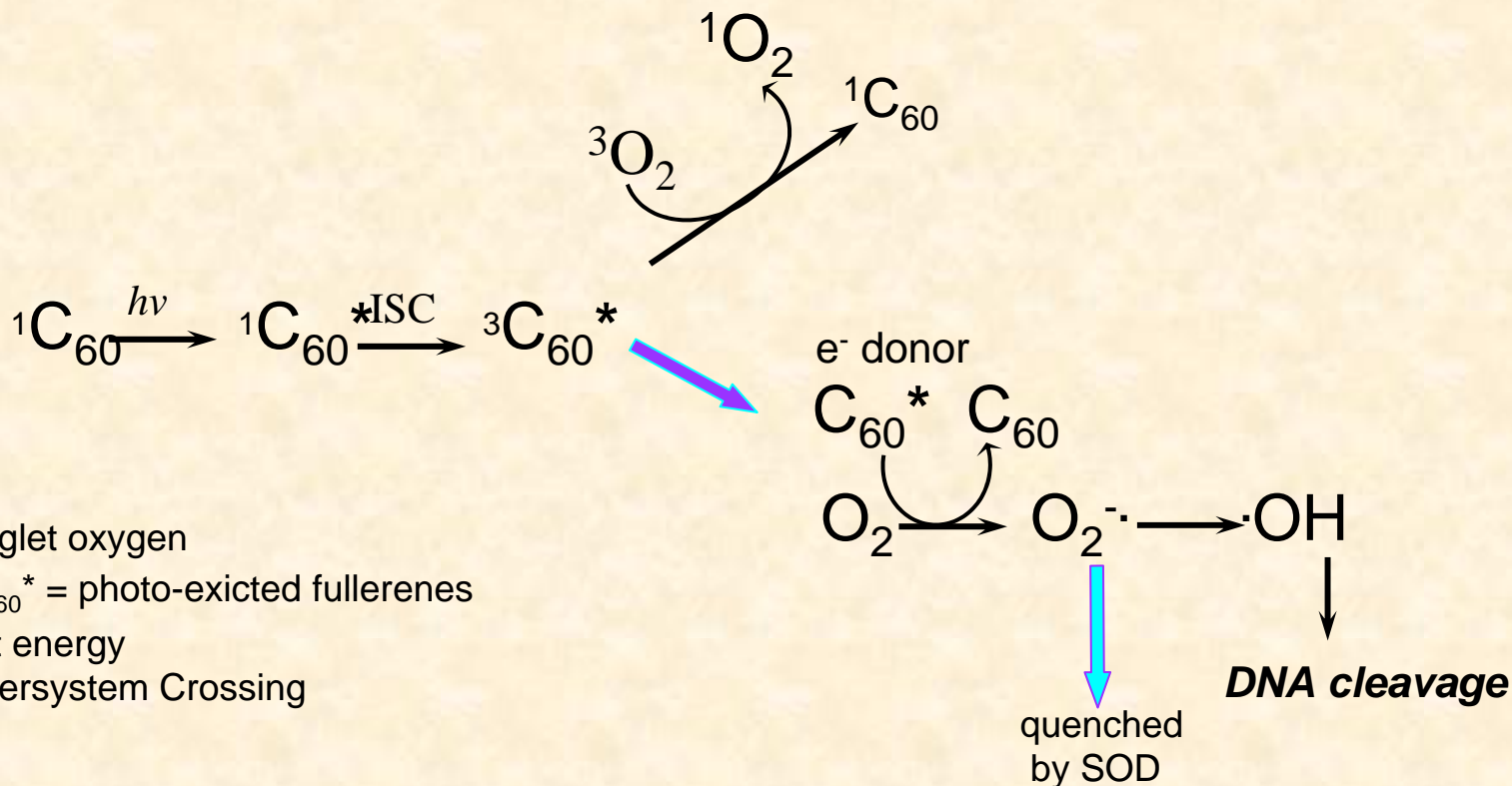
# Quantum dots

- Fluorescent crystalline semiconductors
- Broad excitation spectrum
- Narrow emission spectrum
- Use; In vivo imaging, drug delivery



Bull's-eye. Red quantum dots injected into a live mouse mark the location of a tumor.

# Fullerenes ( $C_{60}$ ) are Re-dox Active



$^1O_2$  = singlet oxygen

$^1C_{60}^*$ ;  $^3C_{60}^*$  = photo-excited fullerenes

$h\nu$  = light energy

ISC = Intersystem Crossing

# ***Other redox active nanomaterials***

## **metal Qdots are redox active**

*(ex: Derfus et al. 2004. NanoLett 4(1):11-18; Joo et al. 2004. Environ Sci Technol. 38(7):2242-2247)*

## **•nano TiO<sub>2</sub> is redox active under UV**

*(Nagaveni et al. 2004 Environ Sci Technol. 38:1600-1604)*

## **•nano TiO<sub>2</sub> causes inflammation and Severe Acute Lung Injury**

*(several studies by G. Oberdörster)*

## **•ambient or laboratory-produced ultrafine particles cause oxidative stress *in vitro***

*(Li et al 2003 EHP 111(4):455-460 ; Brown et al. 2000 OEM 57:685-691, Brown et al. 2001 TAP 175:191-199; Donaldson et al 1998 J Aerosol Sci 29:553-560)*

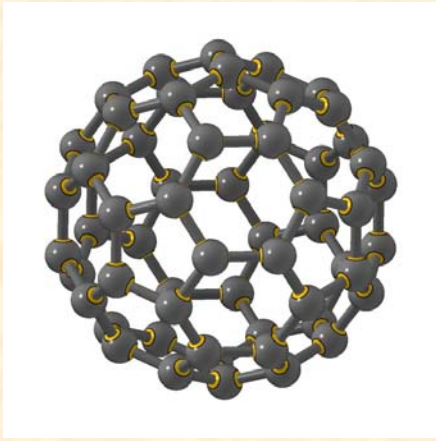
## **•Ultrafine Carbon Black (ufCB) depleted glutathione (GSH) and ATP in macrophages**

### **•ufCB increased TNF $\alpha$ in mouse macrophages**

### **•ufCB induced inflammatory response in rat lung**

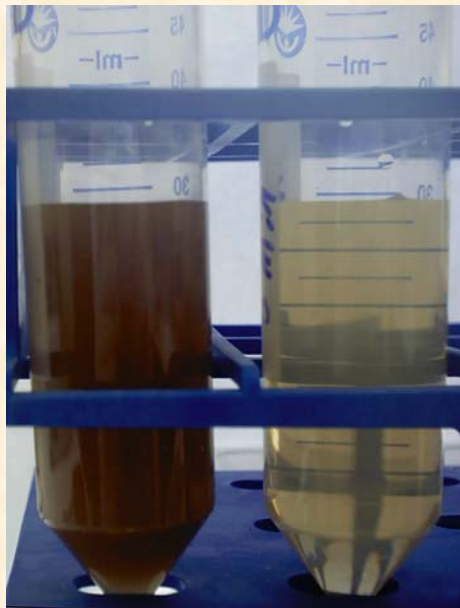
*(Wilson et al. 2002. TAP 184(3):172-179)*

# Materials Prepared for First Studies



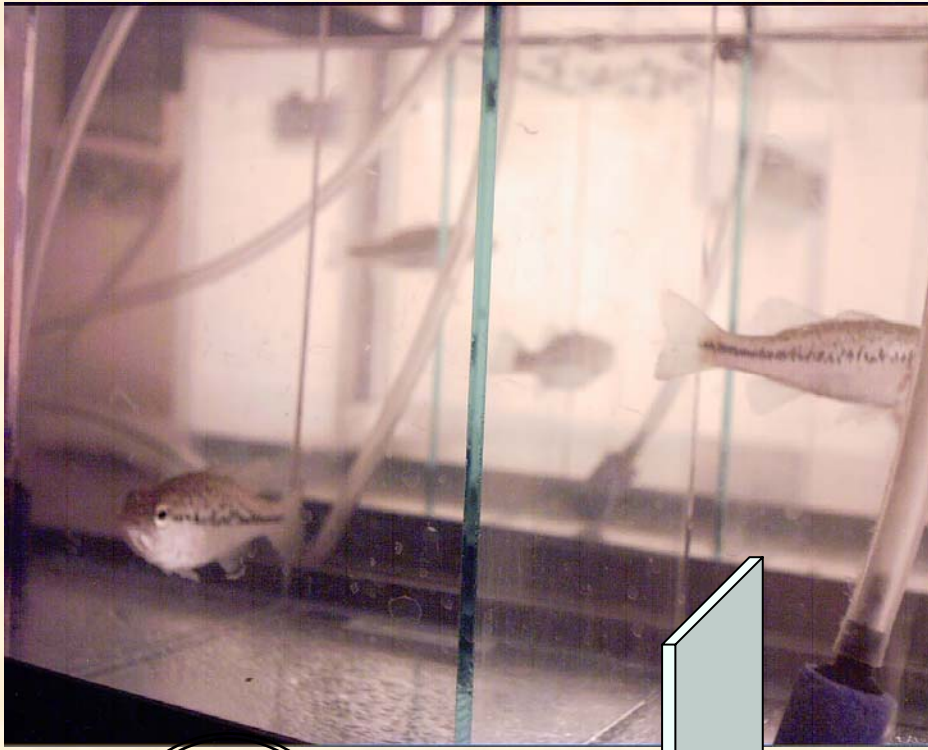
## THF-prepared nC<sub>60</sub>

- 99.5% pure fullerene is dissolved in tetrahydrofuran (THF)
- solution is sparged with nitrogen, stirred overnight, and filtered through a 0.22 μm filter
- MilliQ water is added
- THF is evaporated off
- solution is stored overnight and then filtered through a 0.22 μm nylon filter to yield a working nC<sub>60</sub> suspension in water.



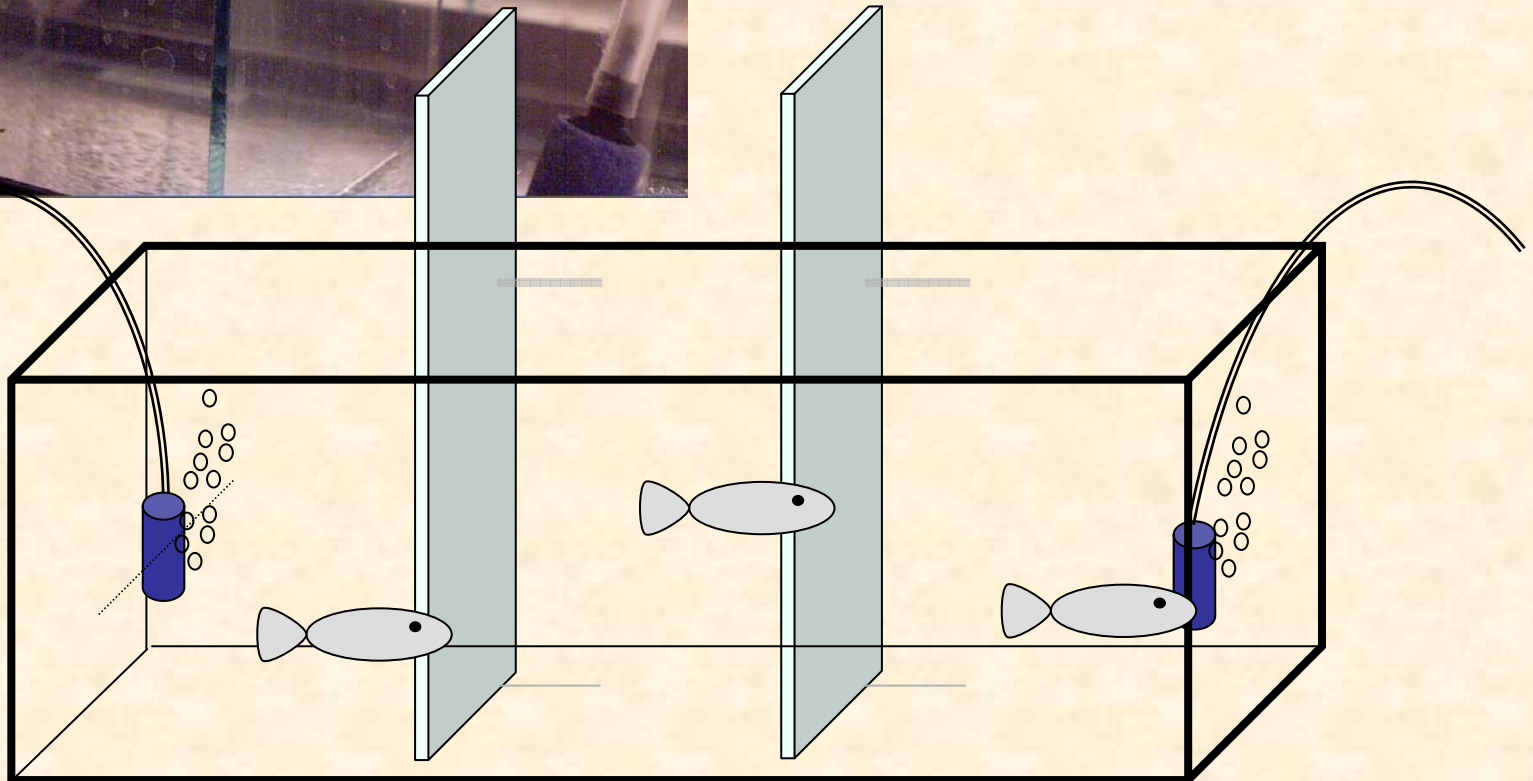
## Water- stirred fullerene

- 99.5% pure fullerene stirred in MilliQ water
- agglomerates into clusters of 10-200 nm diameter
- outside partially hydroxylated, inside neat C<sub>60</sub>
- centrifuged 3X 5000g for 15 minutes



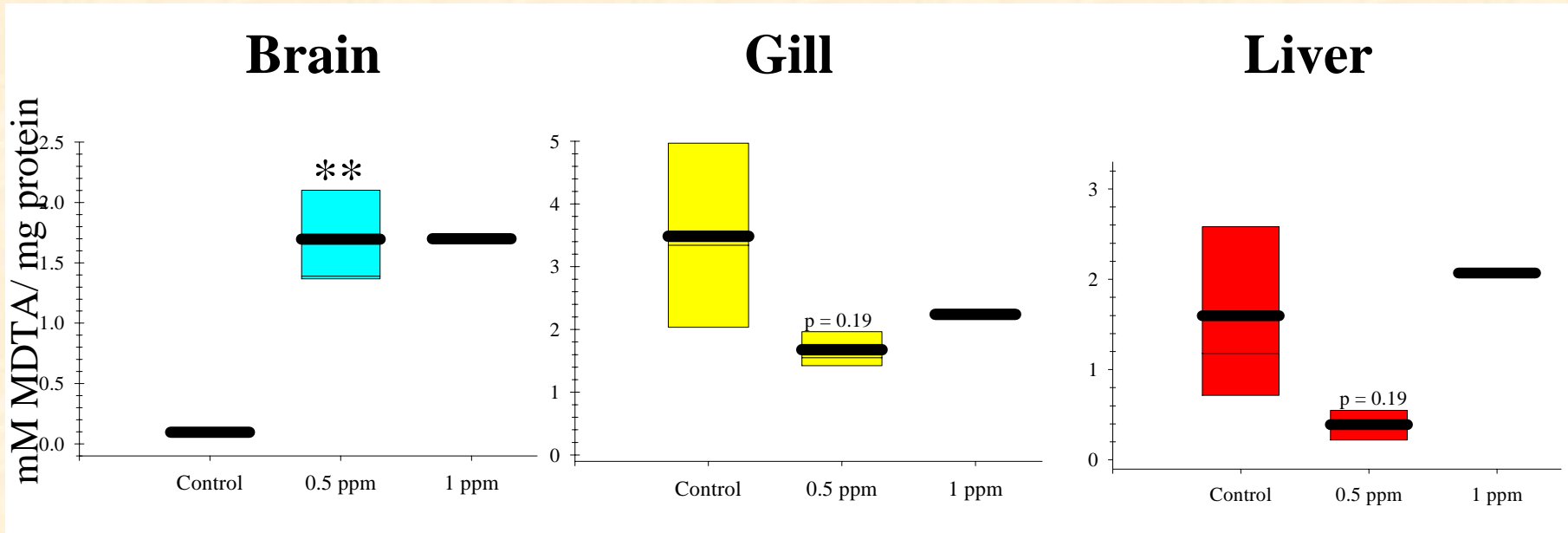
- 10L aquarium, RHW
- partitioned into 3 sections
- water flow between sections
- randomly assigned fish
- 30% volume water change @ 24 hrs

2 separate trials total of:  
3 X control,  
3 X 0.5 ppm fullerene (nC60)  
1 X 1.0 ppm fullerene (nC60)  
2 X 100 mM H<sub>2</sub>O<sub>2</sub> (positive control)



largemouth bass 48 hour nC60 exposure

Lipid Peroxidation

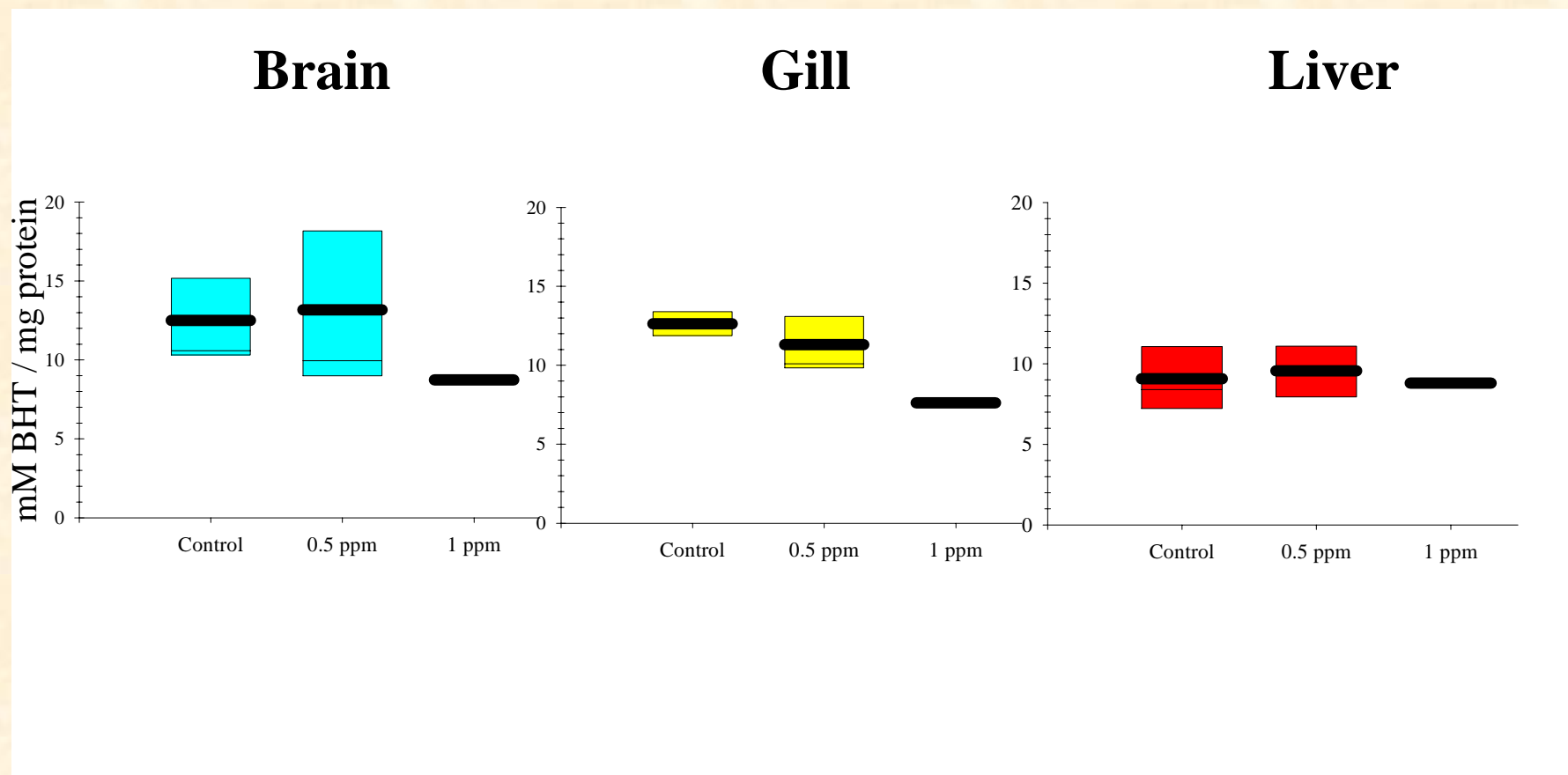


\*\* p < 0.01

data is pooled from two separate trials

largemouth bass 48 hour nC60 exposure

Protein Oxidation



data is pooled from two separate trials

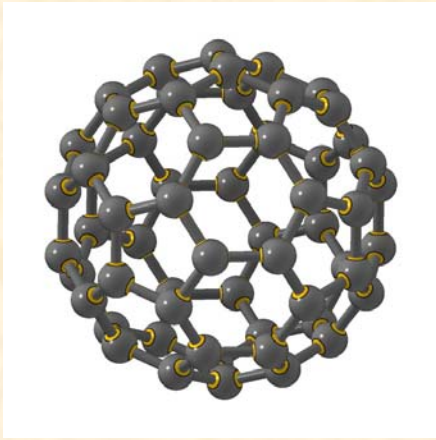
## Subtractive Hybridization Results: Genes that were up-regulated

