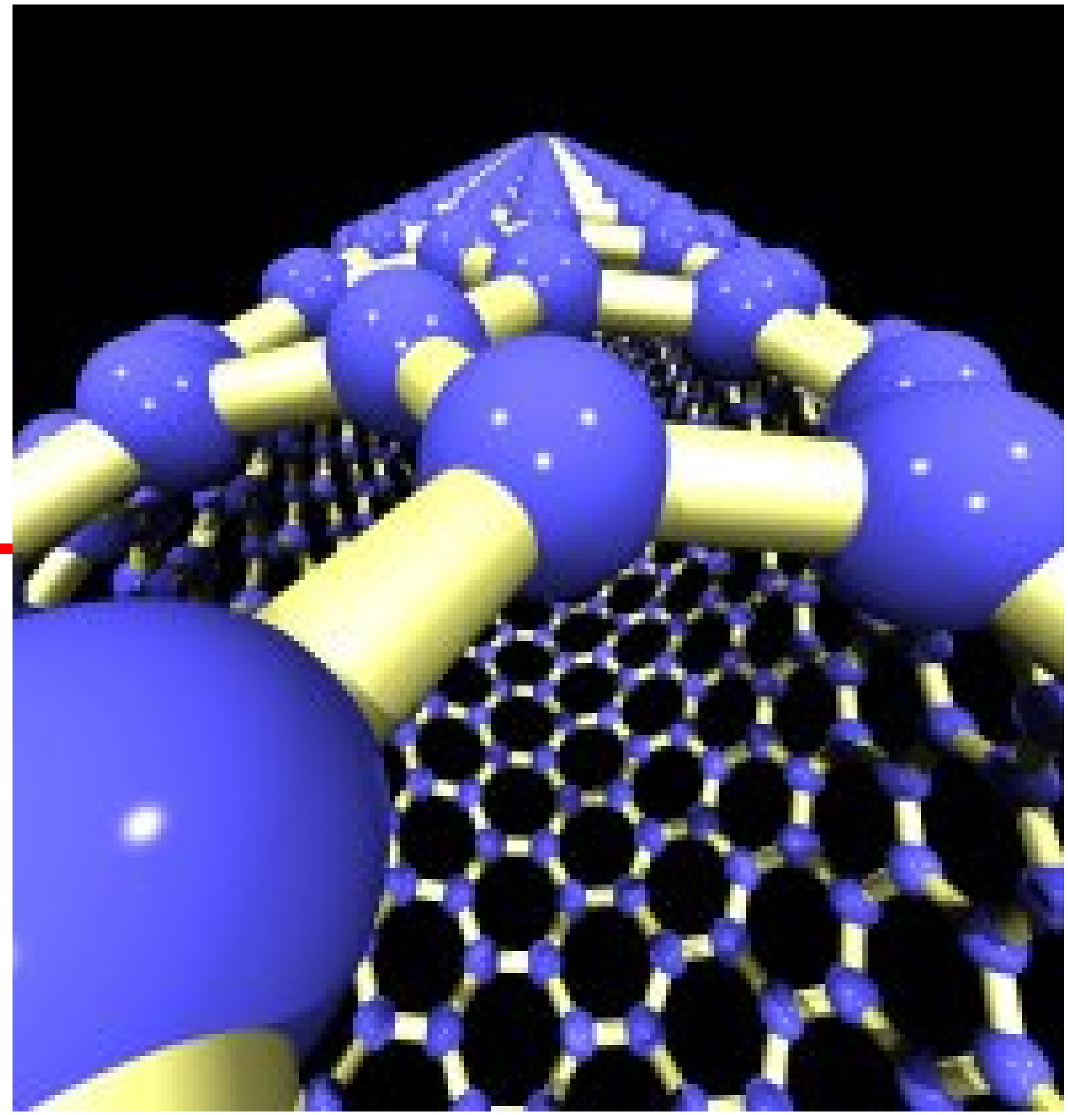
# Microbial Interactions with Fullerenes and Other Engineered Nanoparticles



**2007 NSF Grantees Conf**
**Arlington, VA 12/6/07**

**Pedro J.J. Alvarez**

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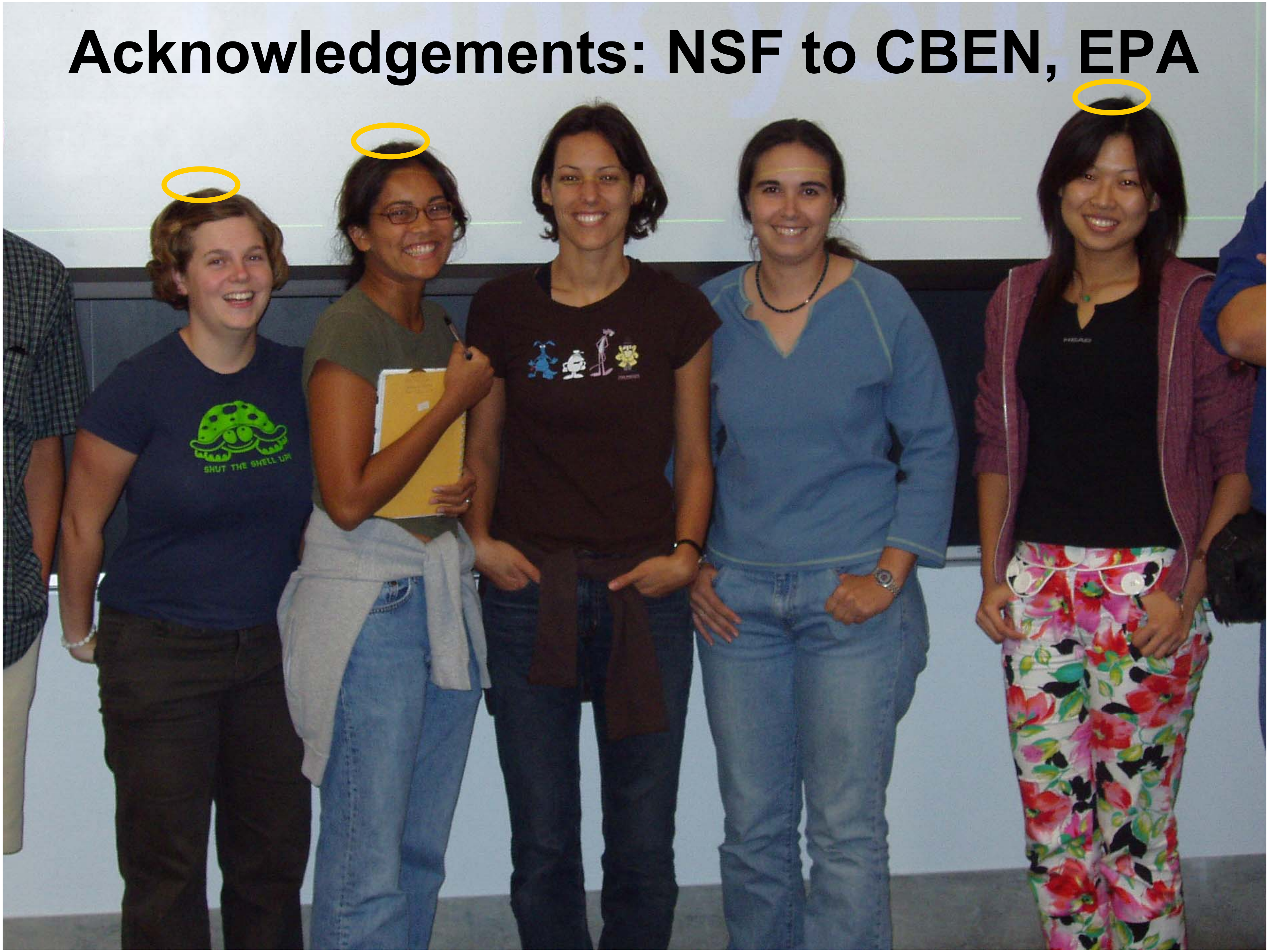
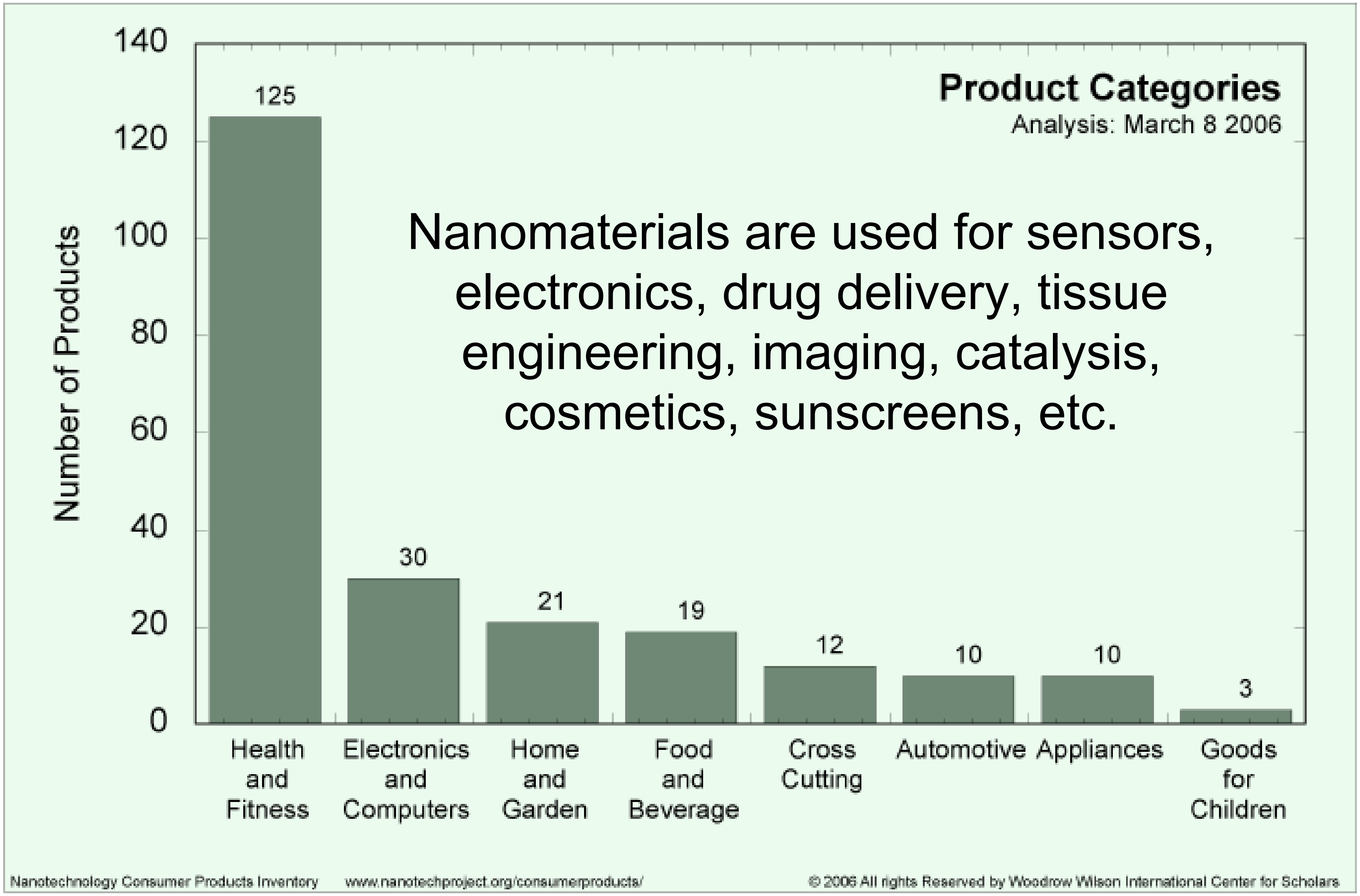
# Acknowledgements: NSF to CBEN, EPA



# > 475 Products Use of Nanomaterials



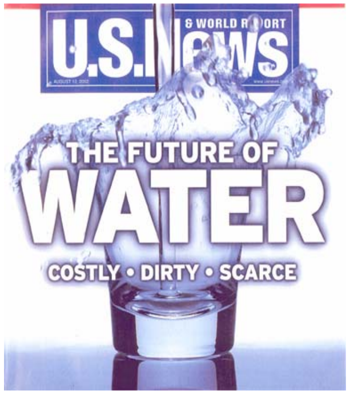

Nanomaterials are used for sensors, electronics, drug delivery, tissue engineering, imaging, catalysis, cosmetics, sunscreens, etc.

# Environmental Concerns & Opportunities

1. **Implications:** Create the information needed to use nanomaterials in an *environmentally responsible* and *sustainable manner*

*CBEN Societal Driver: To enable effective risk management for emerging nanotechnologies.*

2. **Applications:** *Enhanced* or *new* capabilities to address existing and future environmental problems.




*CBEN Systems Goal: To develop effective water treatment systems that exploit engineered nanoparticles*

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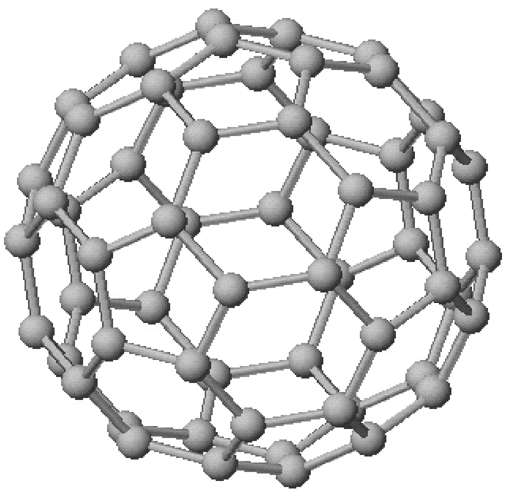
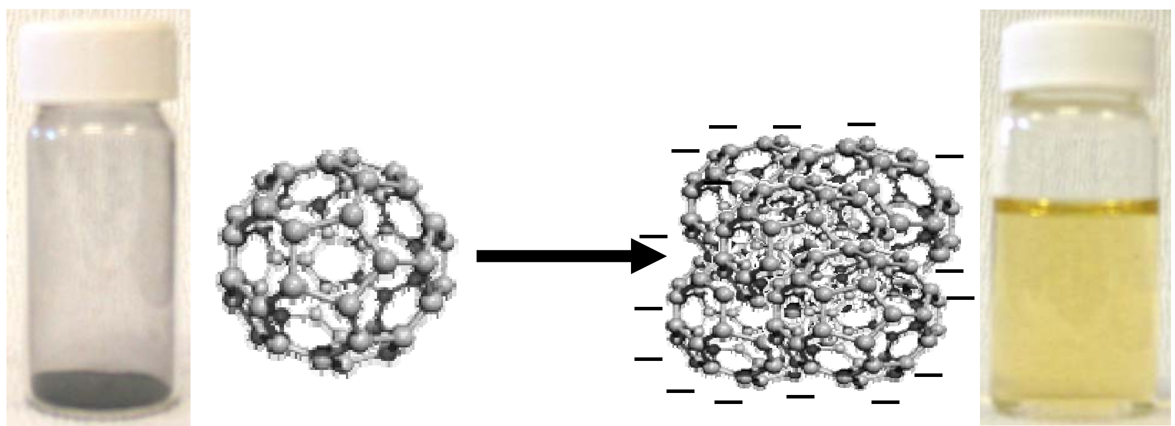
# $C_{60}$ (buckminsterfullerene)

Photocatalyst and Antioxidant (sp2 hybridized)



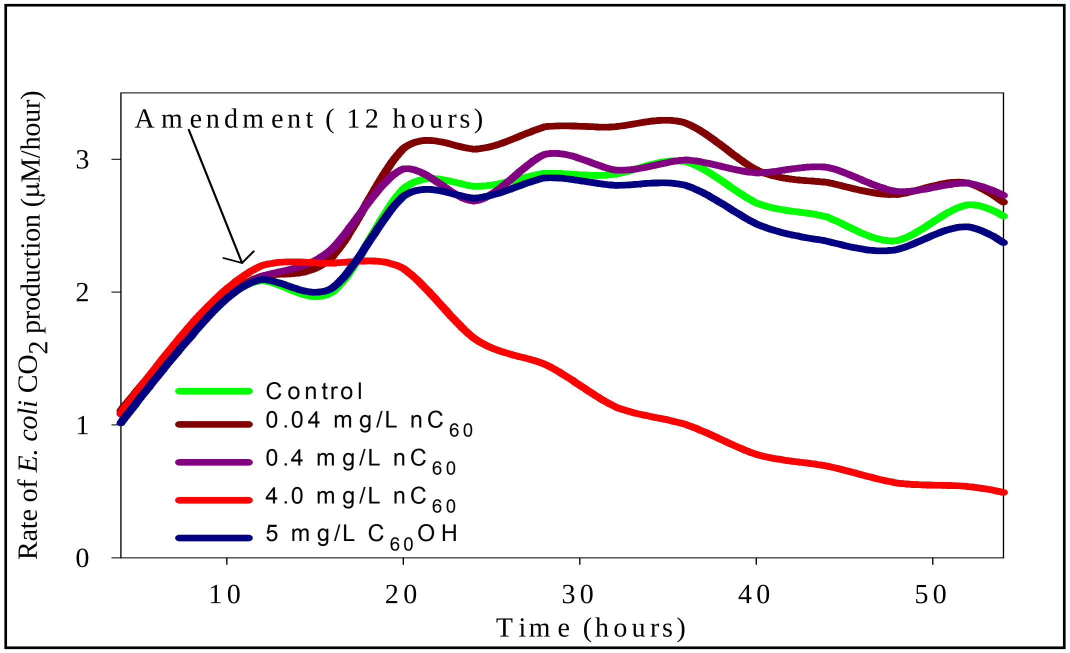
R. Buckminster Fuller (Bucky)



Solid or solution

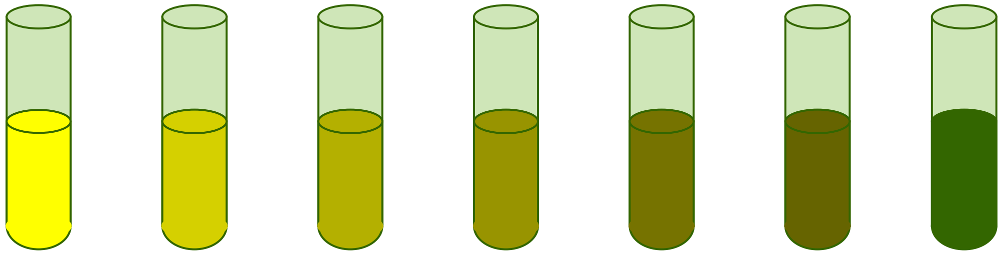
$nC_{60}$ (20-200 nm)