- CYP2K4 = oxidoreductase
- CYP11B1 = oxidoreductase
- ***wide variety of proteins related to immune response are elevated***
  - macrophage stimulating factor = response to inflammation and tissue damage
  - alpha-2-HS-glycoprotein = differentiation of monocytes and macrophages
  - complement component C5-1 = part of classical complement pathway
- ***wide variety of proteins related to tissue repair are elevated***
  - fibrinogen beta chain coagulation factor IX
  - coagulation factor X precursor Urokinase-type plasminogen activator
  - putative hepatocyte growth factor activator

## Subtractive Hybridization Results: Genes that were down-regulated

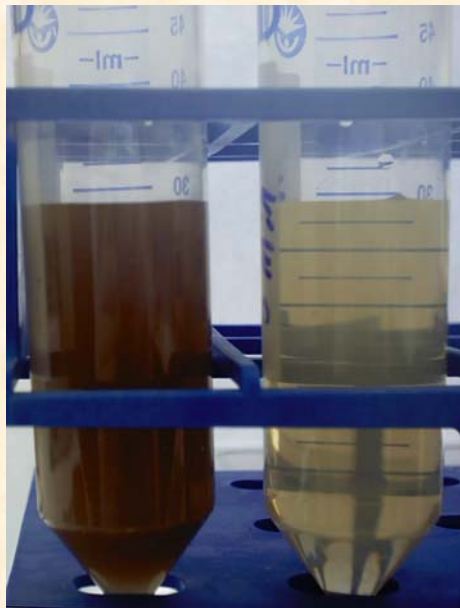
- ***wide variety of proteins related to immune response are suppressed***
  - several chemotaxins = activate macrophages, neutrophils
  - COX 1, 2, 5A, 8H = inflammatory pathways
  - alpha-2-macroglobulin-2 = plasma proteinase inhibitor; potent immune enhan.
  - ovostatin precursor = proteinase inhibitor
- ***proteins related to metabolism and homeostasis are suppressed***
  - glucokinase & hexokinase Fructose-bisphosphate aldolase B
  - organic solute transporter beta O-methyltransferase

# Are we preparing our test materials properly?



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Deguchi et al. 2001

## Second study: Fathead minnows exposed to SWNT and nC<sub>60</sub>

Dose group	Exposure Concentration
Control = RHW	0
Control 2 = RHW + nano-1	0.2 ppm Nano-1 peptide
Nano-1 wrapped SWCNT	0.2 ppm
un-wrapped SWCNT	0.2 ppm
nC60 (THF prep.)	0.5 ppm
nC60 (H <sub>2</sub> O stirred)	0.5 ppm



adult male fathead minnow  
(*Pimephales promelas*)

purchased from Aquatic Bio Systems, Fort Collins, CO

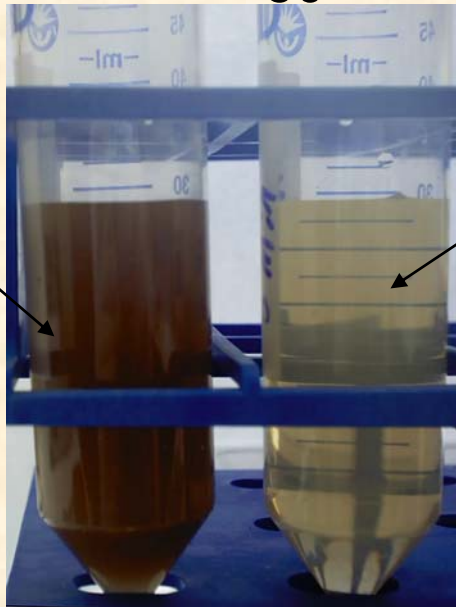
n = 10 (5 for nC60)  
individual aquaria

10 different tissues stored  
+ livers for gene chips

# Fathead minnows exposed to SWCNT and nC60

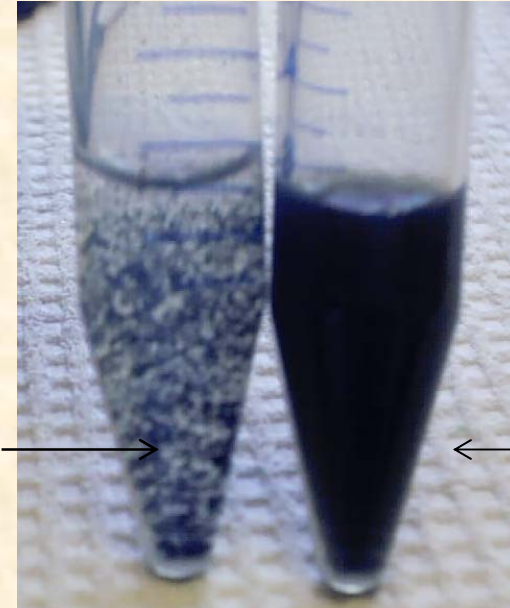
## C<sub>60</sub>

Stirred for 2 wks in MQ water



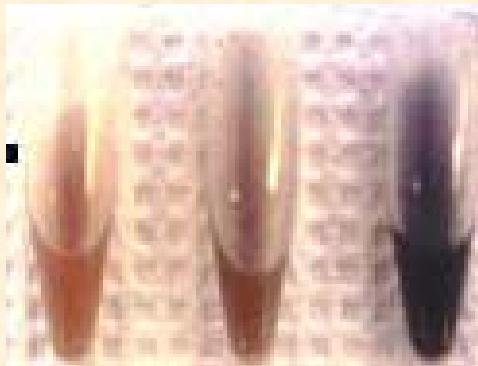
Centrifuged 2x at 4000xg = nC60

## SWCNT



Uncoated

W-SWCNT



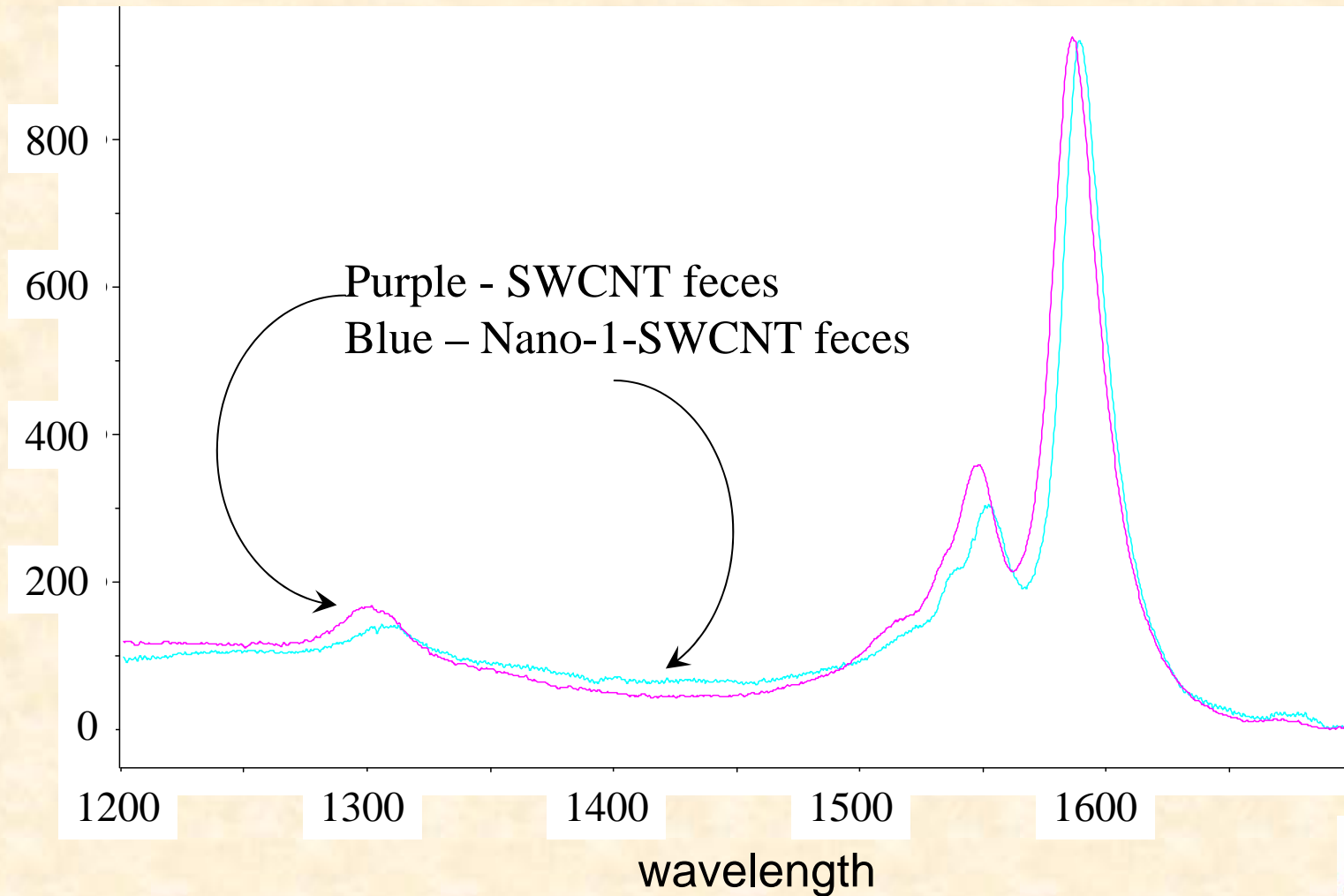
control

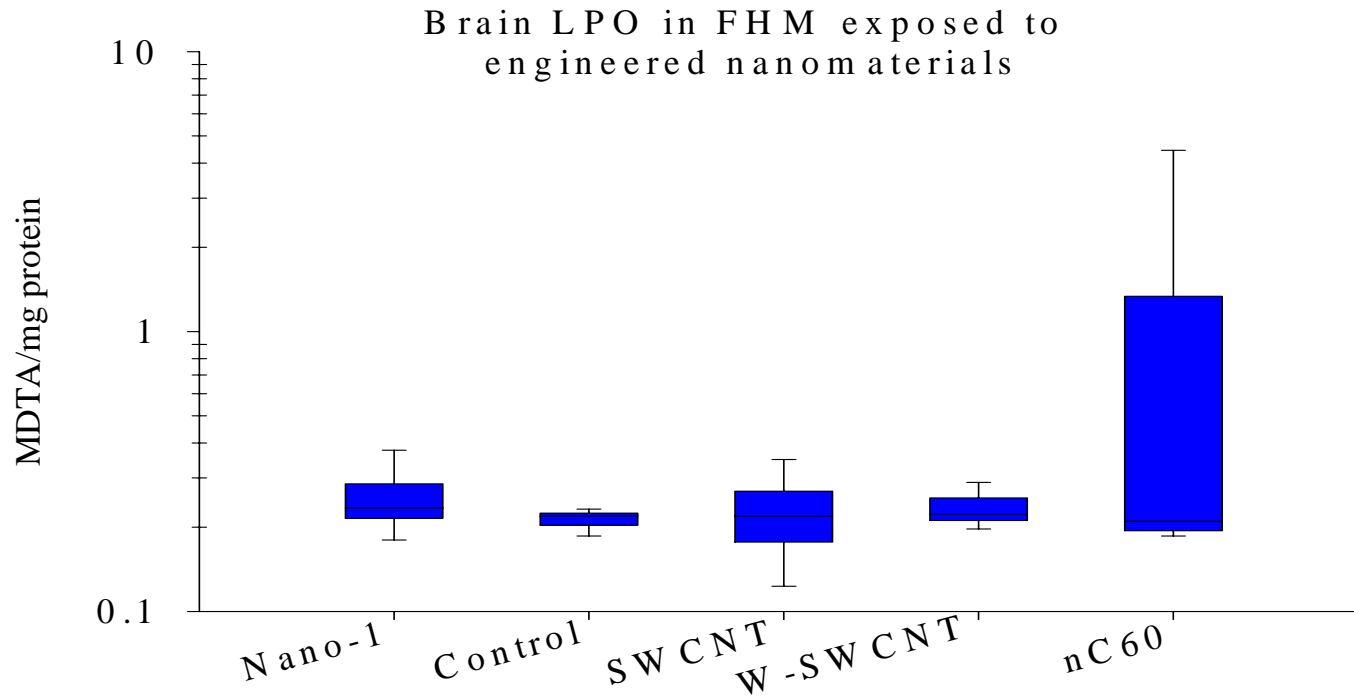
SWCNT  
un-coated

SWCNT  
wrapped w/  
nano-1 peptide

Homogenized fecal pellets recovered from the digestive tract of fathead minnows after 48 hour exposure to 0.2 ppm of un-coated or peptide-wrapped SWCNT. The dark black color and darker brown of pellets from the wrapped- and un-coated SWCNT-exposed fish indicates the presence of SWCNT, which was confirmed by Raman spectroscopy, measuring the G-line.

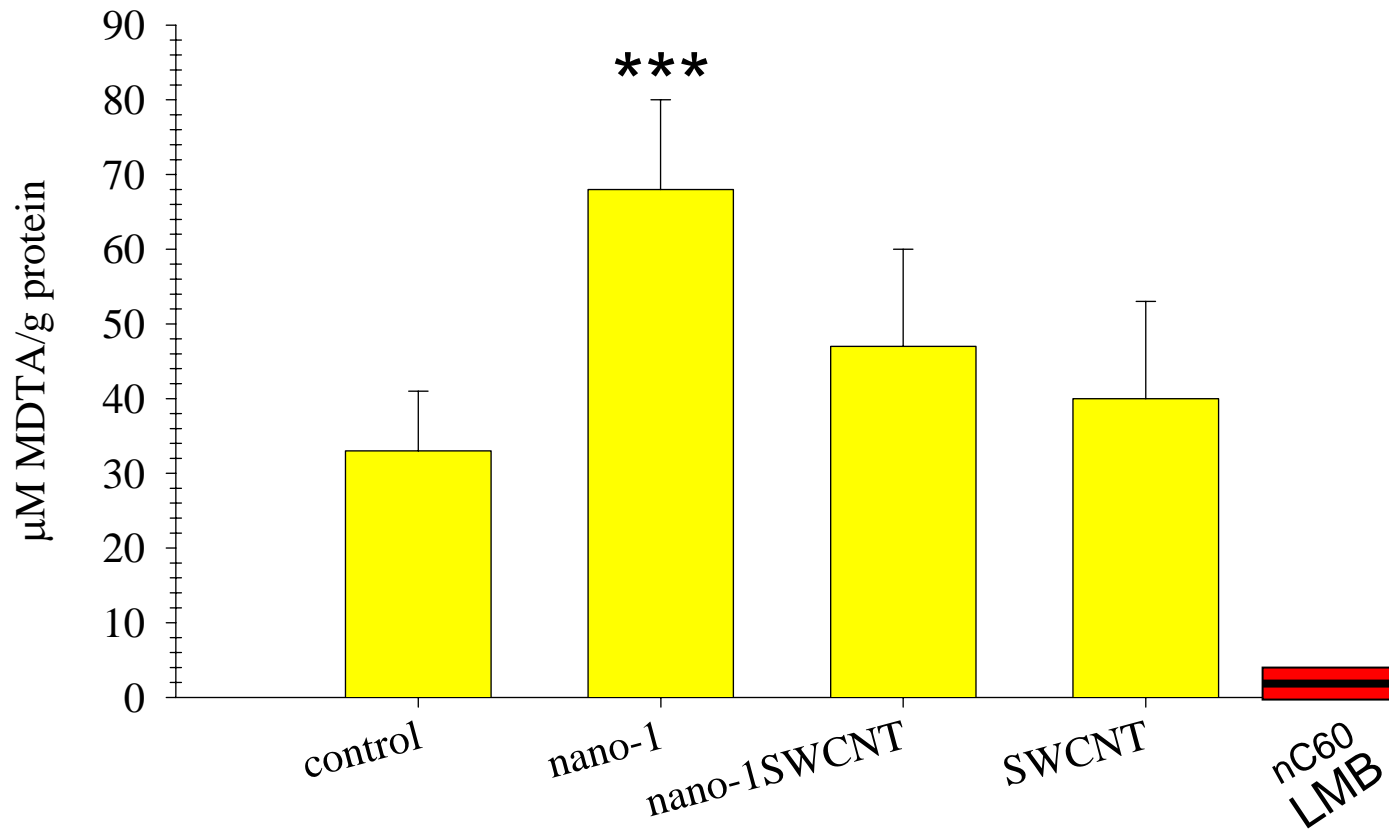
# Raman spectroscopy verifies presence of SWCNT in DG-tract fecal pellets in FHM



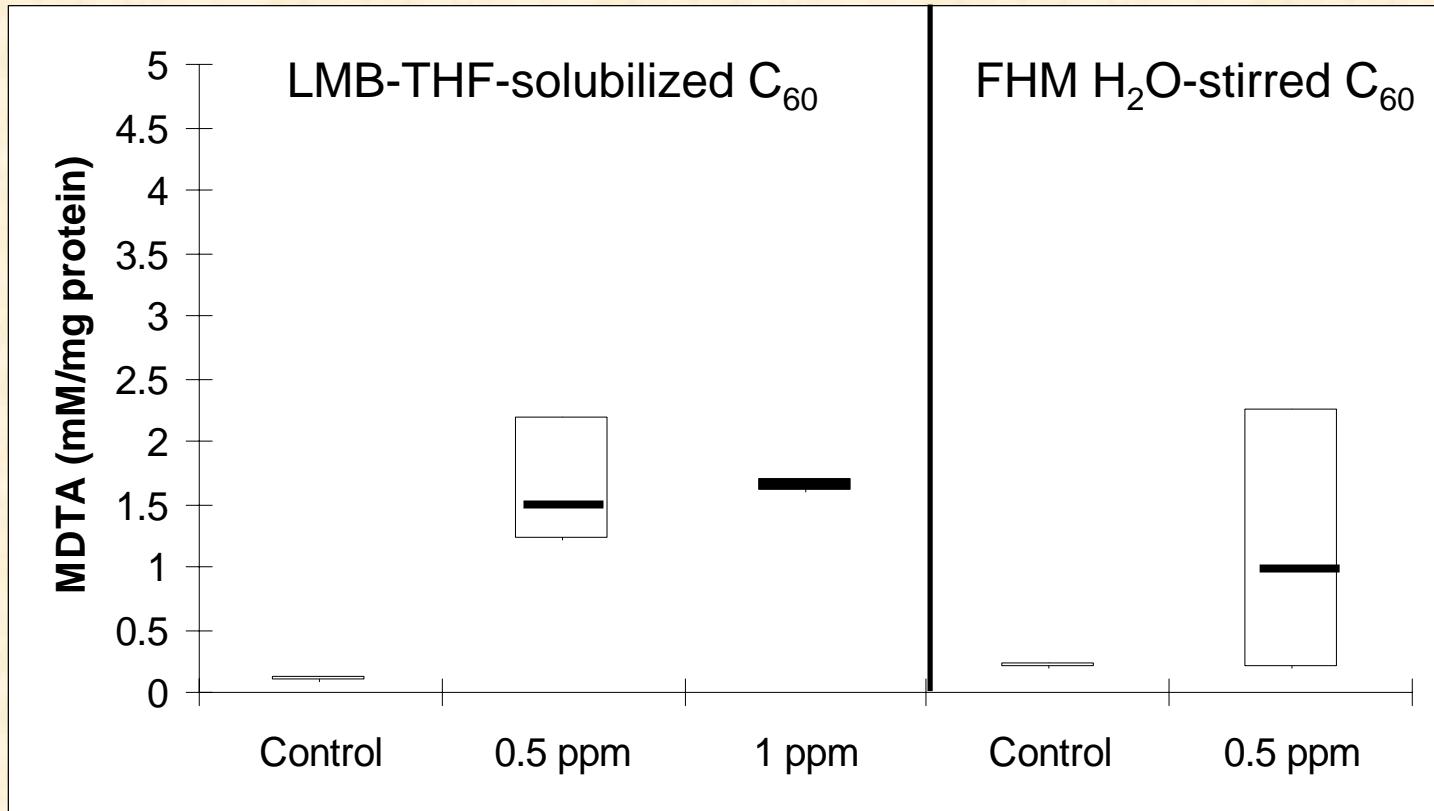


Brain lipid peroxidation in male fathead minnows (FHM) exposed to engineered nanomaterials. Nano-1 = 0.2 ppm nano-1 peptide; SWCNT = 0.2 ppm un-coated purified SWCNT; W-SWCNT = 0.2 ppm nano-1 peptide wrapped SWCNT, nC<sub>60</sub> = 0.5 ppm stirred C<sub>60</sub>. N = 5 for each exposure group.

## LPO in liver of FHM



data from single trial; n = 5 to 10



Brain LPO in large mouth bass and fat head minnow exposed to C<sub>60</sub>

# Fish exposures to 0.5 and 1 ppm stirred nC<sub>60</sub>, 96 hours

Assessed levels of liver:

- CYP1A
- CYP2M1/2
- CYP2K1/2

•PMP70



mRNA and protein in FHM  
n = 10-11

***PMP70 downregulated in FHM (both mRNA and protein)***

## Acute water-soluble nC<sub>60</sub> toxicity

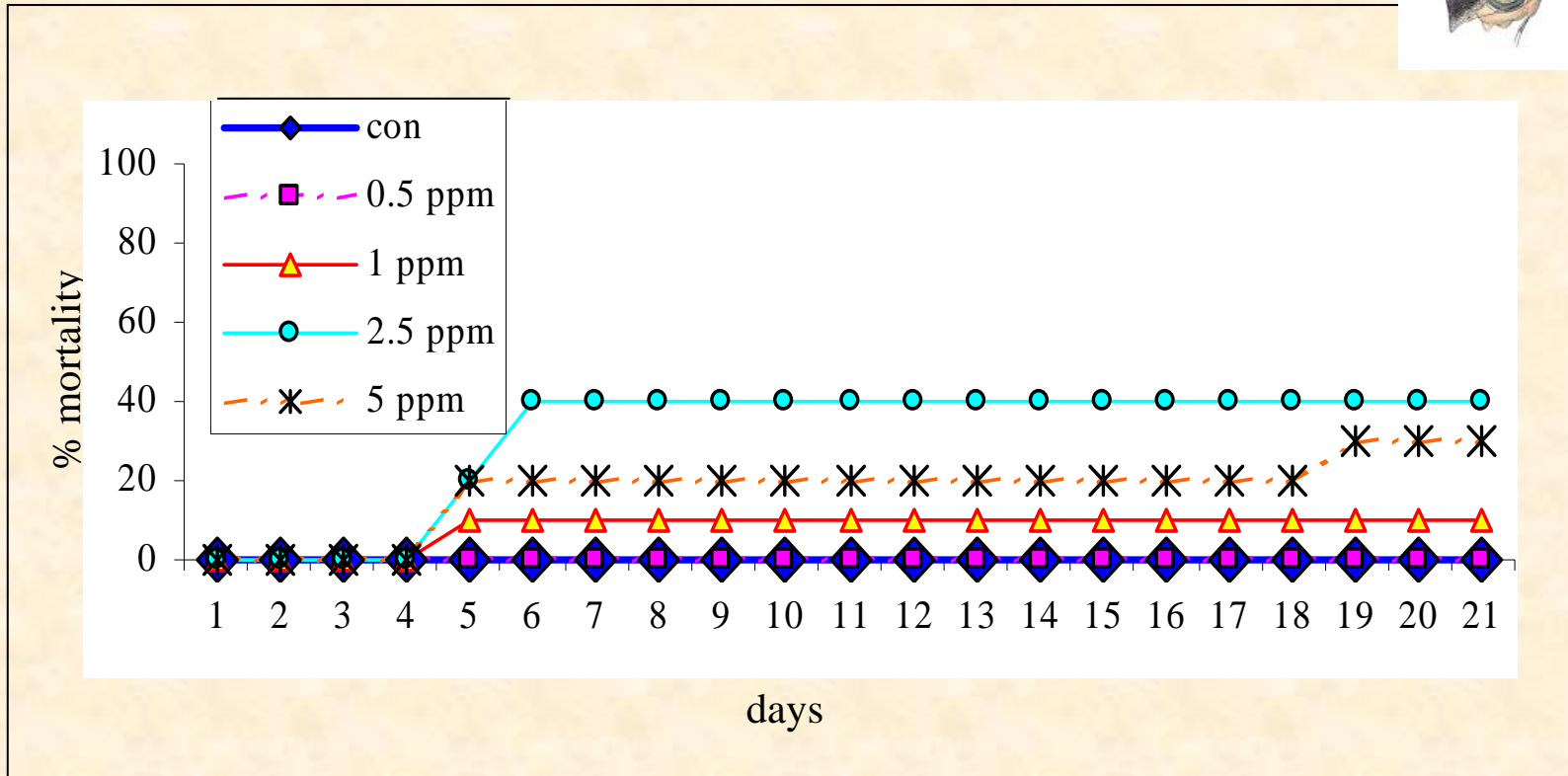
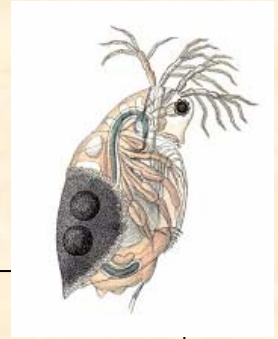


**THF-fullerene:**

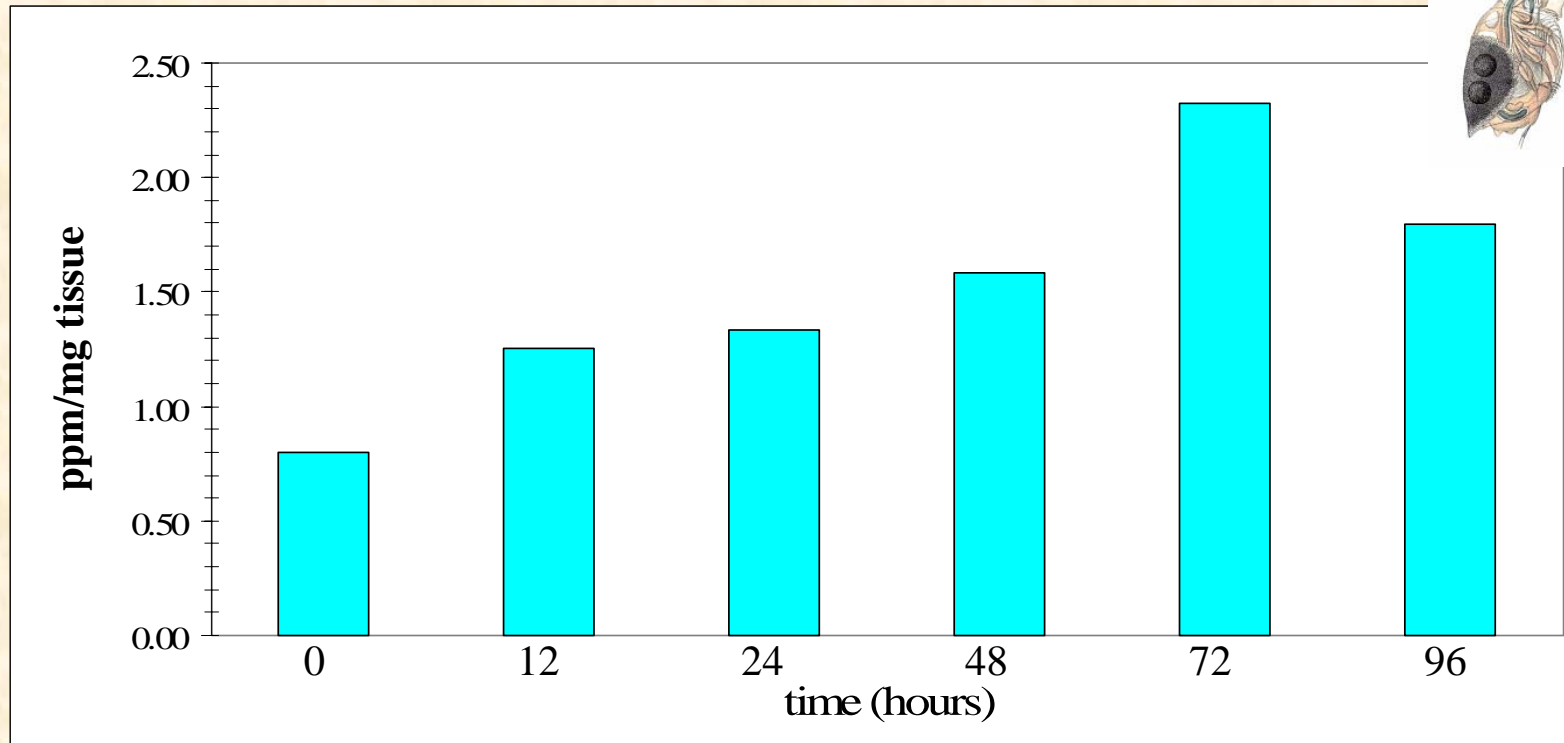
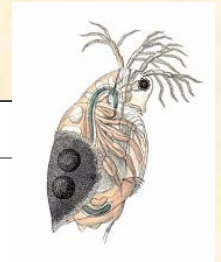
**48-hour LC<sub>50</sub> = 0.8 ppm**

**Water-stirred-fullerene: 48-hour LC<sub>50</sub> = greater than 35 ppm**

# Mortality per exposure concentration

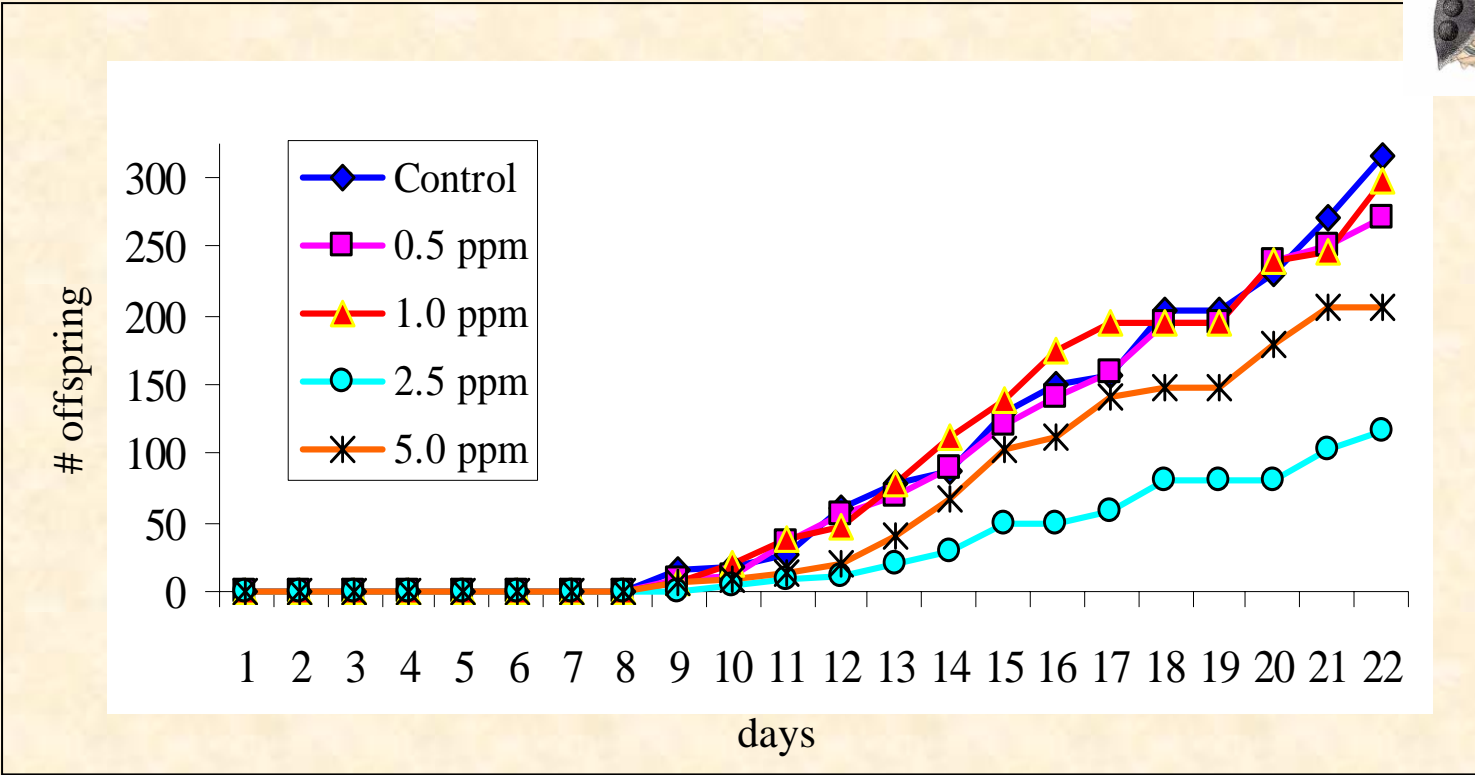


# Uptake of water-stirred nC<sub>60</sub> into *daphnia* (exposed to 30 ppm)



- 12-15 daphnids collected at each time point
- rinsed 5X w/ RHW over course of 1 hour
- wet-weight taken
- oxidized using 1 mL bleach
- extracted O/N into toluene