# $nC_{60}$ is antibacterial




*E. coli* respiration ceases after exposure to $nC_{60}$

<u>Standardized Microtox Assay</u>

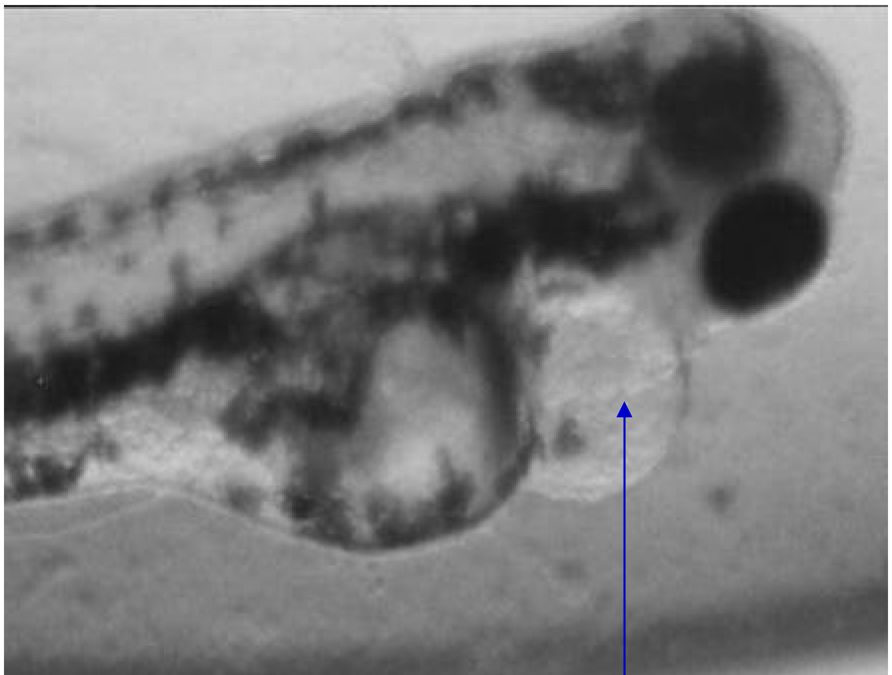


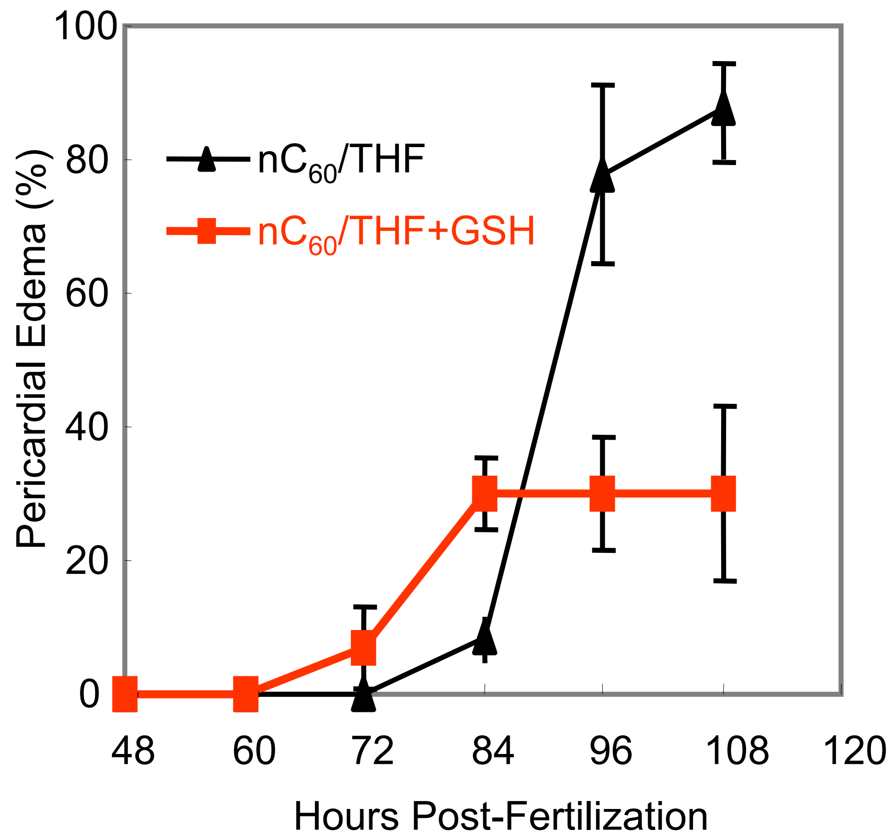
*Vibrio fischeri* (luminescent bacteria) with increasing concentrations of $nC_{60}$

| Compound | $EC_{50}$ (mg/L) |
|---|---|
| $nC_{60}$ | 1.6 |
| Benzene | 2.0 |
| Sodium azide | 43-66 |

# Developmental toxicity of $nC_{60}$ (Zebrafish)

**Mitigation by GSH suggest that toxicity is related to oxidative stress**

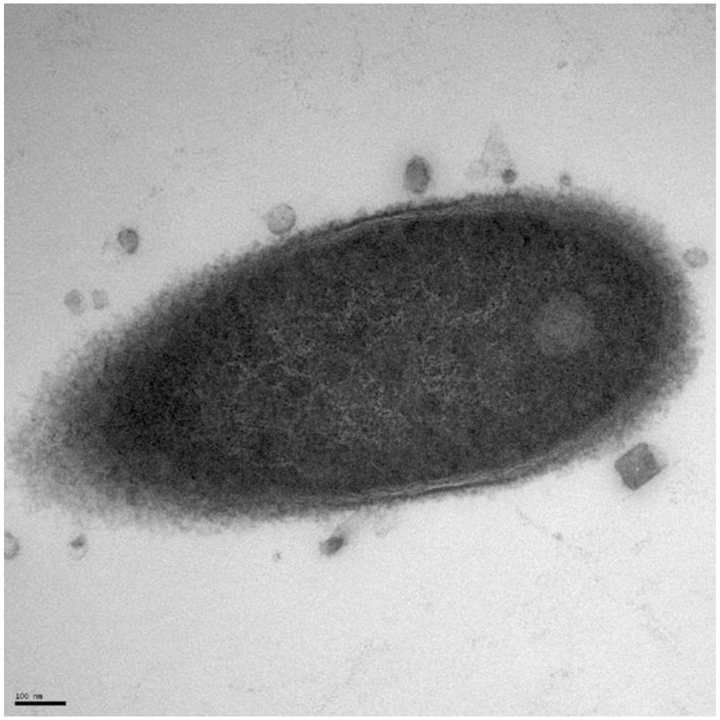





Zebrafish larva with pericardial edema due to $nC_{60}$ exposure (1 mg/L)


X. Zhu, Lin Zhu, Y. Li, Z. Duan, W. Chen and P.J. Alvarez (2006). *Environ. Toxicol. Chem.* 26(5):976-979.

# Possible Antibacterial Mechanisms



1. $nC_{60}$ punctures cells
2. $nC_{60}$ exerts oxidative by generating photocatalytic reactive oxygen species
3. $nC_{60}$ is a direct oxidant, harming membrane proteins and/or serving as "e- sponge" that interrupts electron transport phosphorylation

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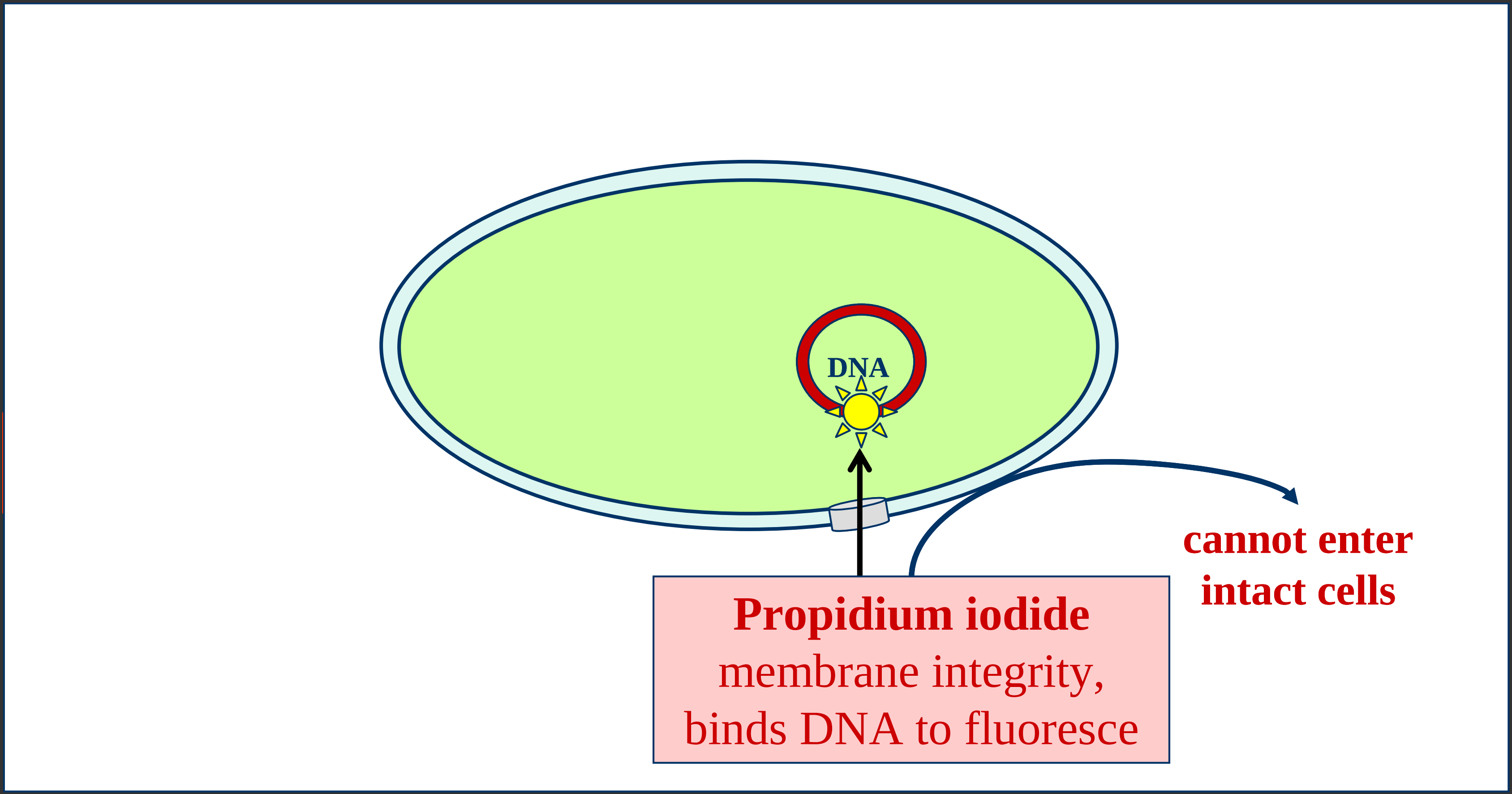
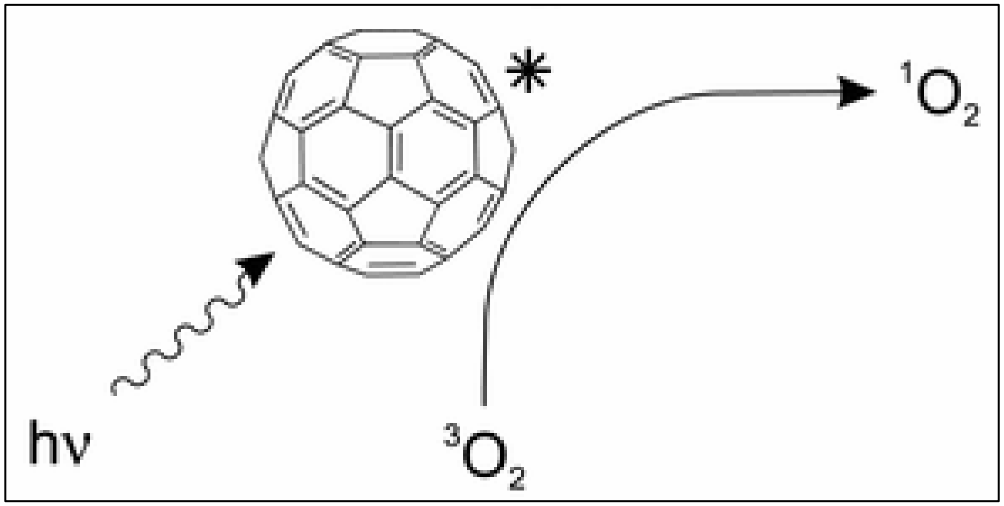
# 1. Does $nC_{60}$ puncture cells?

- Propidium iodide enters permeablized cells and stains nucleic acids



# 2. Oxidative Stress Due to ROS?

Photosensitization



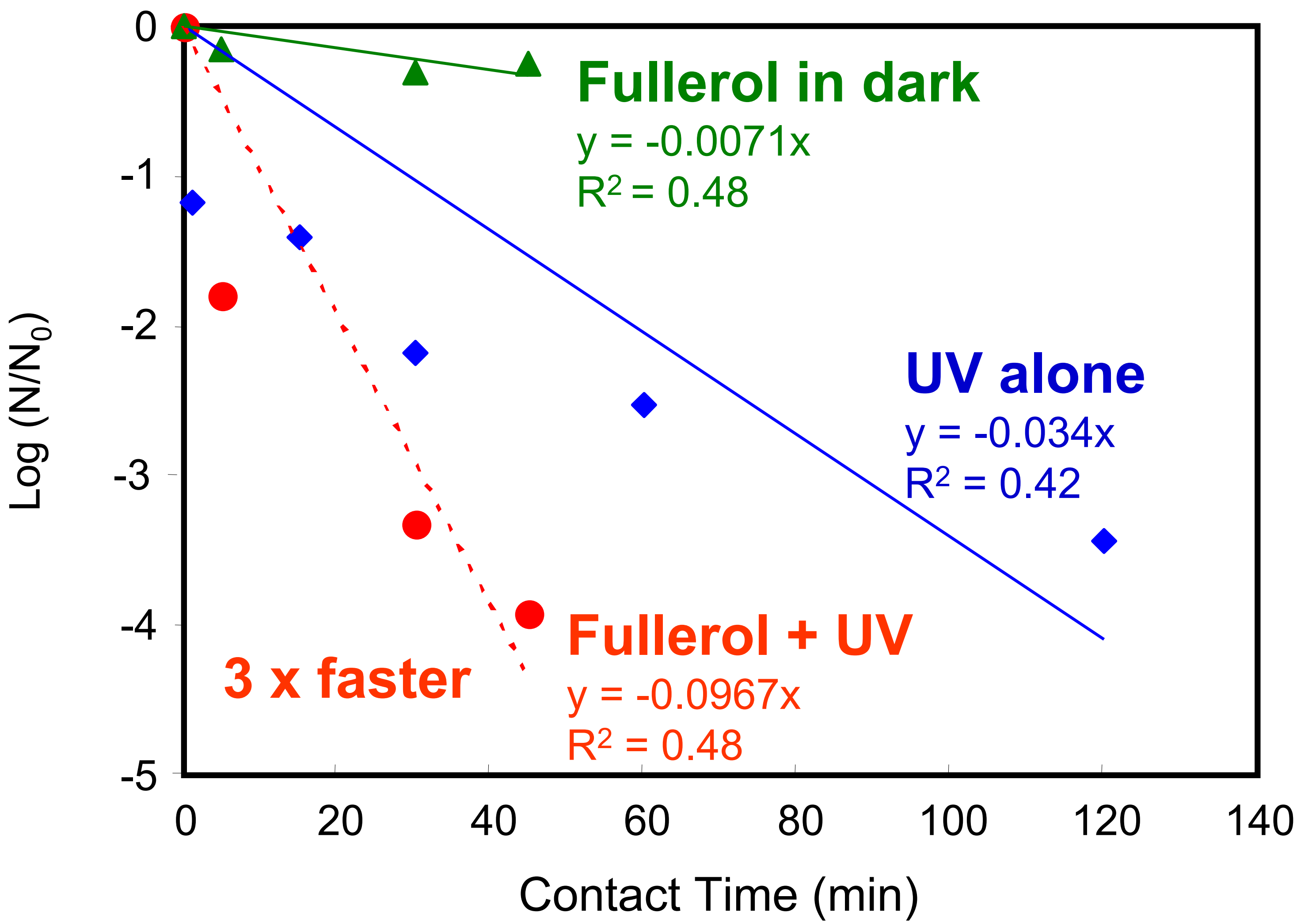

<u>Light</u>:

$C_{60}$ is photosensitive.

$C_{60}$ - high electron affinity

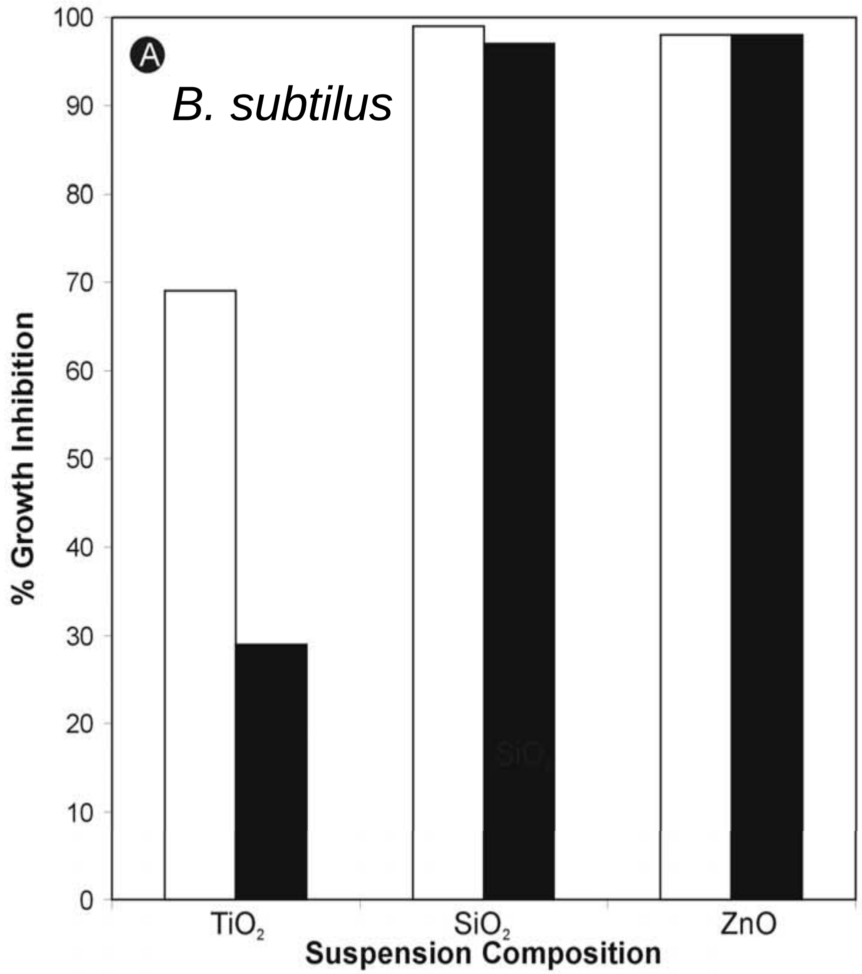
*Do fullerenes produce enough ROS*
*to inactivate bacteria or virus*
*in conjunction with UV disinfection?*

# MS2 virus inactivation by UV and fullerol




Hotze M., A.R. Badireiddy, S. Chelam, P.J.J.Alvarez and M. Wiesner (2007). *Environ. Sci. Technol*. 41: 6627-6632

# Effect of illumination on antibacterial activity of photosensitive inorganic nanomaterials




Toxicity: ZnO > $SiO_2$ > $TiO_2$

ROS production: $TiO_2$ > ZnO > $SiO_2$

(No correlation)

**Cell death also occurs in the dark and in the absence of $O_2$.**

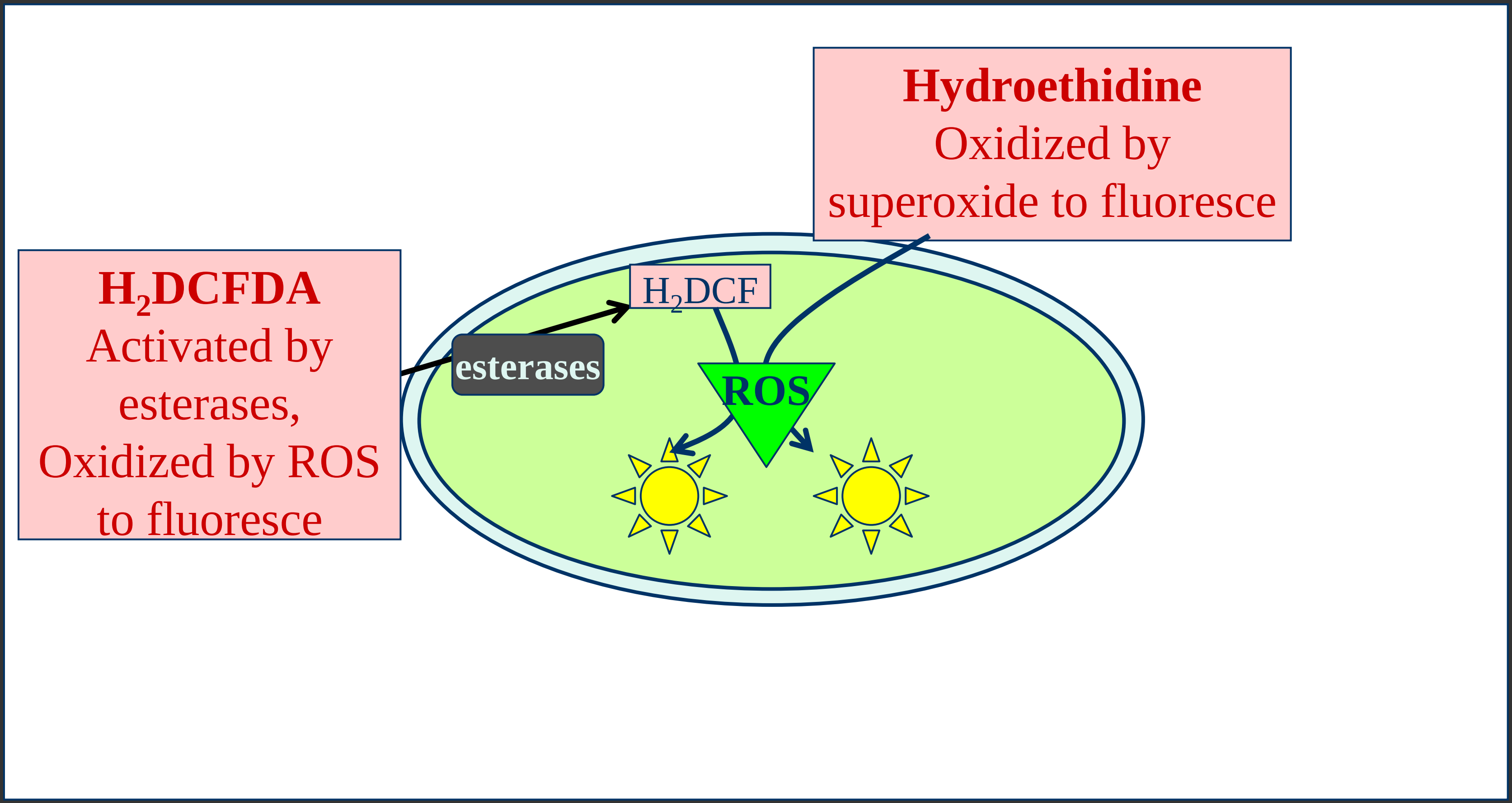
Thus, an additional mechanism besides photocatalytic ROS production is involved.