# Cumulative number of offspring per exposure group



## Water-stirred fullerene toxicity



Freshwater filter feeder: *Daphnia magna*

No LC<sub>50</sub> value at concentrations from 0-35ppm



Benthic freshwater crustaceans: *Hyalella azteca*

No LC<sub>50</sub> value at concentrations from 0-7ppm



Marine harpacticoid copepod

No LC<sub>50</sub> value at concentrations from 0-22.5ppm

# Nano-Iron Studies

## Surface Stabilized iron slurry

-Hc = 458

- $\sigma_s = 136.7$  emu/g

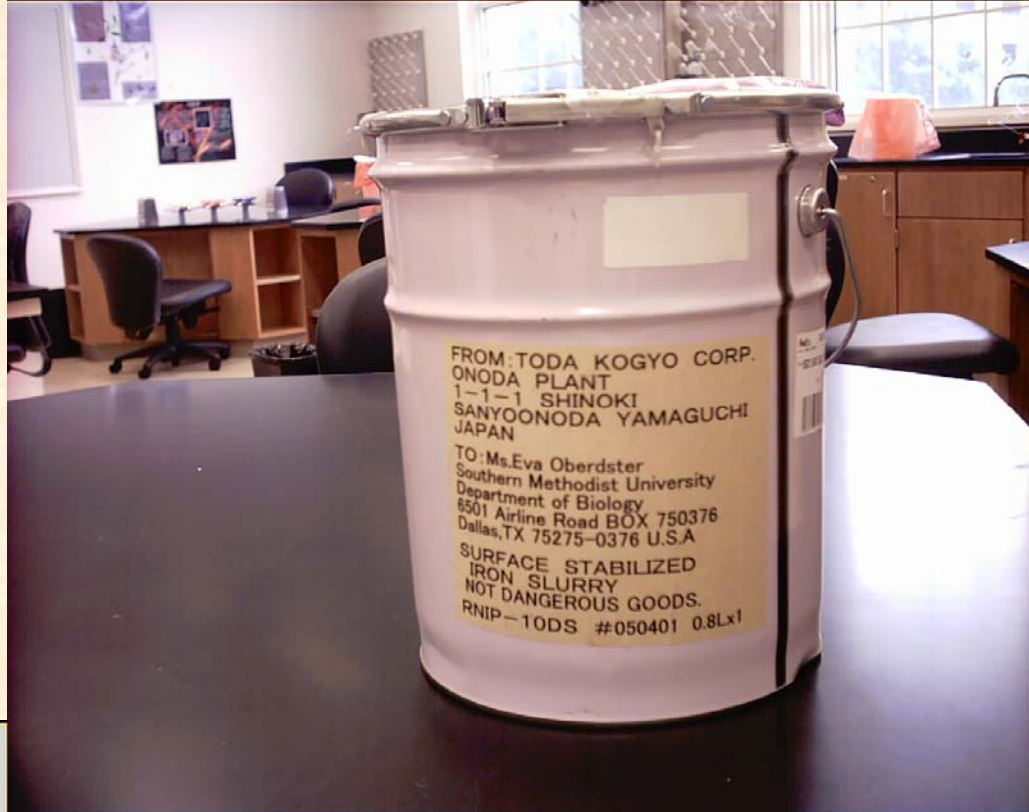
- $\sigma_r/\sigma_s = 0.180$

-pH = 10.5

-BET = 30.7 m<sup>2</sup>/g

-D110 = 279 Å

-CM  $\mu$  s/cm = 2.61 E2



## Ingredients:

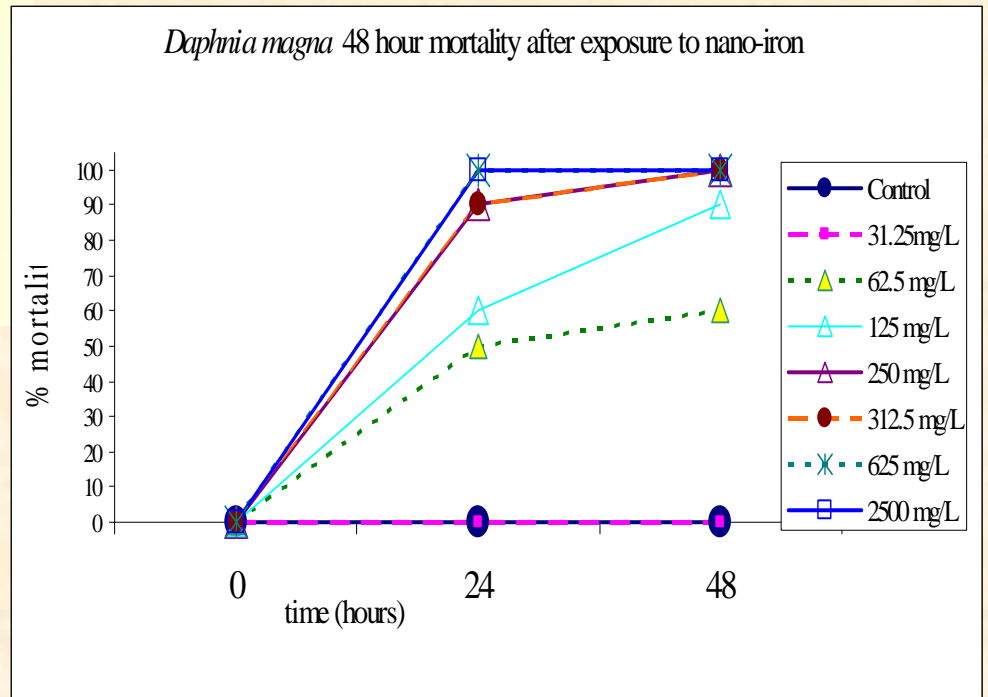
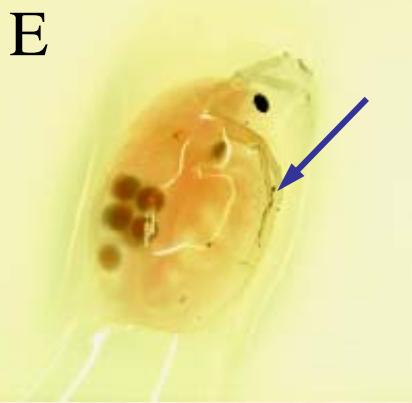
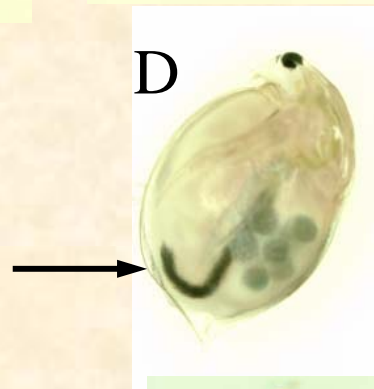
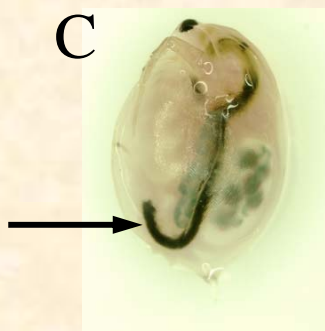
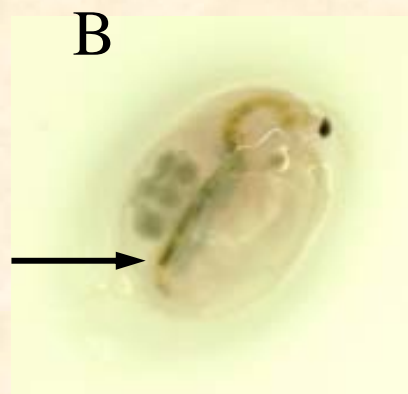
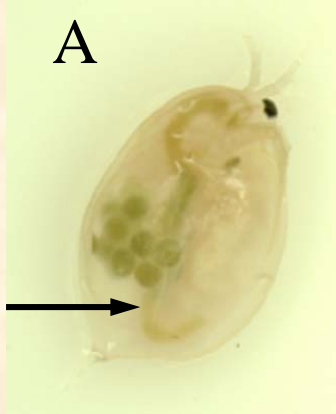
Fe: 16.5 %

Fe<sub>3</sub>O<sub>4</sub>: 8.5%

H<sub>2</sub>O: 75%

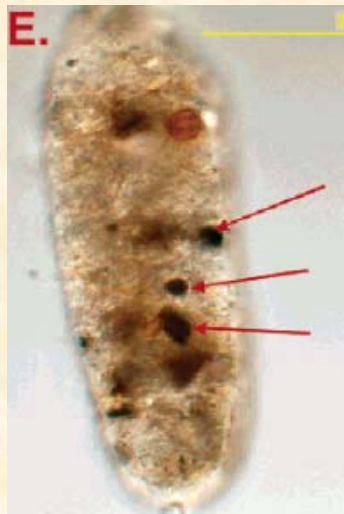
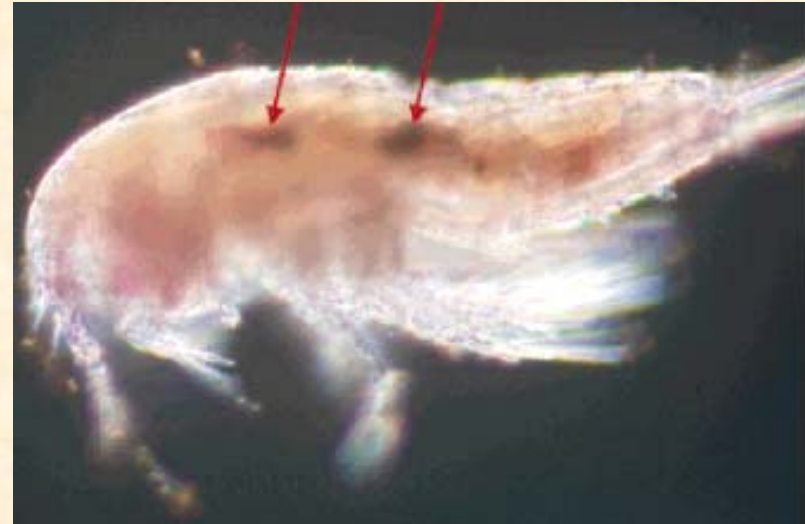
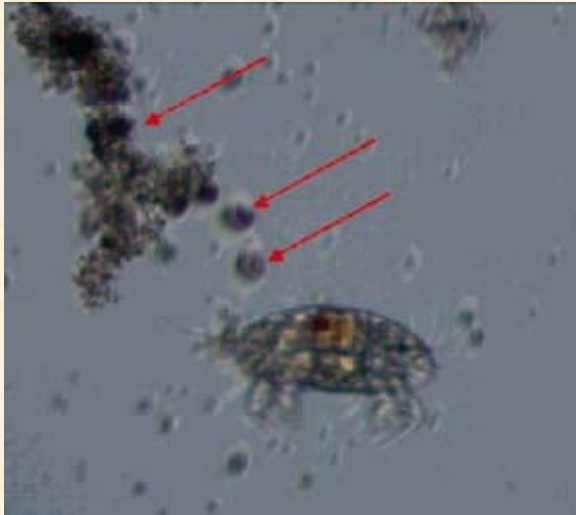
specific gravity: 1.25

Courtesy of Dr. E. Oberdörster

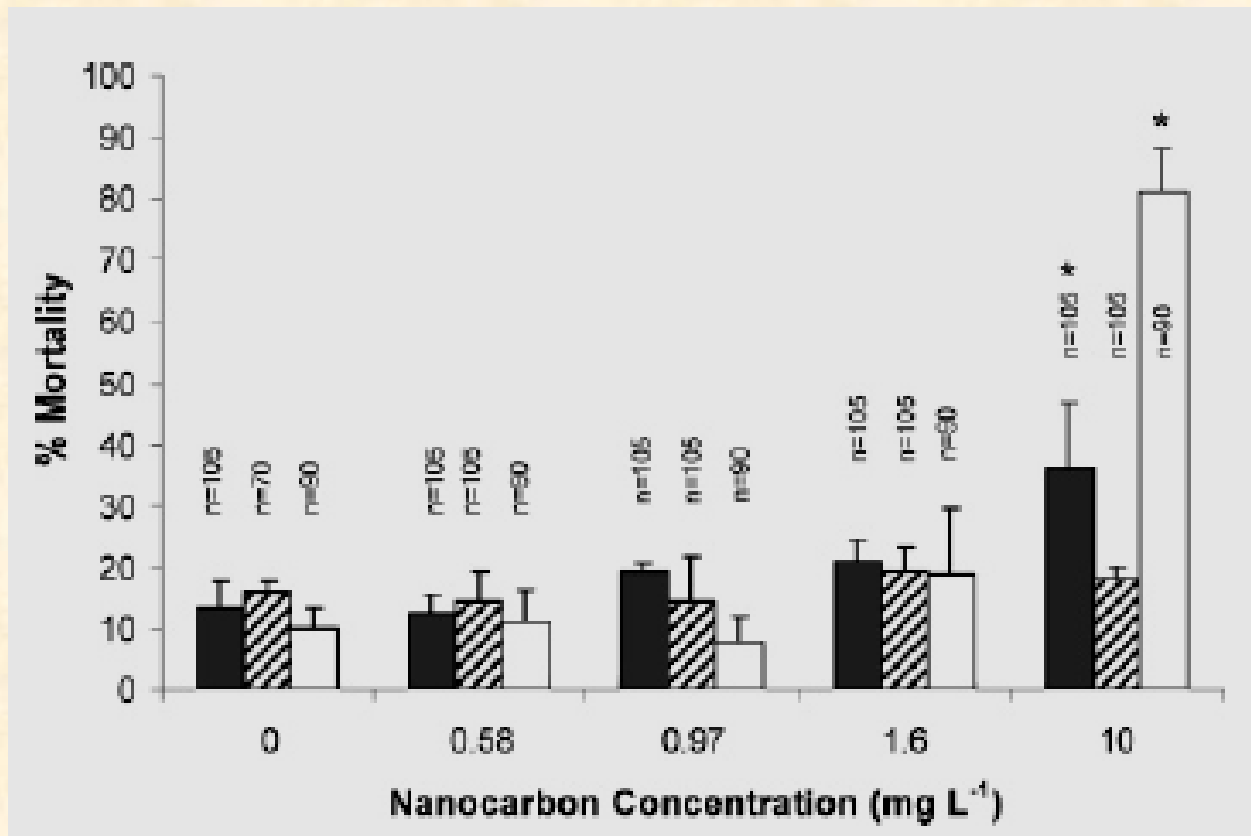


Daphnia exposed to various concentrations of nano-iron used in remediation. A = control; B = 3 mg/L; C = 7.5 mg/L; D = 15 mg/L; E = 30 mg/L; F = 125 mg/L (dead daphnid). All daphnids shown are 21 days old and eggs are visible in their brood pouches (green circles). Note the darkening of the digestive tract from A (normal greenish color) to D with increased ingestion of nano-iron particles (black arrows). Antennae become clogged with nano-iron in E and F (blue arrows). The 24 and 48 hour mortality curve is shown on the right.

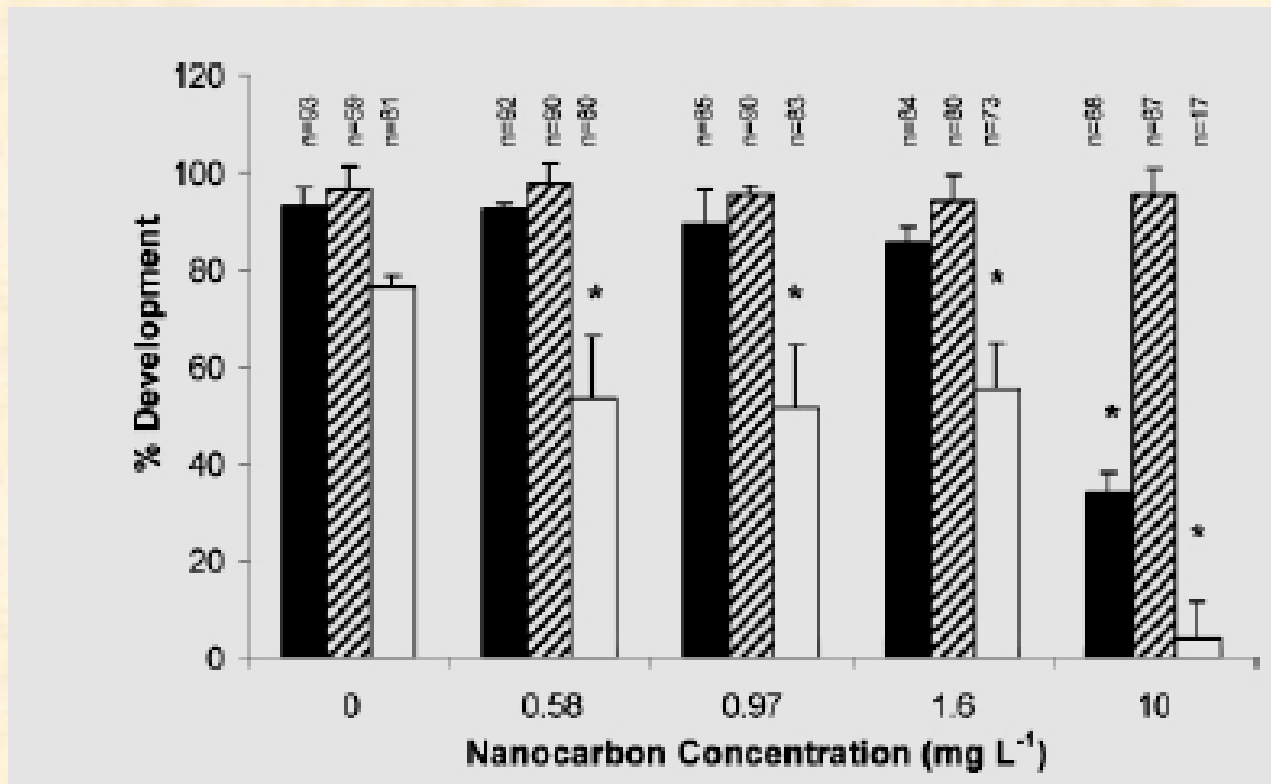
# Uptake cycle of pure SWCNT



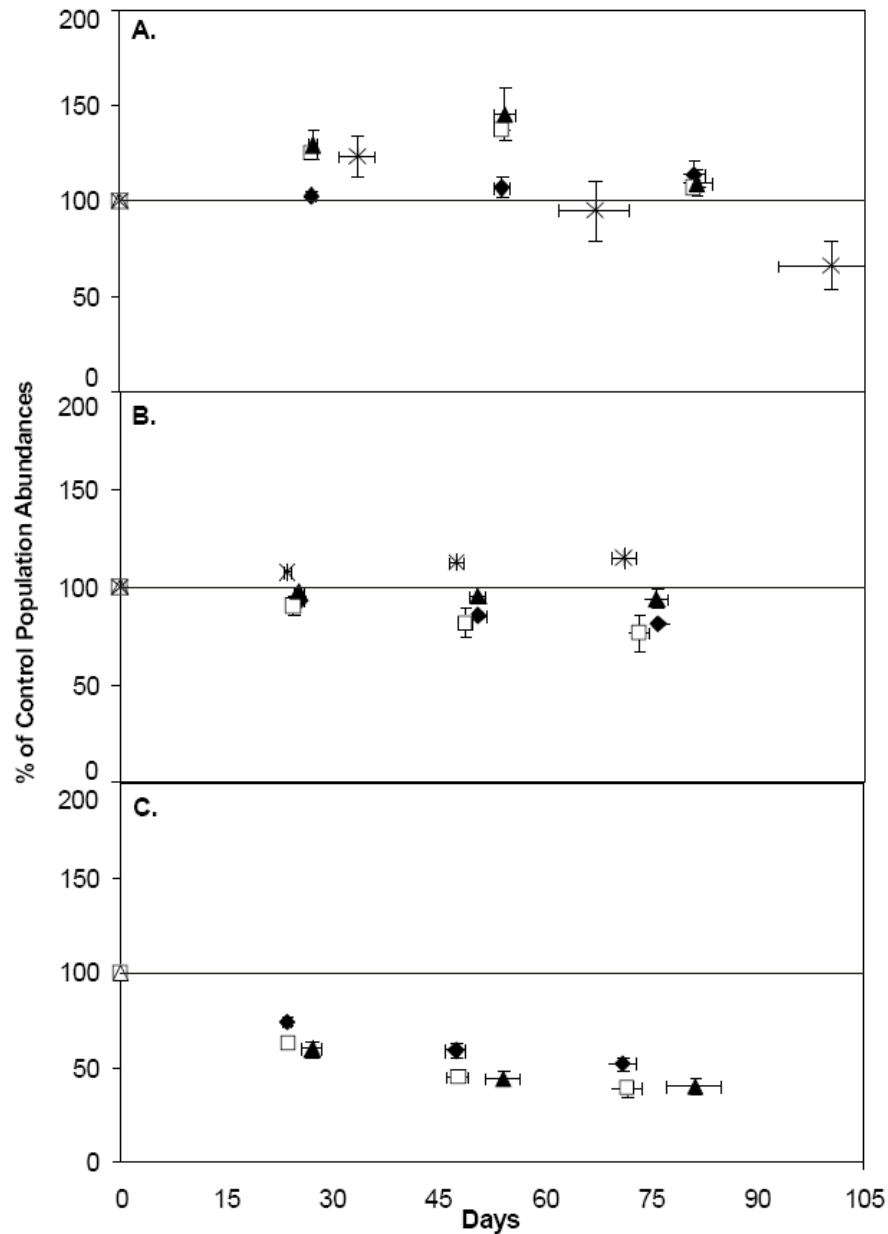
Templeton et al., 2006 ES&T 40(23): 7387-7393.



Life-cycle mortalities for each population. AP-SWCNTs (solid black bars), pure SWCNTs (hatched bars) and fluorescent nanocarbon byproducts (unfilled bars).



Mean percentage of individuals from each population that successfully developed from the nauplius stage to the adult stage. AP-SWCNTs (solid black bars), pure SWCNTs (hatched bars) and fluorescent nanocarbon byproducts (unfilled bars).



Experimentally-based exposure-specific projected population abundances through three generations for treatments with **A)** AP-SWCNTs, **B)** pure SWCNTs, and **C)** fluorescent nanocarbon byproducts. Control normalized population projections are shown by ◆ for 0.58 mg-L-1 exposures, □ for 0.97 mg-L-1 exposures, ▲ for 1.6 mg-L-1 exposures, and \* for 10 mg-L-1 exposures.