*OS by direct contact with cell?*

*ROS produced by the (eukaryotic) cell's immune response system?*

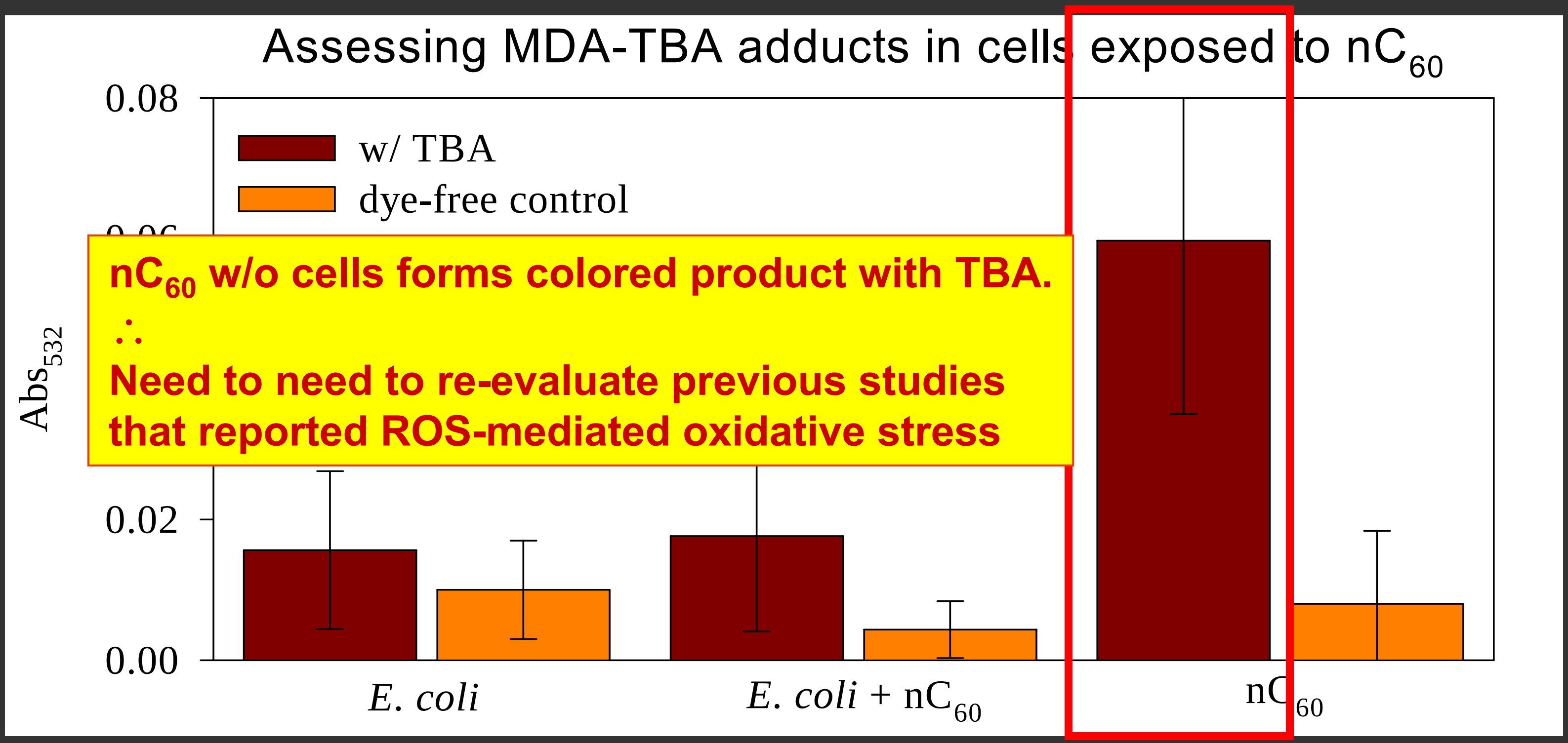
Adams L.K. D. Y. Lyon, and P.J.J. Alvarez (2005). *Wat. Res.* 40(19):3527-3532.

# Does $nC_{60}$ produce ROS in bacteria?

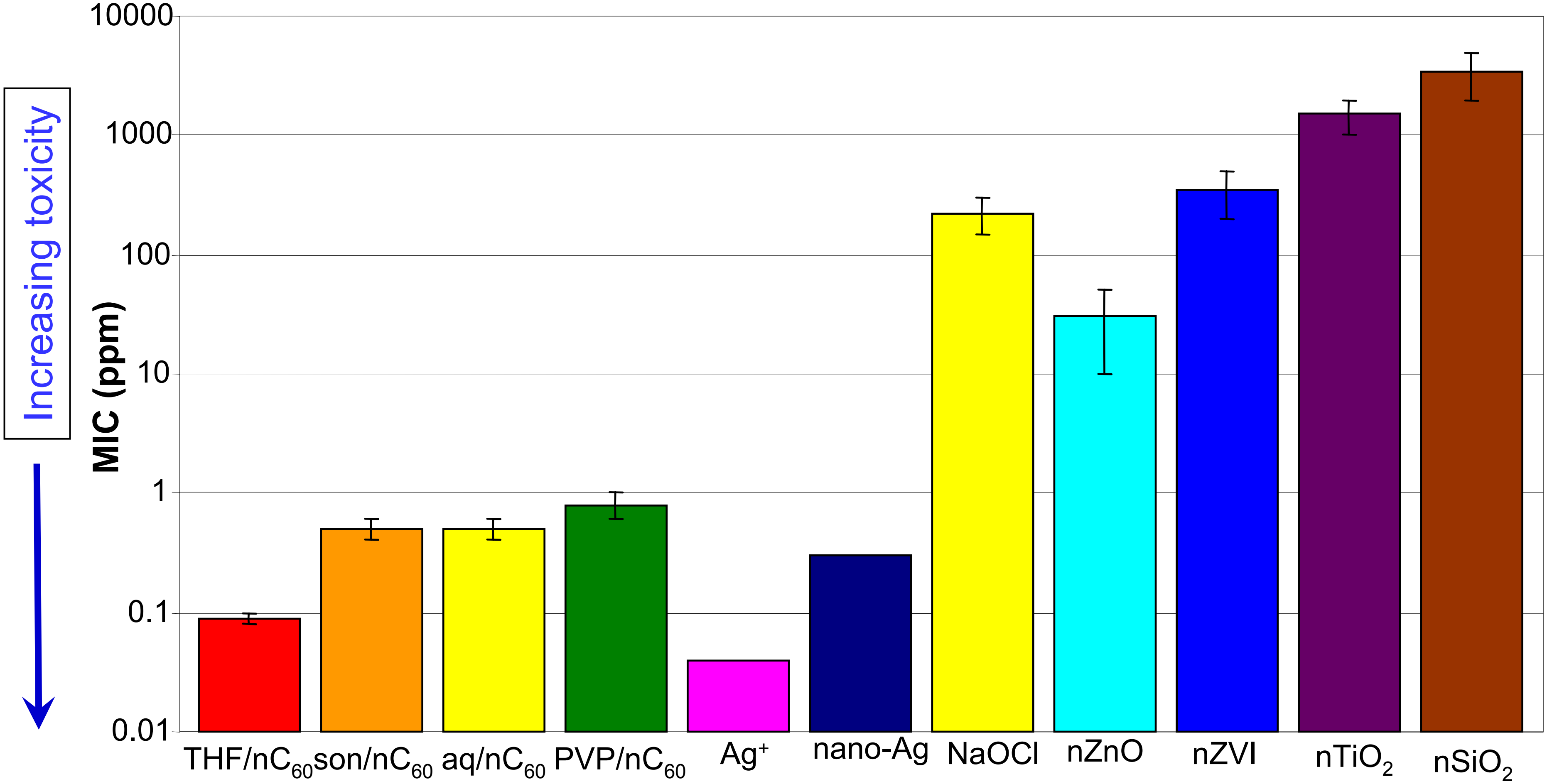


# Looking for lipid peroxidation as evidence of ROS damage

- Hallmark of lipid peroxidation is malonedialdehyde (MDA)
- MDA forms colored adducts with thiobarbituric acid (TBA)