# Behavior of $C_{60}$ in sea water?



Mix water-stirred  $C_{60}$  with 20 ppt sea water

$\leq 200$  nm\*

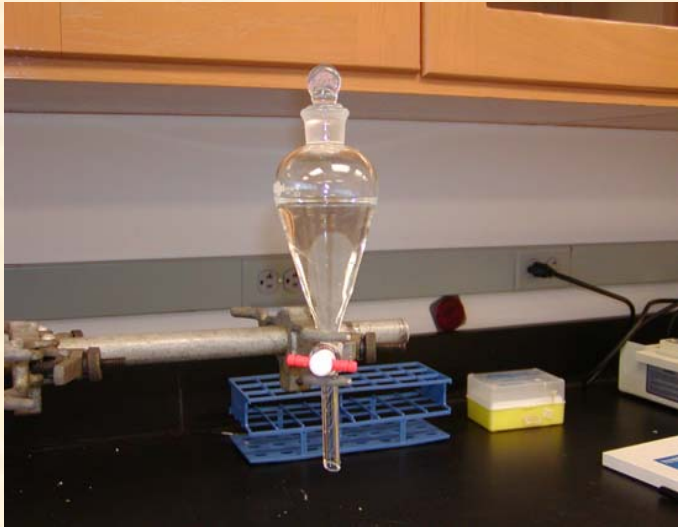


Diffuse aggregates float on top

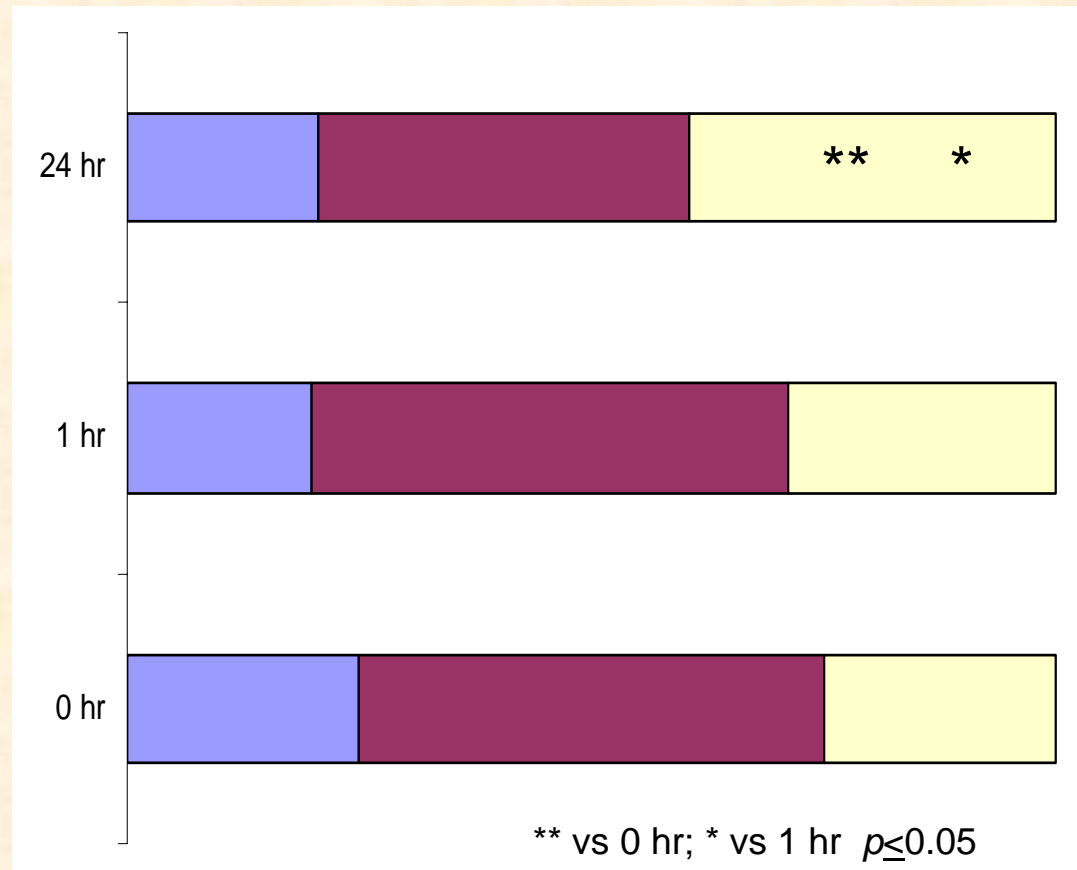


Large aggregates form on bottom

# Partitioning of C<sub>60</sub> in Sea Water



Water-stirred C<sub>60</sub> was added to 20 ppt sea water. The samples were gently mixed for 0, 1 or 24 hours then allowed to settle for 2 hours.

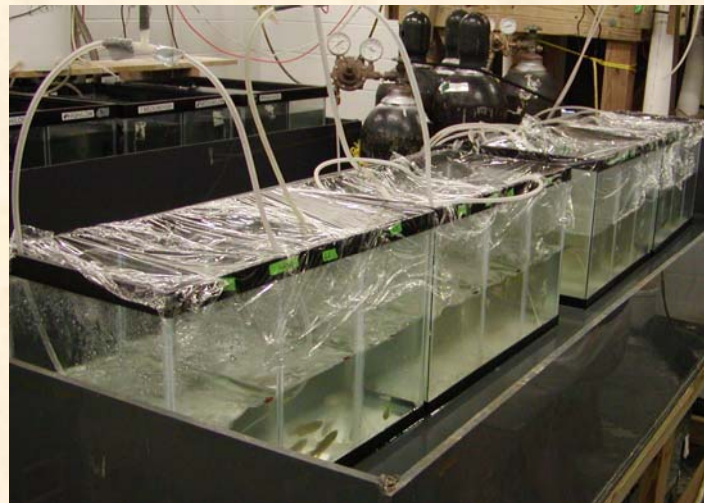


# Exposure of *Fundulus heteroclitus* to water-stirred C<sub>60</sub>

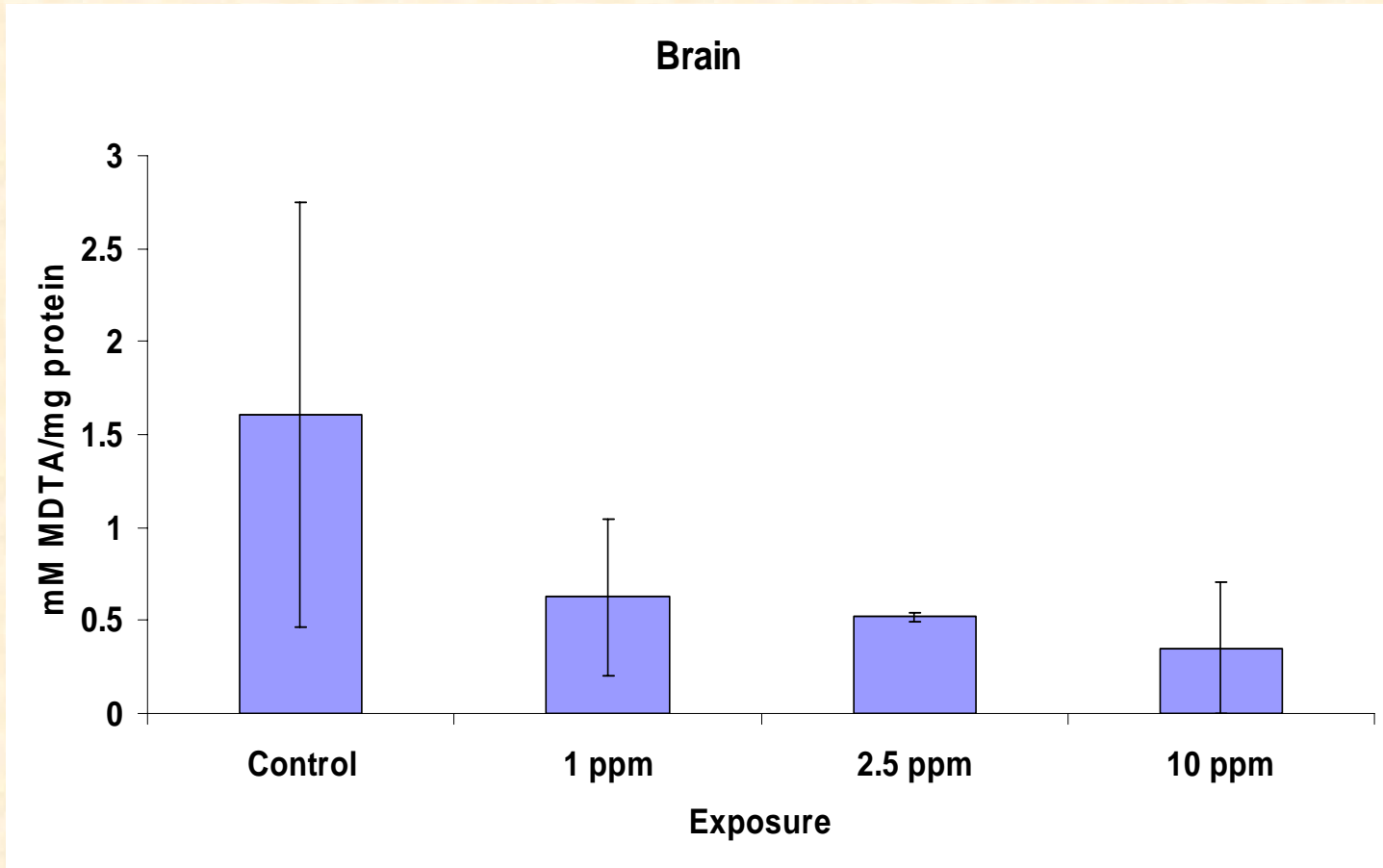


*Fundulus* were exposed to 0, 1, 2.5 or 10 ppm water stirred C<sub>60</sub> for 3 days.

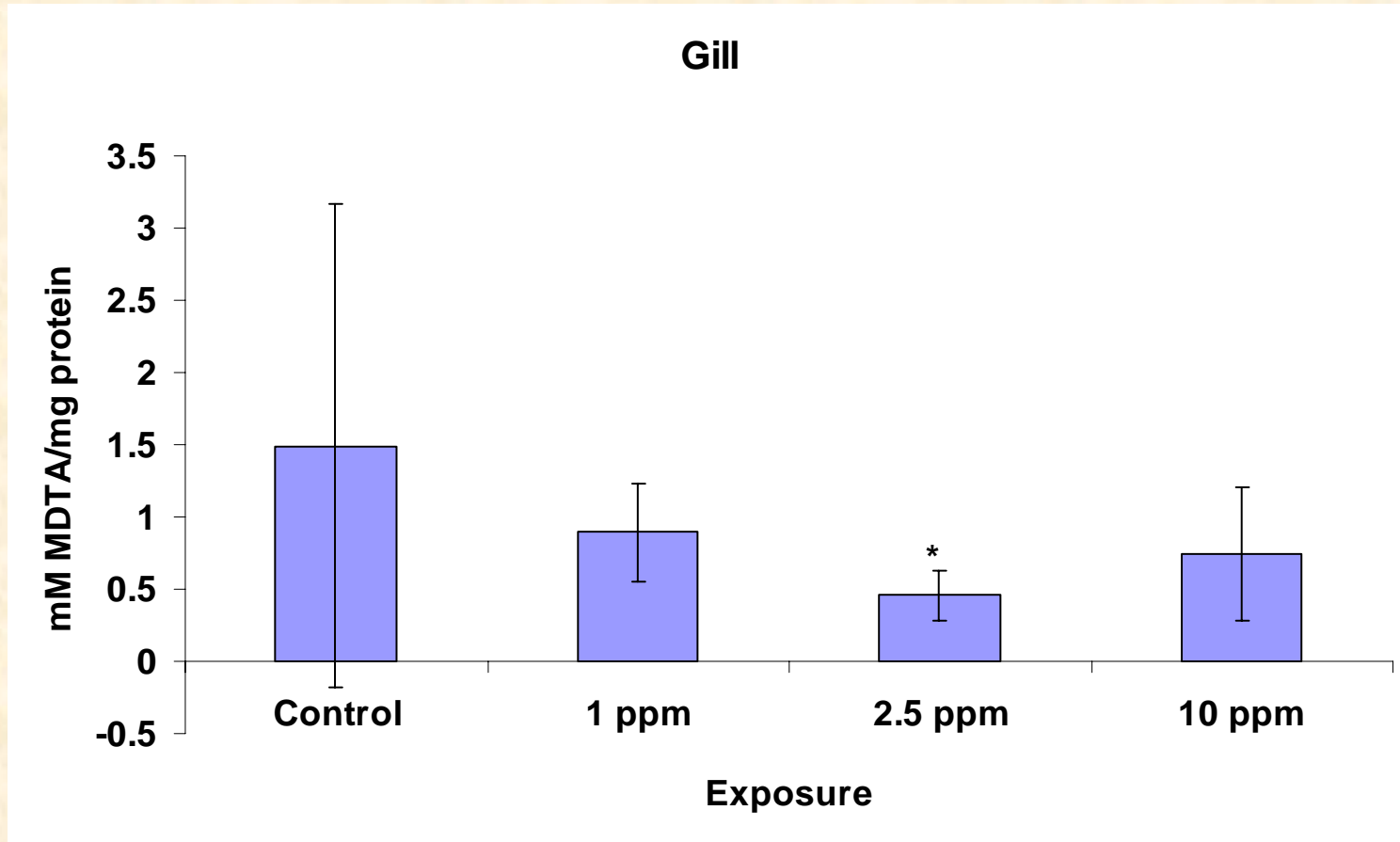
Analyzed effects in brain, gill, gut and liver.



# Lipid Peroxidation in tissues of *Fundulus* exposed to increasing concentrations of C<sub>60</sub>

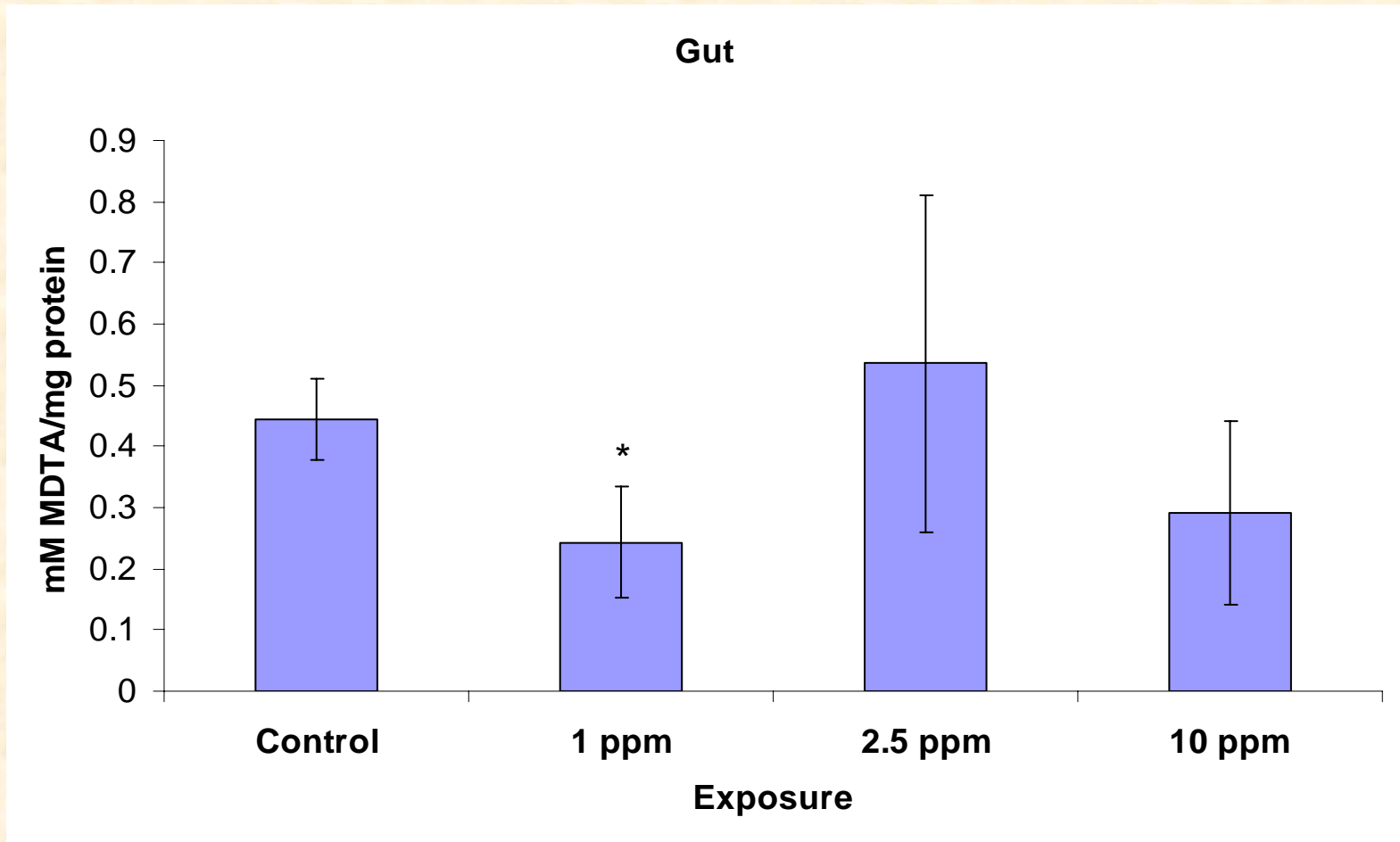


# Lipid Peroxidation in tissues of *Fundulus* exposed to increasing concentrations of C<sub>60</sub>



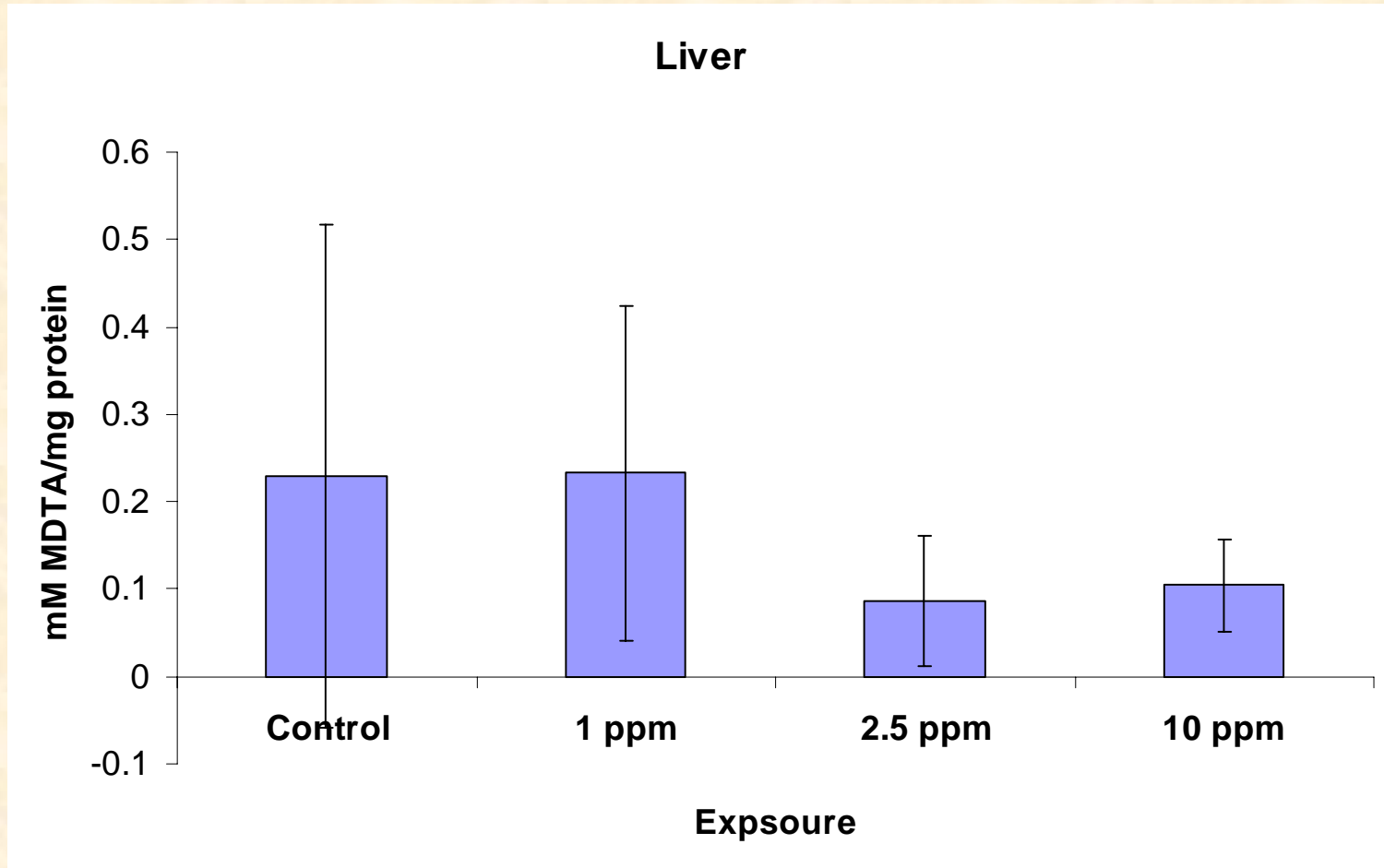
\* (statistically different from 1 ppm at  $p \leq 0.05$ )

# Lipid Peroxidation in tissues of *Fundulus* exposed to increasing concentrations of C<sub>60</sub>

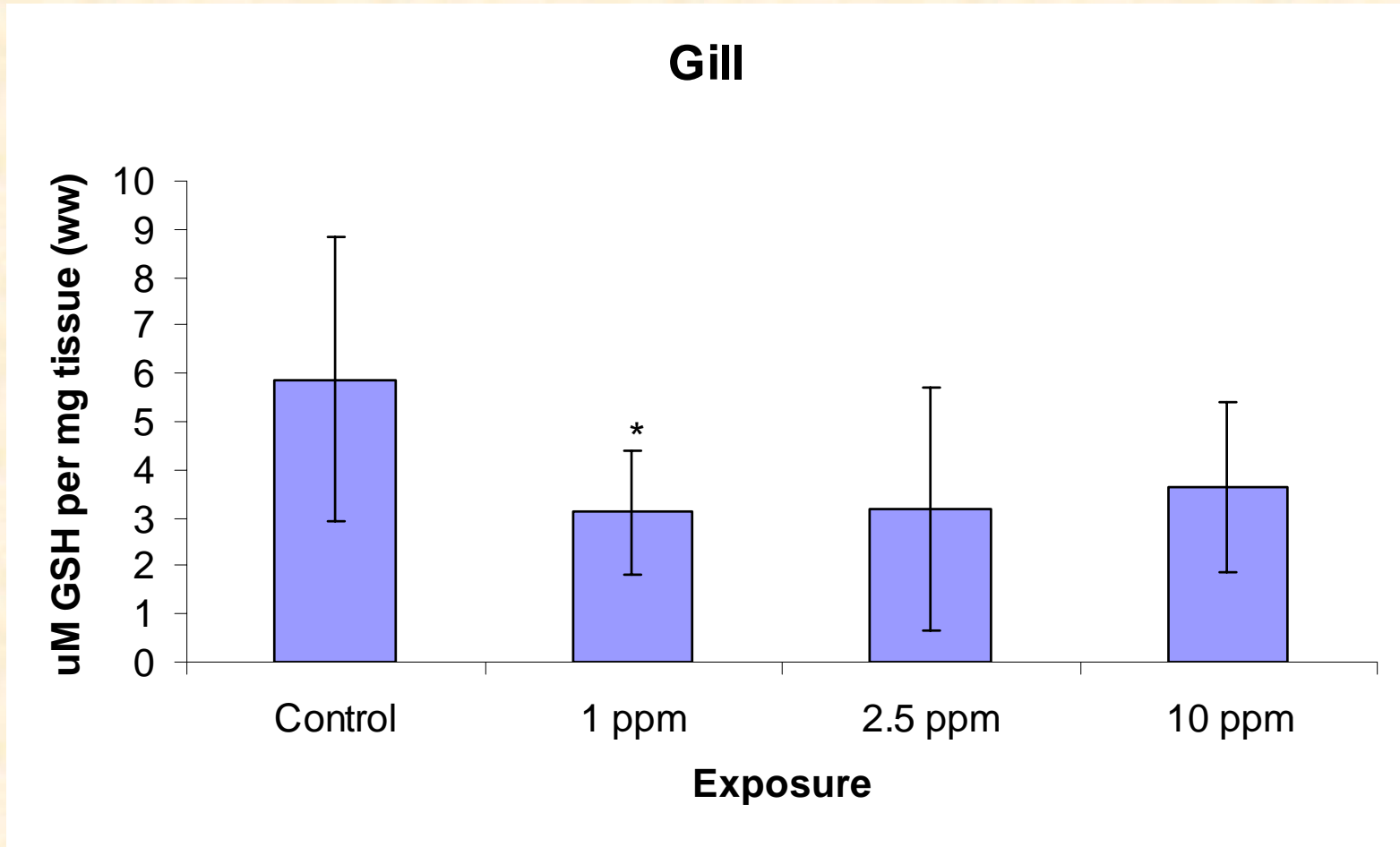


\*(statistically different from control  $p \leq 0.05$ )

# Lipid Peroxidation in tissues of *Fundulus* exposed to increasing concentrations of C<sub>60</sub>

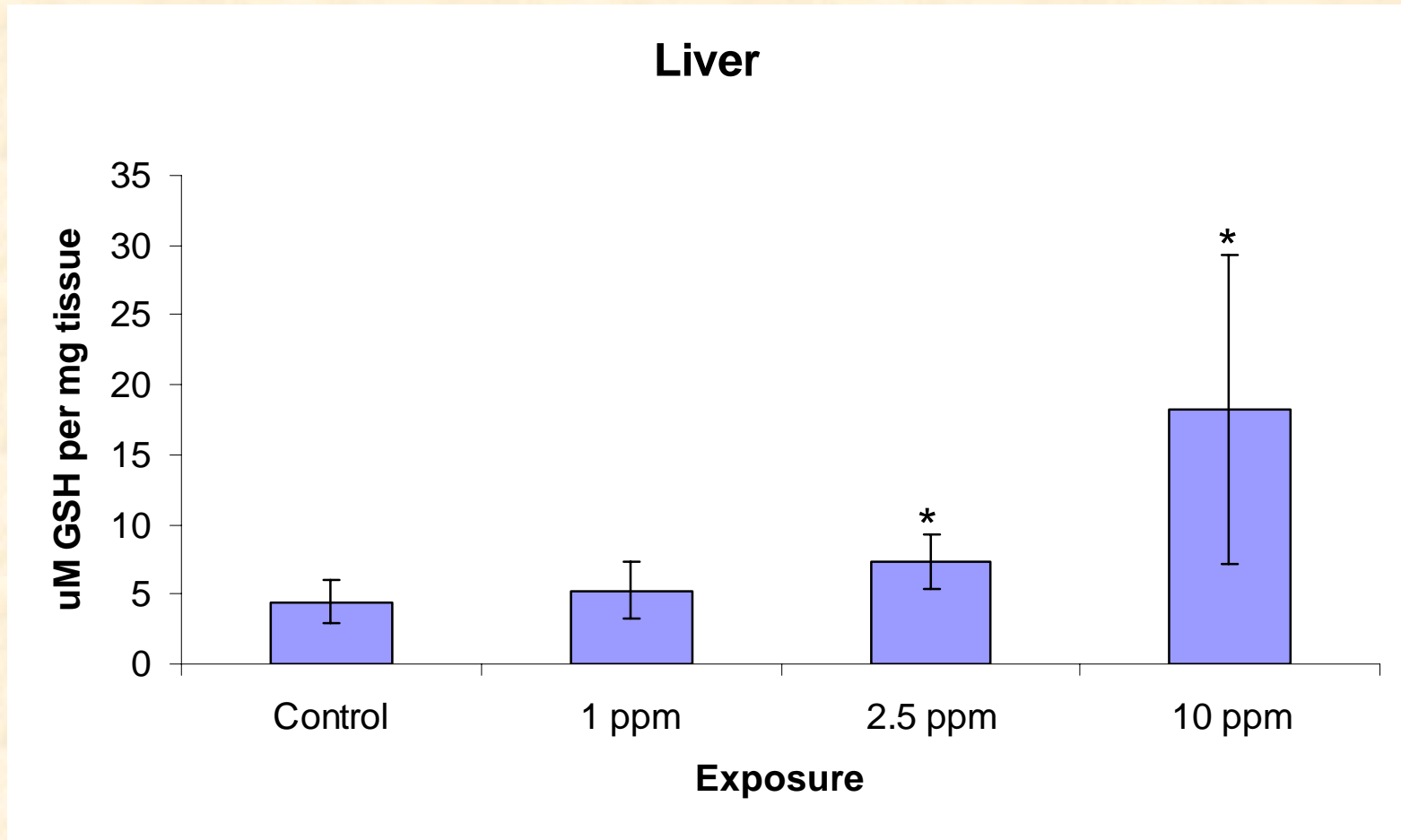


# GSH levels in tissues of *Fundulus* exposed to increasing concentrations of C<sub>60</sub>



\* (not statistically different at  $p \leq 0.05$  but is different at  $p \leq 0.1$ )

# GSH levels in tissues of *Fundulus* exposed to increasing concentrations of C<sub>60</sub>



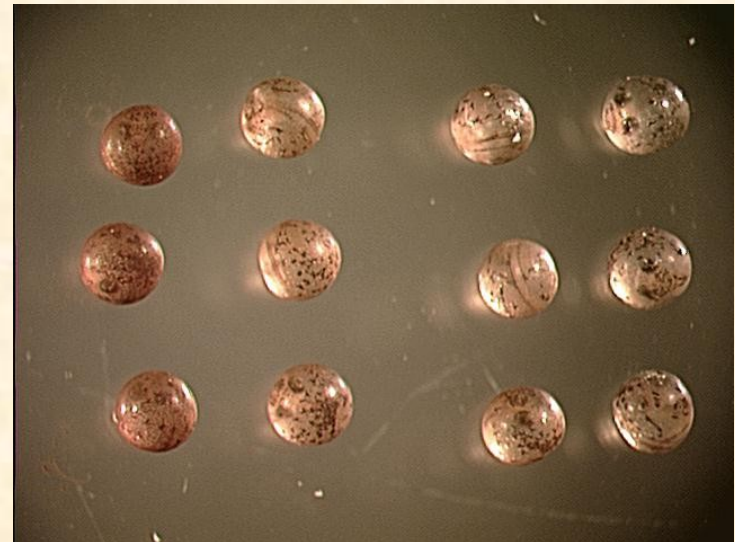
\* (statistically different from controls at  $p \leq 0.05$ )

# Exposure of Embryos to water-stirred C<sub>60</sub>

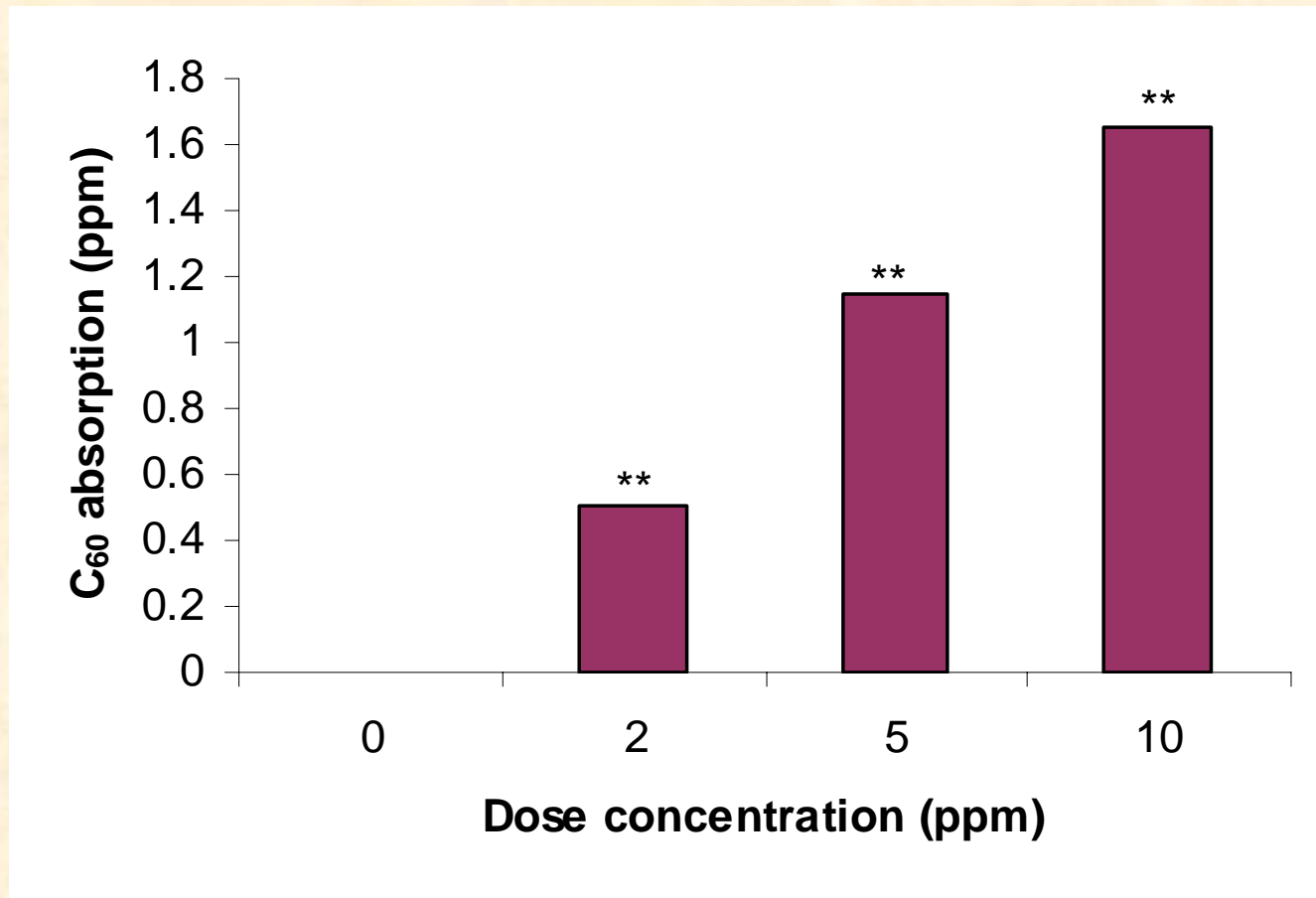


10      5      2      0  
(ppm)

*Fundulus* embryos (6 per replicate) were exposed to increasing concentrations of water-stirred C<sub>60</sub> for 6 days in 20 ppt sea water.

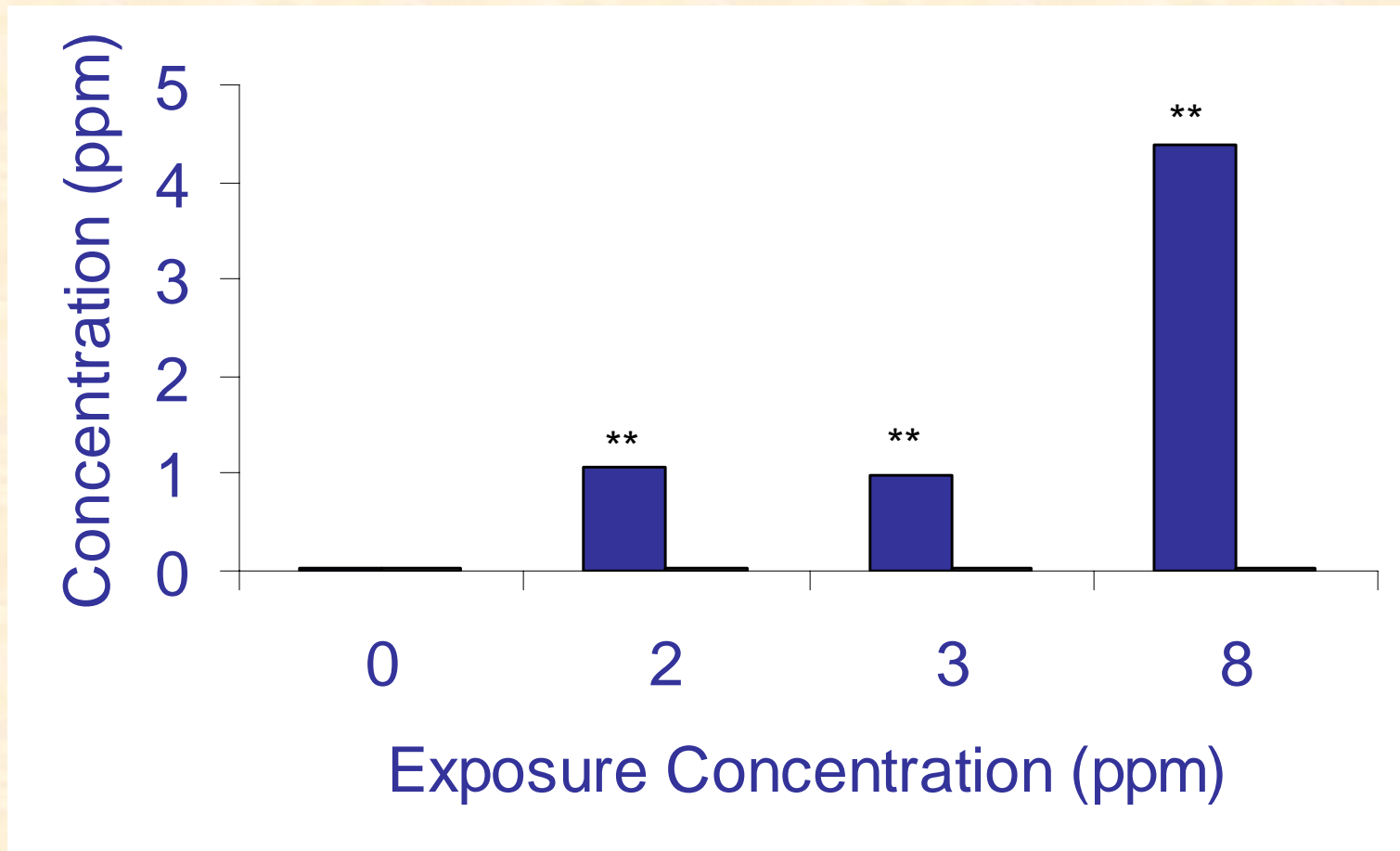


# Uptake by Embryos in water-stirred C<sub>60</sub>



\*\* (statistically different from control at  $p \leq 0.05$ )

# Uptake by Embryos to water-stirred C<sub>60</sub> (partition into fry)

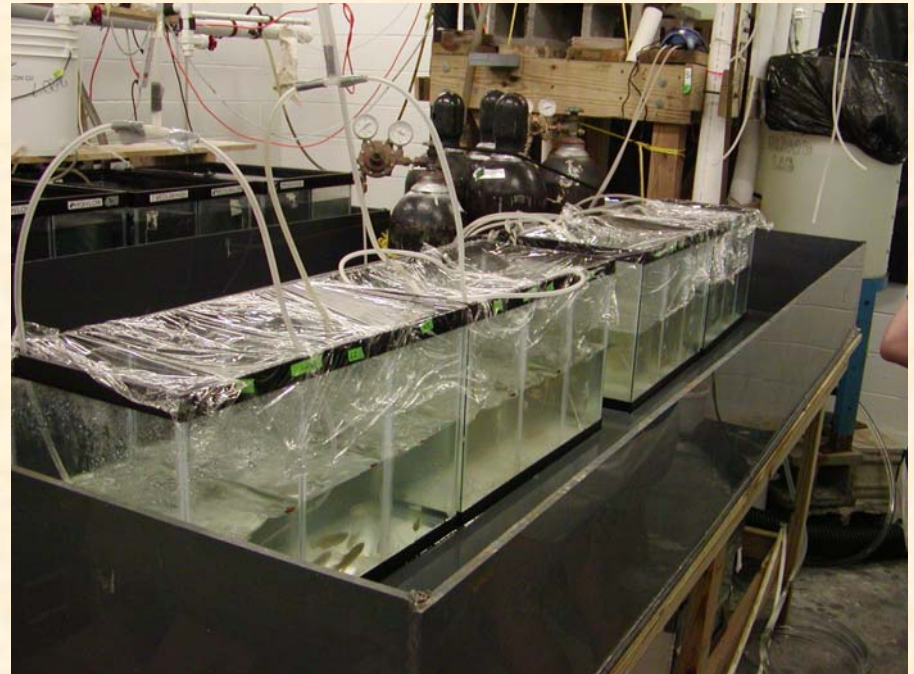


\*\* (statistically different from control at  $p \leq 0.05$ )