# $nC_{60}$ is more toxic to bacteria than many other common nanomaterials

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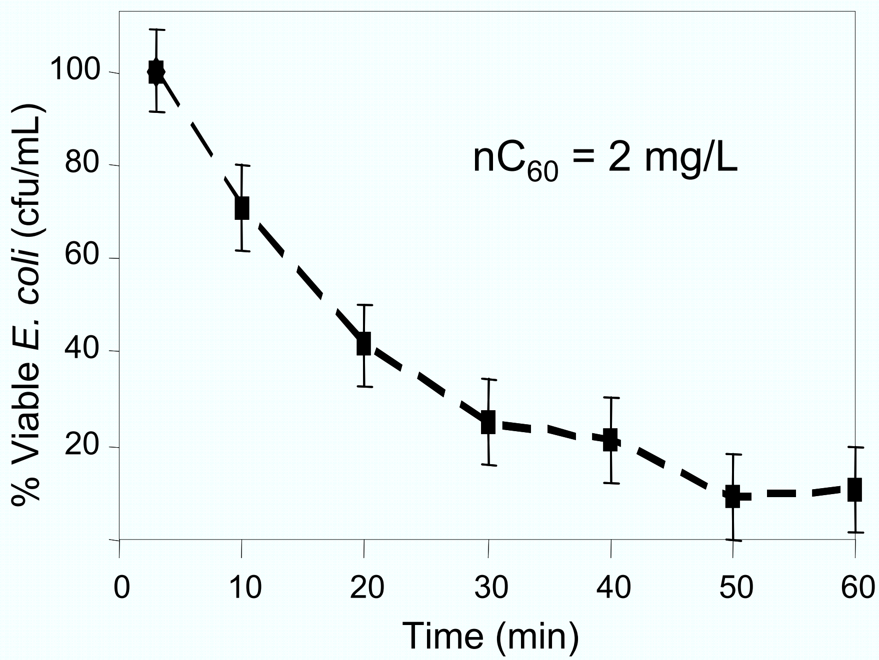
# $nC_{60}$ is a broad-spectrum antibacterial agent

Ct (mg/l * min) for 99% kill:

- 0.03-0.05  for free chlorine
- ~ 100 for $nC_{60}$
- 95-180 for chloramines




| Bacteria | MIC (mg/L) |
|---|---|
| *Bacillus subtilis* | 0.01-0.05 |
| *Burkholderia cepacia* | 0.0125 - 0.025 |
| *Desulfovibrio desulfuricans* | 0.1-0.2 |
| *Escherichia coli* | 0.01 - 0.05 |
| *Pseudomonas aeruginosa* | 0.05 - 0.066 |
| *Ralstonia pickettii* | 0.025 - 0.0375 |
| *Streptomyces albus* | <0.05 |

- *High activity*, as indicated by MIC's (stronger than azide!)
- Kills Gram +, Gram -, facultative aerobes, anaerobes

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# Potential Leapfrogging Opportunities

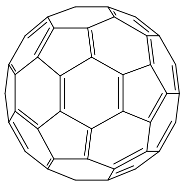
# What factors affect $nC_{60}$'s toxicity to bacteria?

- Motivation
  - Elucidate factors that attenuate or amplify toxicity, to prevent unintentional ecosystem damage and/or exploit its antibacterial properties In engineered systems.

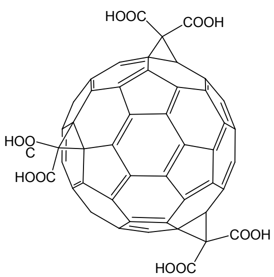
- Factors considered
  - $C_{60}$ Derivatization
  - Particle size
  - Salt (ionic strength)
  - Sorption

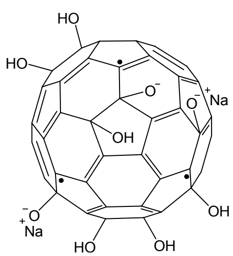
# Dose Response Curve for Fullerenes

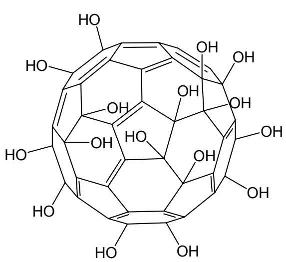
## Colvin, West & co-workers, Rice University

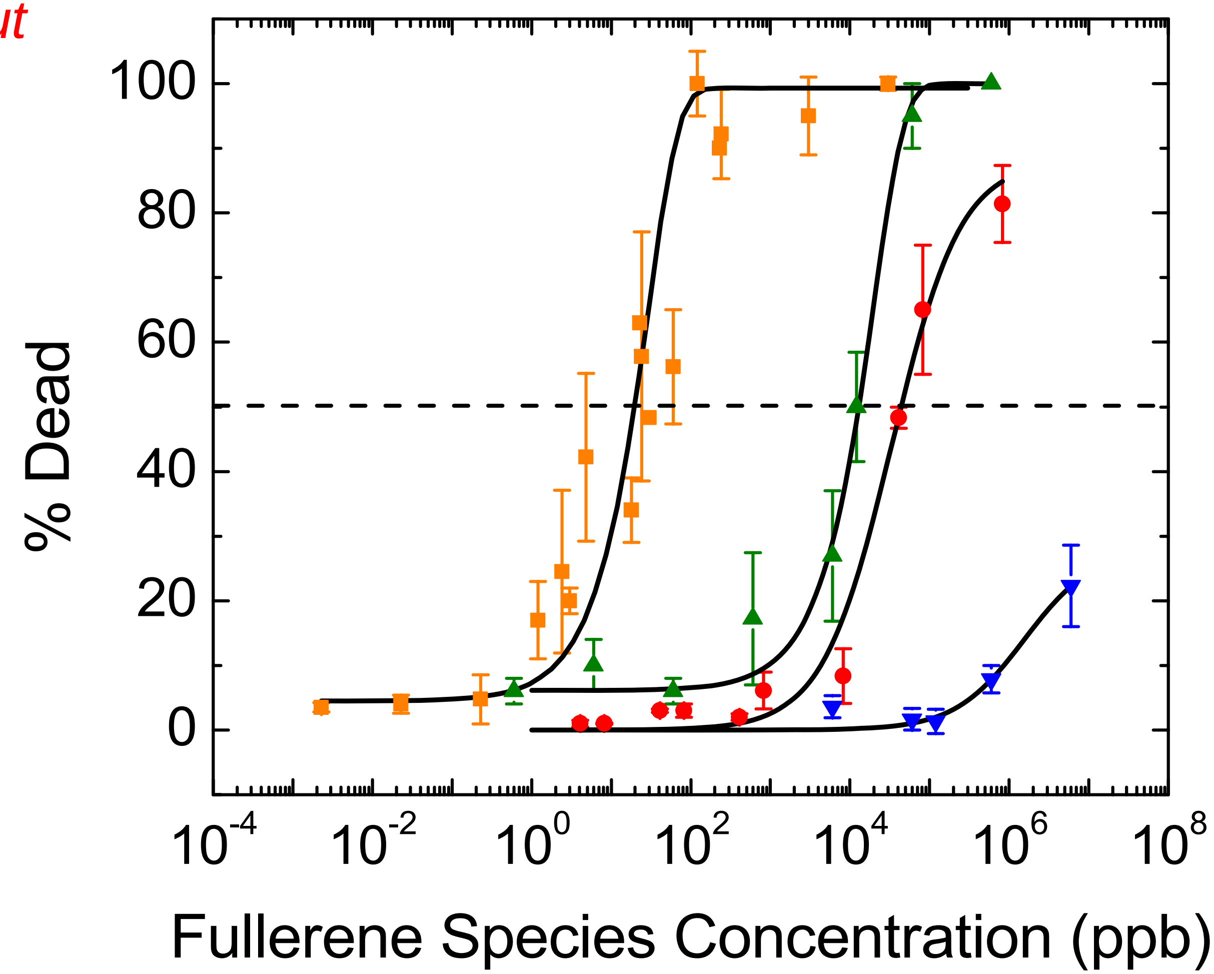


*Less toxic but more mobile*



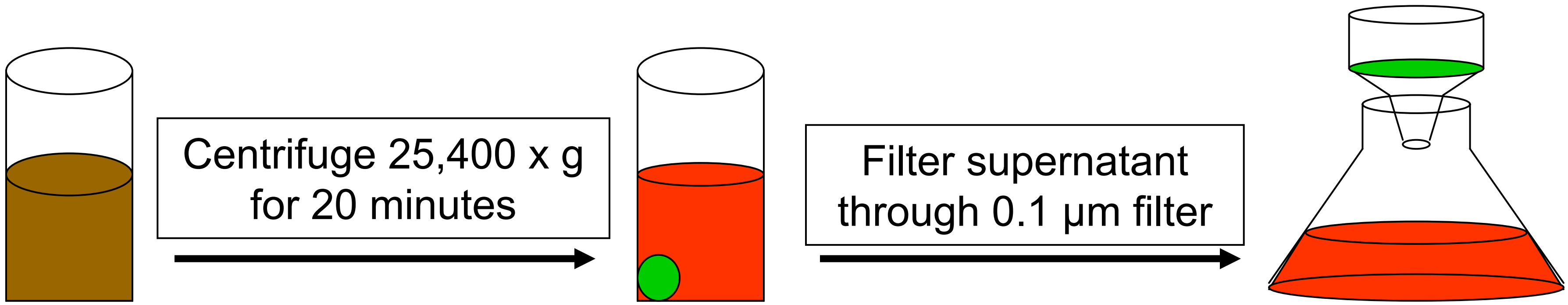



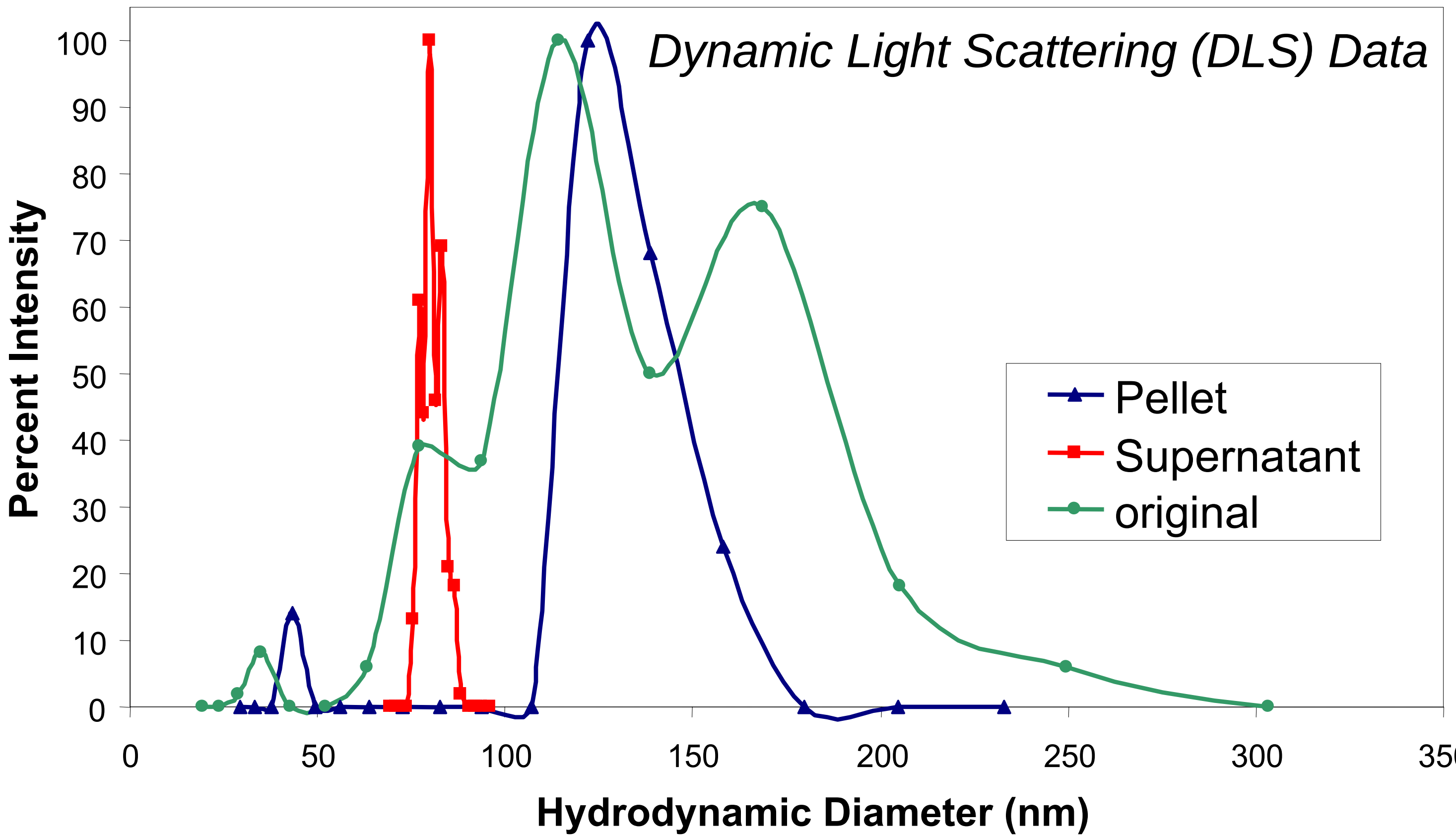



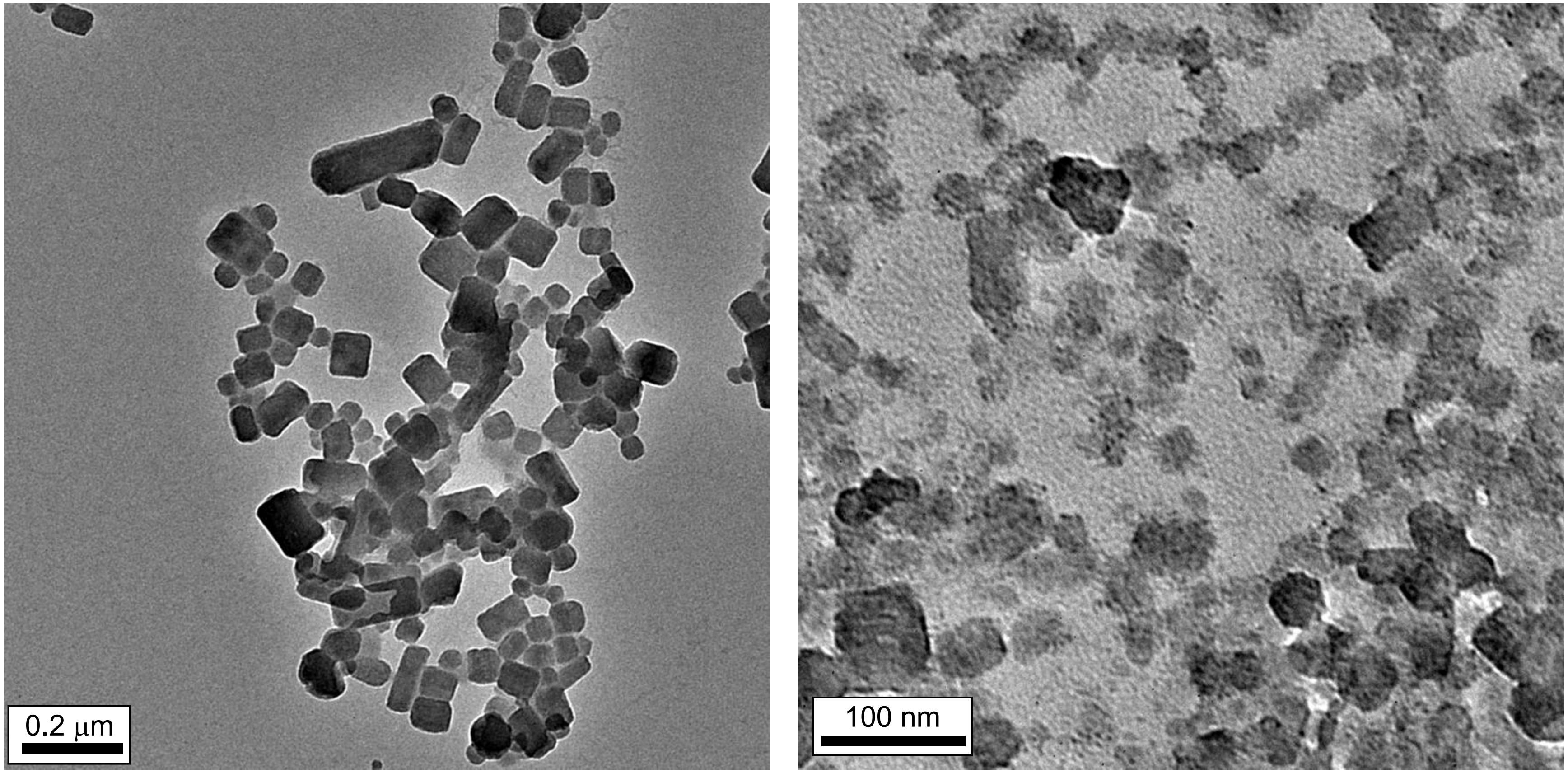

Sayes et al,. *NanoLetters* **2004**, *4*, 1881-1887

# Effect of $nC_{60}$ Particle Size?



# TEM of Size-separated $nC_{60}$




**Large $nC_{60}$ (pellet) crystalline**

**Small $nC_{60}$ (filtered supernatant) amorphous**

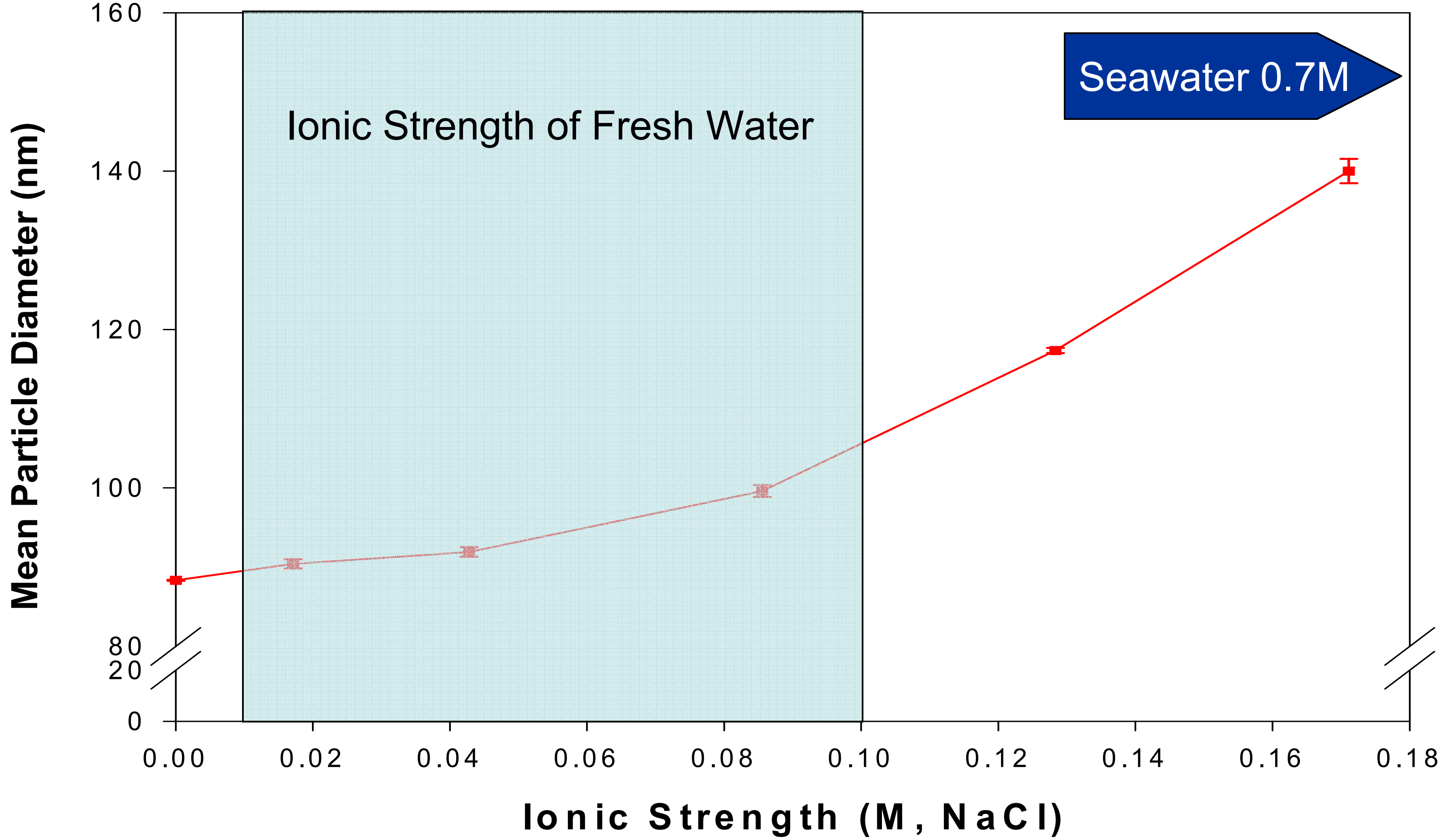
Lyon* D. Y., L. K. Adams, J. C. Falkner, and P.J.J. Alvarez (2006). Environ. Sci. Technol. 40 (14):4360-4366