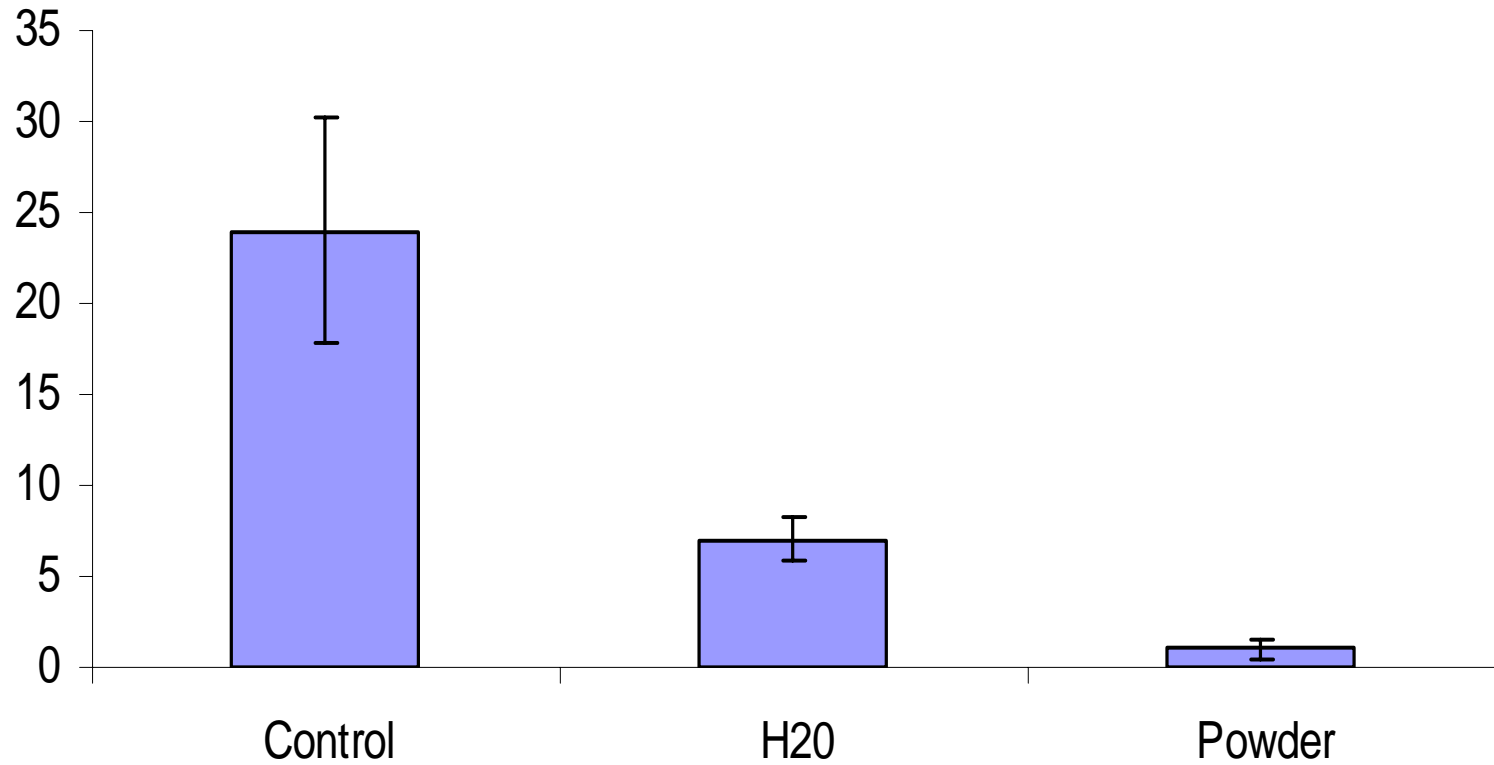
# *Fundulus* Feeding Study

## Experimental Design

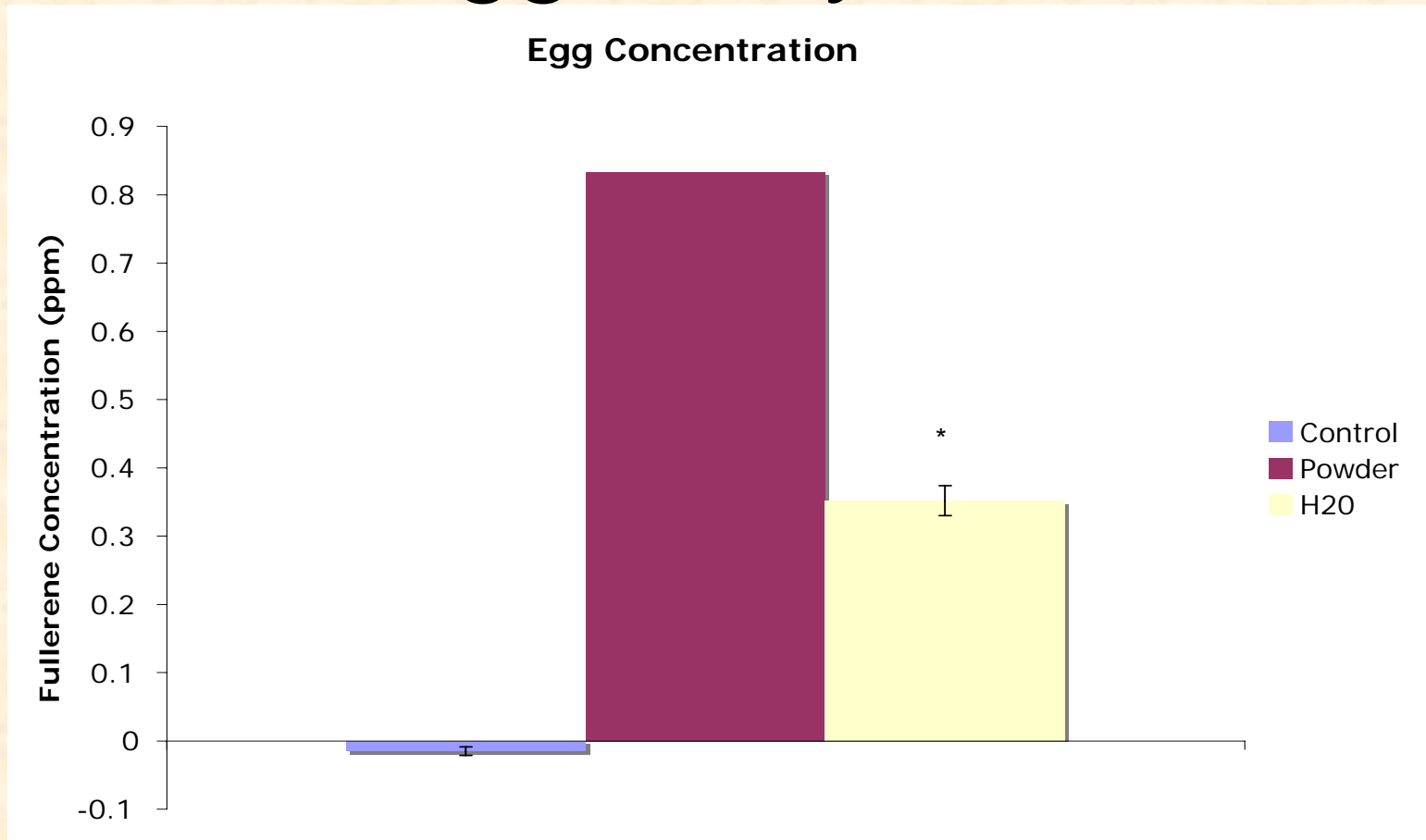
- ▶ 18 female fish split into 3 groups
- ▶ Fed food cubes twice daily (Powder, Water Stirred [2 ppm each], & Control)
- ▶ Eggs collected daily. 1<sup>st</sup> spawn discarded and 2<sup>nd</sup> spawn analyzed.



## Egg Number

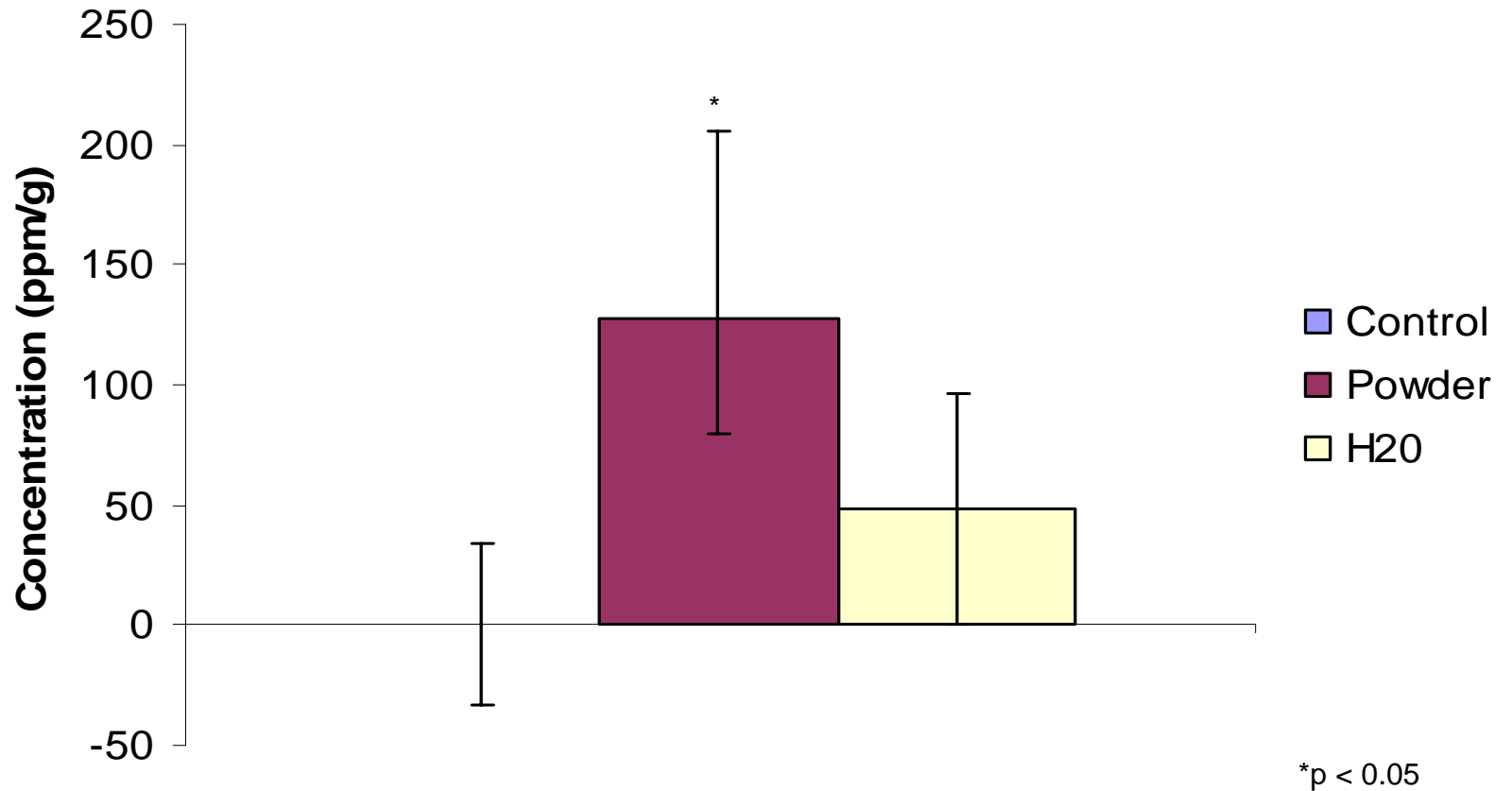


# Egg Analysis



\*p < 0.05

## Liver Concentration



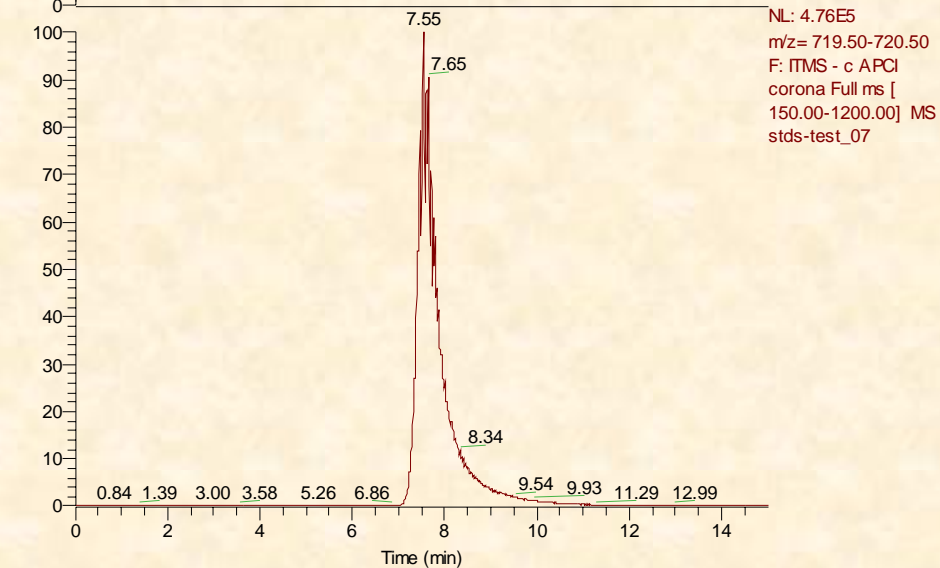
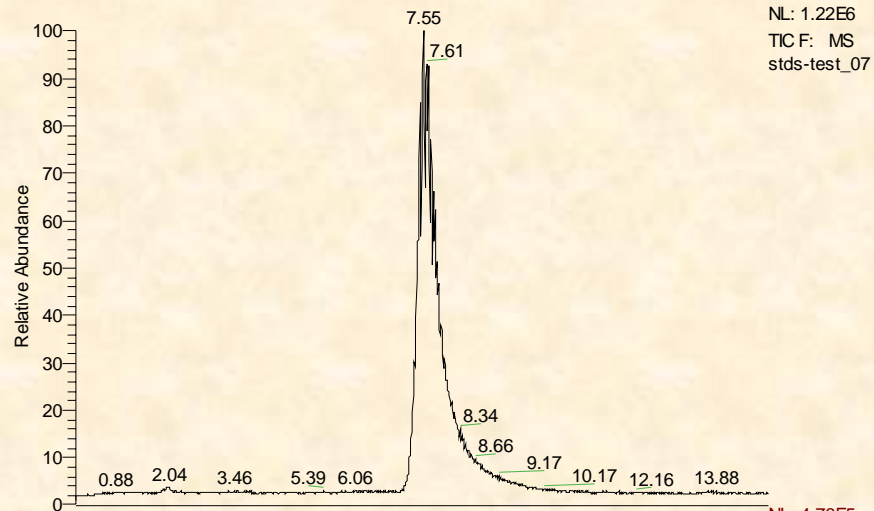
A similar relationship is observed when calculated on a mg protein basis.

# LC-MS of C<sub>60</sub> (toluene)

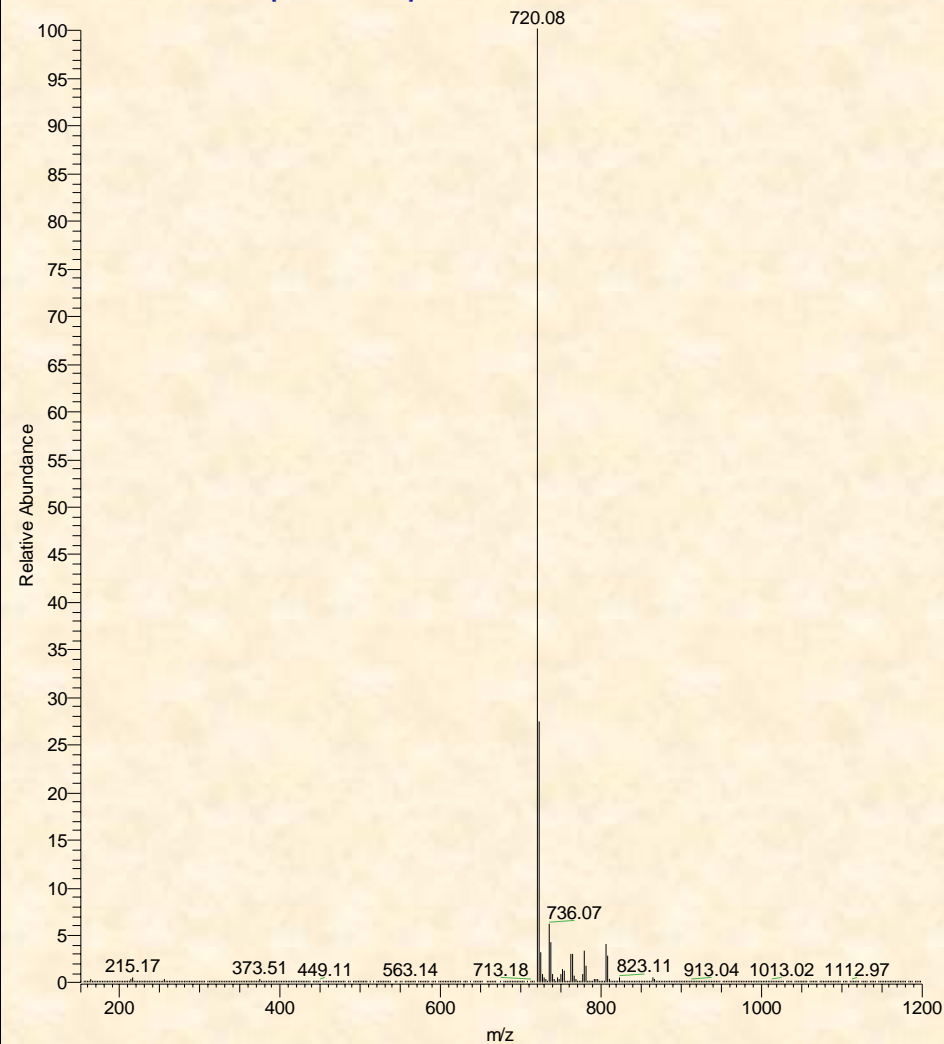
N. Deighton, NCSU

C:\Xcalibur\...stds-test\_07  
100ppm in acetonitrile

RT: 0.00 - 14.99



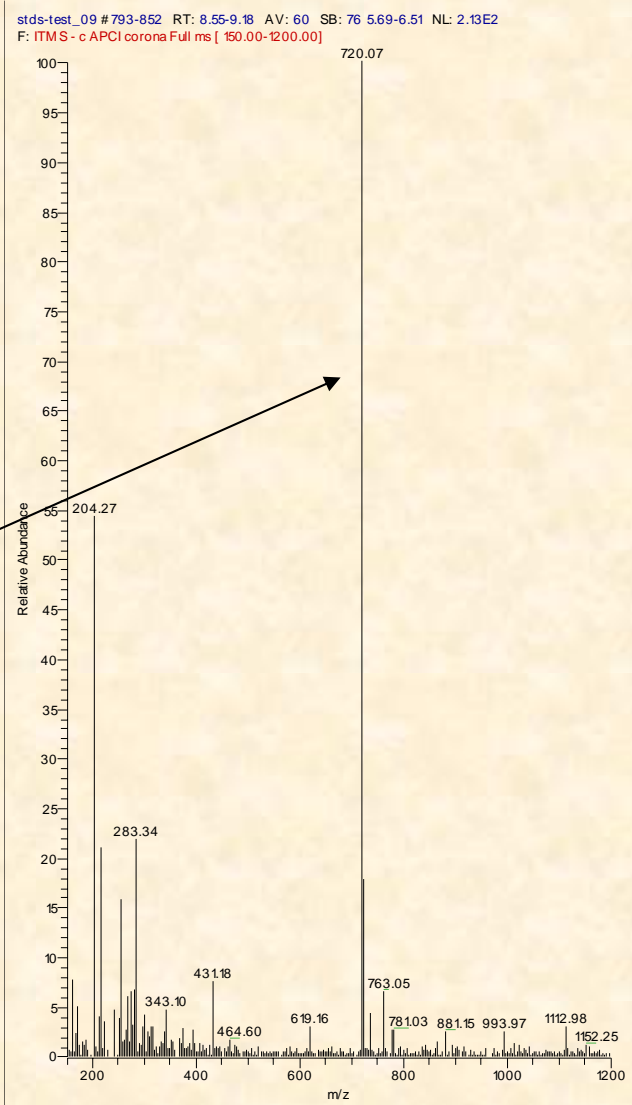
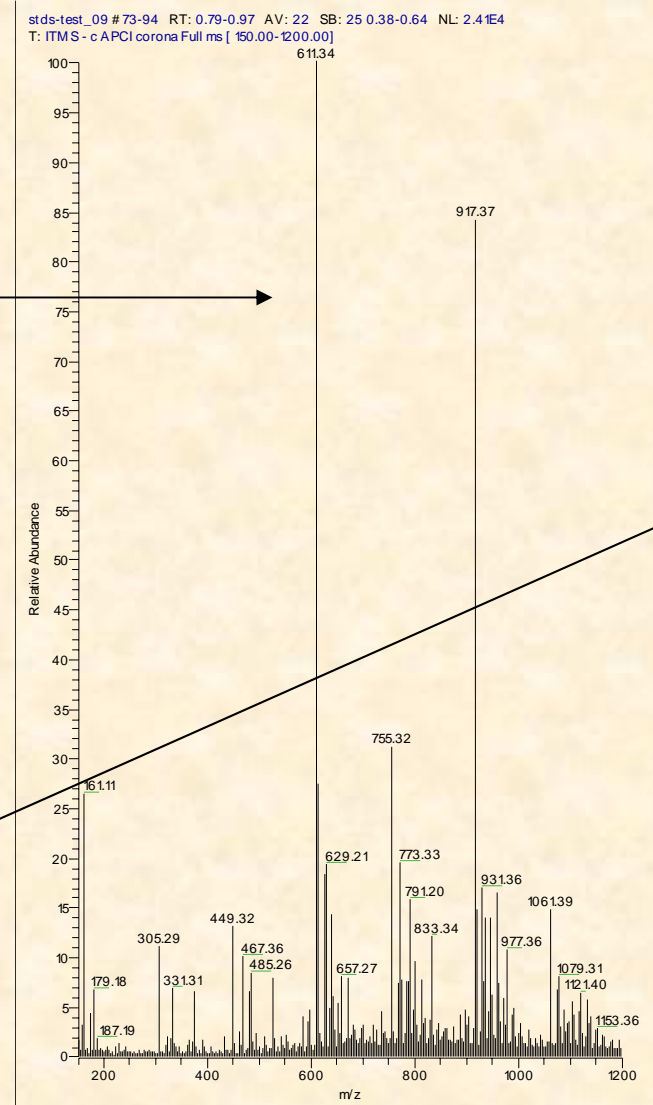
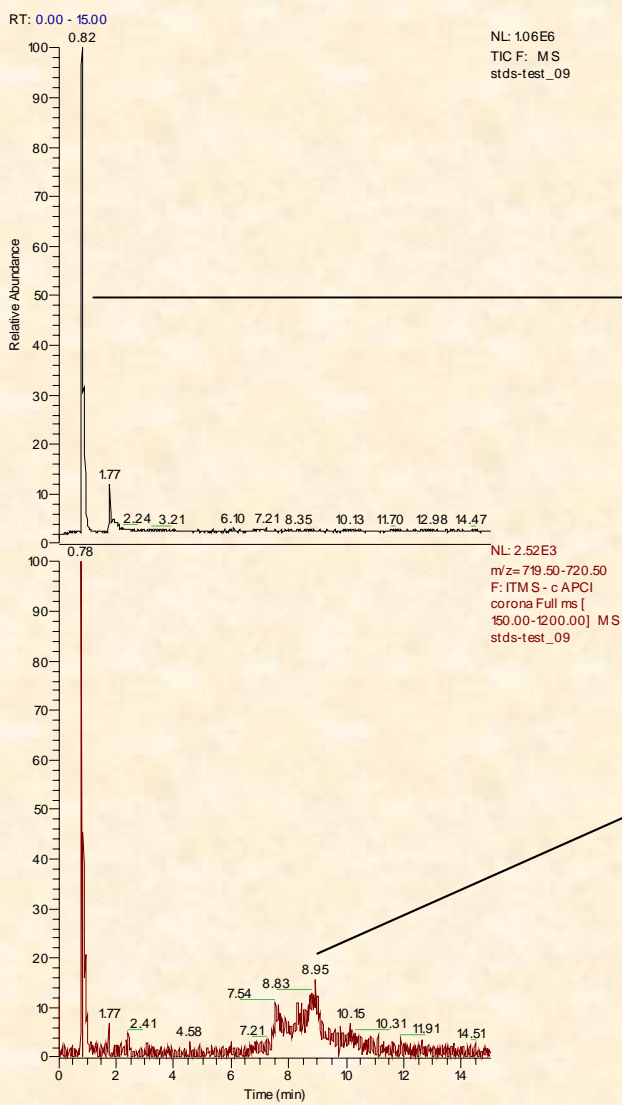
stds-test\_07 #693-742 RT: 7.45-7.77 AV: 50 NL: 3.39E5  
T: ITMS - c APCI corona Full ms [ 150.00-1200.00]



# LC-MS of aqua-C<sub>60</sub> (H<sub>2</sub>O-stirred)

N. Deighton, NCSU

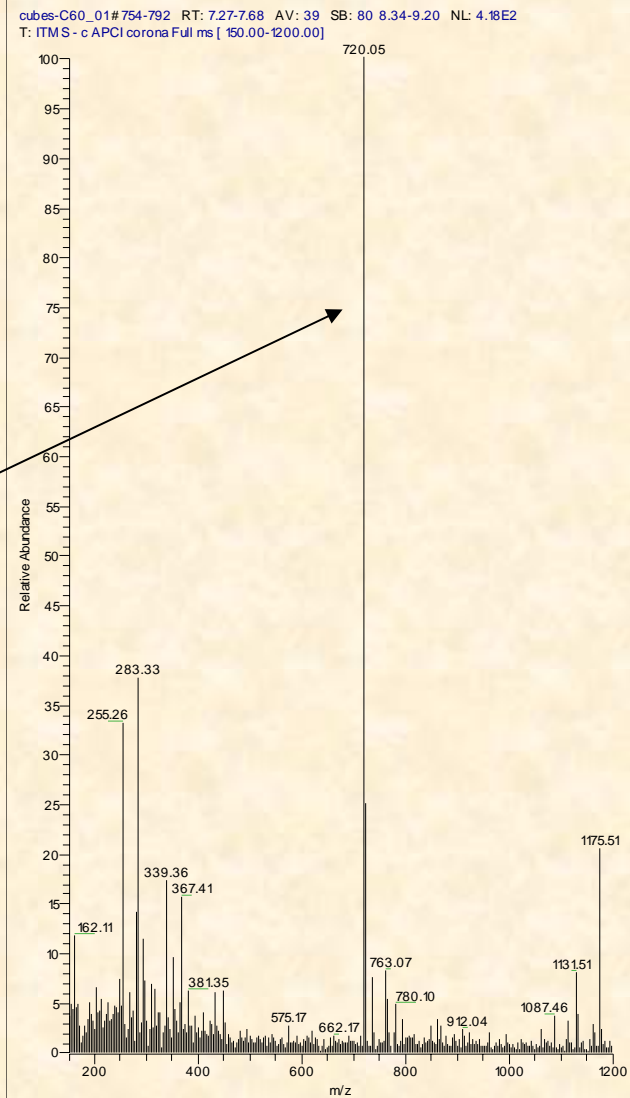
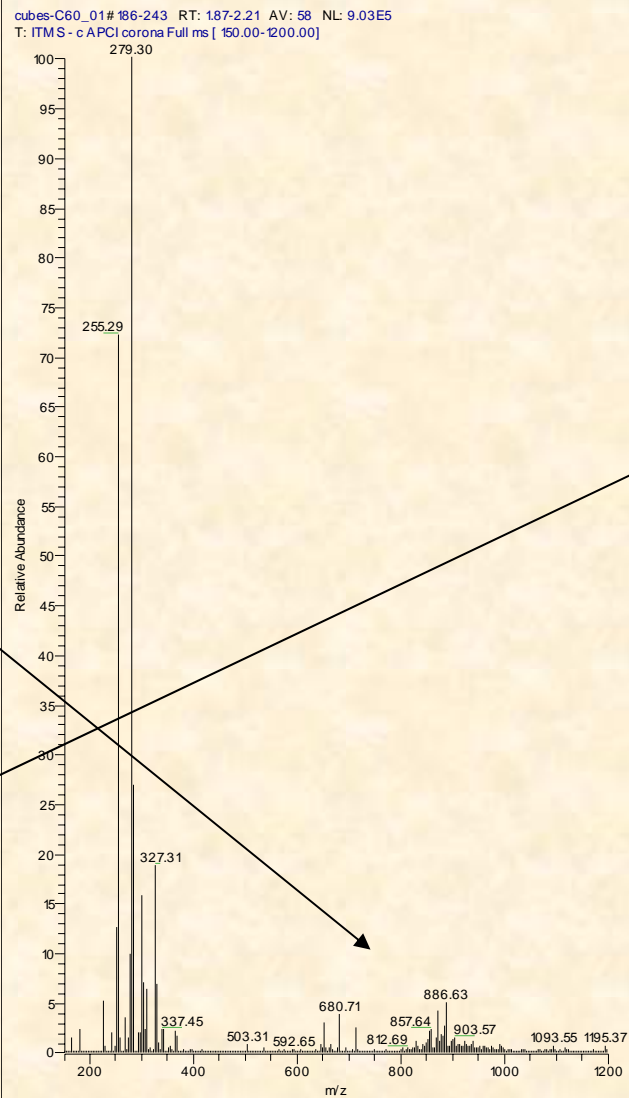
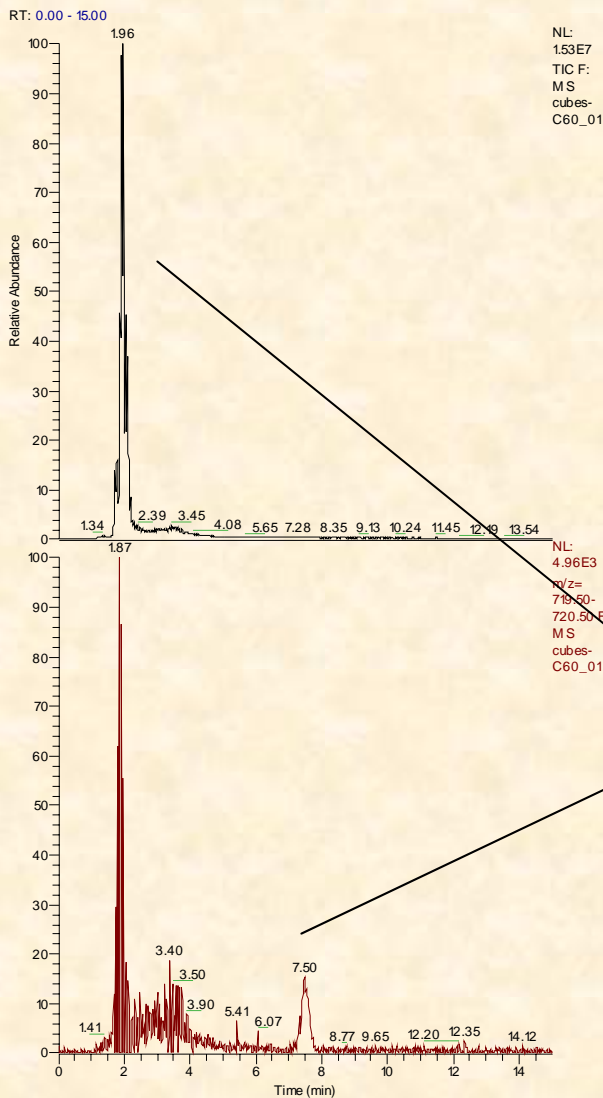
C:\xcalibur...\stds-test\_09  
as supplied



# LC-MS of H<sub>2</sub>O stirred -C<sub>60</sub> in food cubes

N. Deighton, NCSU

C:\xcalibur\...cubes-C60\_01  
i-PrOH extract



# Environmental Nanotoxicology is Dependent On:

- **Size (Surface Area \* Mass<sup>-1</sup>):** Porosity; Weathering (UV, microbial)
- **Chemistry:** Surface Properties (Area, charge, redox activity, coating); Air Exposure (coating); Covalent Modifications
- Agglomeration State: (Singlets vs. Aggregates)
- Particle Number Per Unit Measure and also Per Unit Time
- Biokinetic Behavior
- Biopersistence (species-specific physiological clearance and particle specific bio-durability as physical-chemical processes); fiber bio-durability depends on dissolution as well as mechanical breaking and splitting.
- **Life History Habits of Organism:** Filter Feeder (specific surface chemistry preference), Detritus Feeder, Food Source, Water Column Position/Sediment Contact
- Exposure Route: Inhalation, dermal, ingestion

# Environmental Nanotoxicology is Dependent On:

- **Contact Tissue Surface Characteristics:** Mucous layer (protection, sloughing); Protective Layer (waxy cuticle, shell, scale, carapace); Porosity; Entry Portal to Circulatory System (blood, lymph); Entry Portal to Metabolism (digestive tract, antioxidant capacity, mitochondrial resilience/capacity/reserve, Entry Portal to Immune System, Entry Portal to CNS (olfactory neurons, lateral line)
- **Susceptibility of Organism:** Health, adaptability, energy reserves.
- Mechanisms of Uptake/Distribution/Translocation/Elimination
- **Metabolism**

# So.....What do we specifically know?

- Previous research shows us that **HOW** we prepare nanomaterials matters.
- **NO** acute toxicity was observed in exposed *Fundulus* and many invertebrate species.
- Unlike the LMB study, exposure to water-stirred C<sub>60</sub> does not increase LPO in brain (decreases LPO). Do see an increase in GSH in liver.
- Aggregates increase with time in 20 ppt sea water which plays havoc with exposure. C<sub>60</sub> adheres tightly to biologic material (eggs).

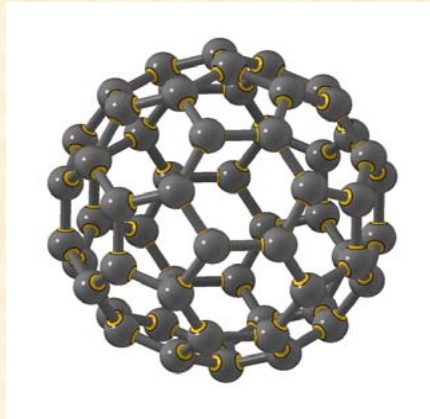
# A few other important references

- Conova, S. 1999. Role of particle wettability in capture by suspension-feeding crab (*Emerita talpoida*). *Mar. Biol.* 133: 419-428.
- Fortner, J. D., D. Y. Lyon, C. M. Sayes, A. M. Boyd, J. C. Falkner, E. M. Hotze, L. B. Alemany, Y. J. Tao, W. Guo, K. D. Ausman, V. L. Colvin, and J. B. Hughes. 2005. C60 in water: Nanocrystal formation and microbial response. *Environ. Sci. Technol.* 39: 4307-4316.
- Henry, T. B., F-M. Menn, J. T. Fleming, J. Wilgus, R. N. Compton and G. S. Sayler. 2007. Attributing effects of aqueous C60 nano-aggregates to tetrahydrofuran decomposition products in larval zebrafish by assessment of gene expression. *Environ. Hlth. Perspec.* 115:1059-1065.
- Kashiwada, S. 2006. Distribution of nanoparticles in the see-through medaka (*Oryzias latipes*). *Environ. Hlth. Perspec.* 114: 1697-1702.
- Lovern, S. and Klaper, R. 2006. *Daphnia magna* mortality when exposed to titanium dioxide and fullerene (C60) nanoparticles. *Environ. Toxicol. Chem.* 25:1132-1137.
- Lovern, S., Strickler, J.R. and Klaper, R. 2007. Behavioral and physiological changes in *Daphnia magna* when exposed to nanoparticle suspensions (titanium dioxide, nano-C60 and C<sub>60</sub>HxC<sub>70</sub>Hx). *Environ. Sci. Technol.* 41:4465-4470.
- Federici, G., Shaw, B.J. and R.D. Handy. In press. Toxicity of titanium dioxide nanoparticlesto rainbow trout (*Oncorhynchus mykiss*): Gill injury, oxidative stress and other physiological effects. *Aquat. Toxicol.* doi: 10.1016/j.aquatox.2007.07.009.

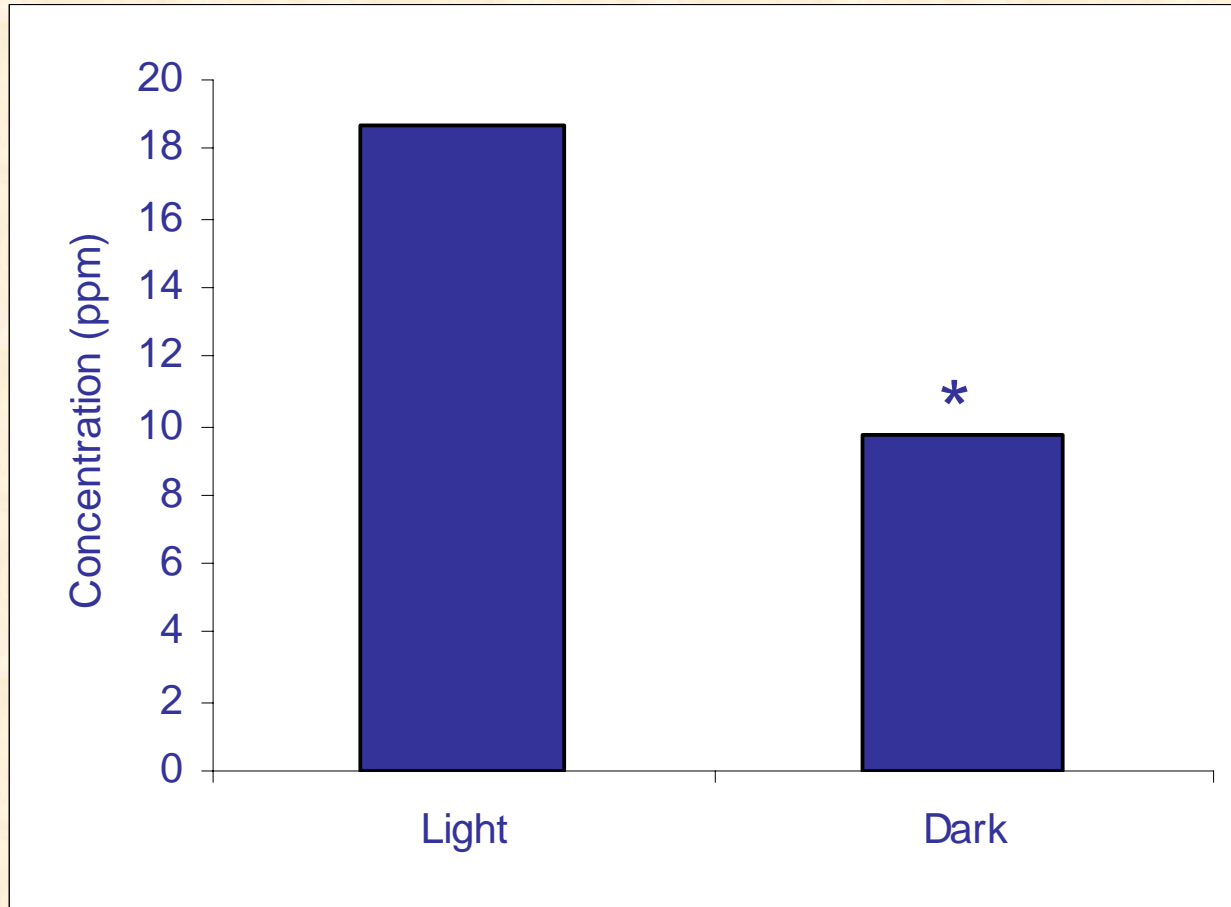
# Thanks to.....

- Dr. Eva Oberdörster, SMU
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- Shiqian Zhu, Univ. Miss.
- Benjamin LaRoque, UNC-CH
- Weston Smith, UNC-CH
- Brett Fair, Duke Univ.

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# Effect of Light on solubility of C<sub>60</sub>



C<sub>60</sub> was prepared by stirring in DiH<sub>2</sub>O for 30 days in the presence or absence of light.