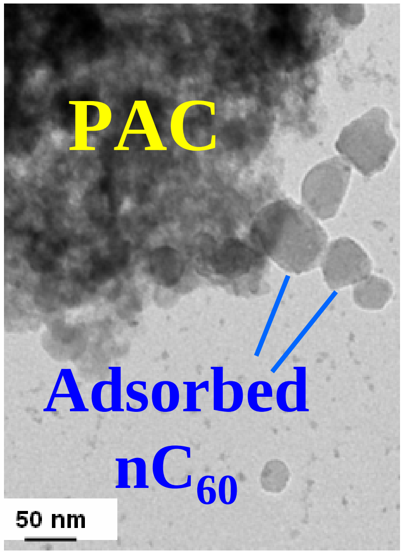
# $nC_{60}$ Particle Size vs Toxicity

| | *B. subtilis* MIC (mg/L) | Average Diameter (nm) | Surface Area:Volume |
|---|---|---|---|
| $nC_{60}$ | 0.75 - 1.0 | 100 | 0.06 |
| >100 nm particles | 7.5 - 10 | 110 | 0.055 |
| | x100 | | x 2 |
| <100 nm particles | 0.01-0.1 | 50 | 0.12 |

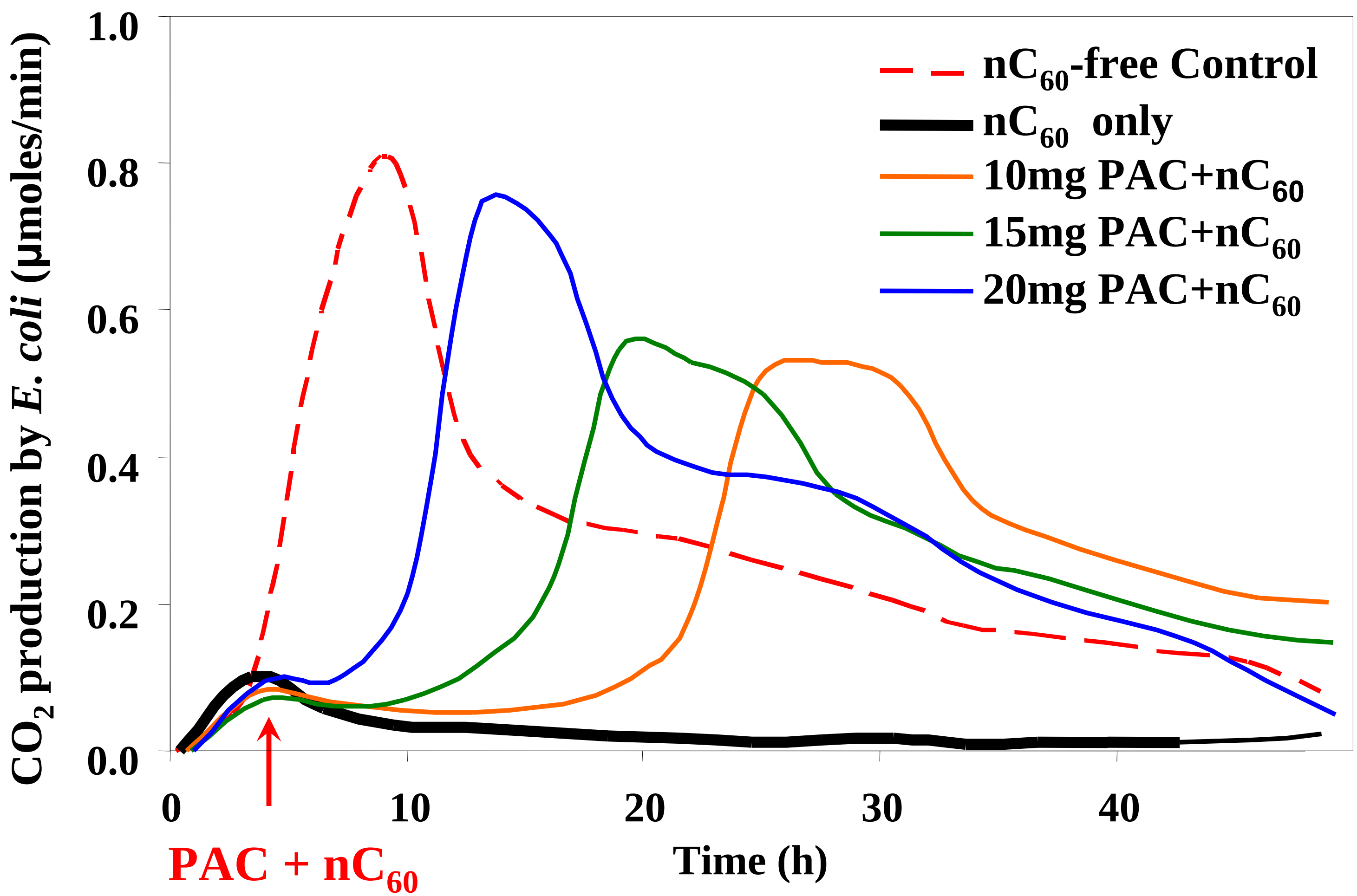
Lyon* D. Y., L. K. Adams, J. C. Falkner, and P.J.J. Alvarez (2006). Environ. Sci. Technol. 40 (14):4360-4366

# Salts promote coagulation & precipitation = less toxicity

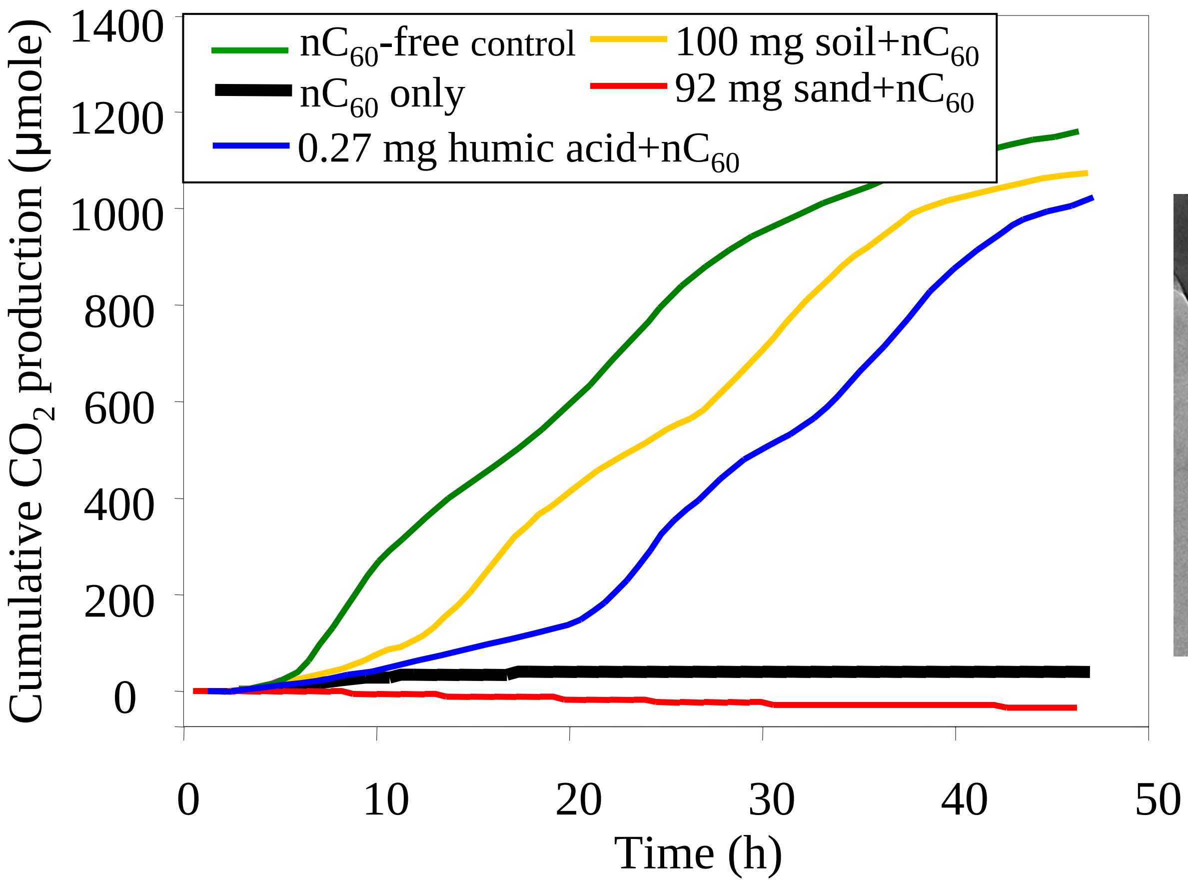




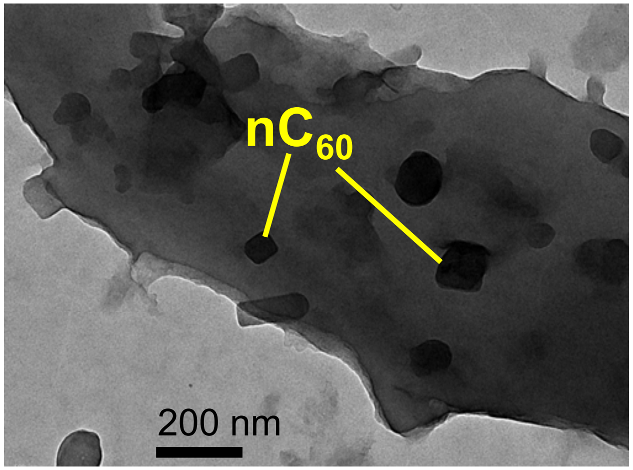

# Sorption of $nC_{60}$ onto PAC reduced its bioavailability and toxicity when added concurrently at the time of exposure (more PAC added = more attenuation).



# NOM reduces bioavailability & toxicity of $nC_{60}$



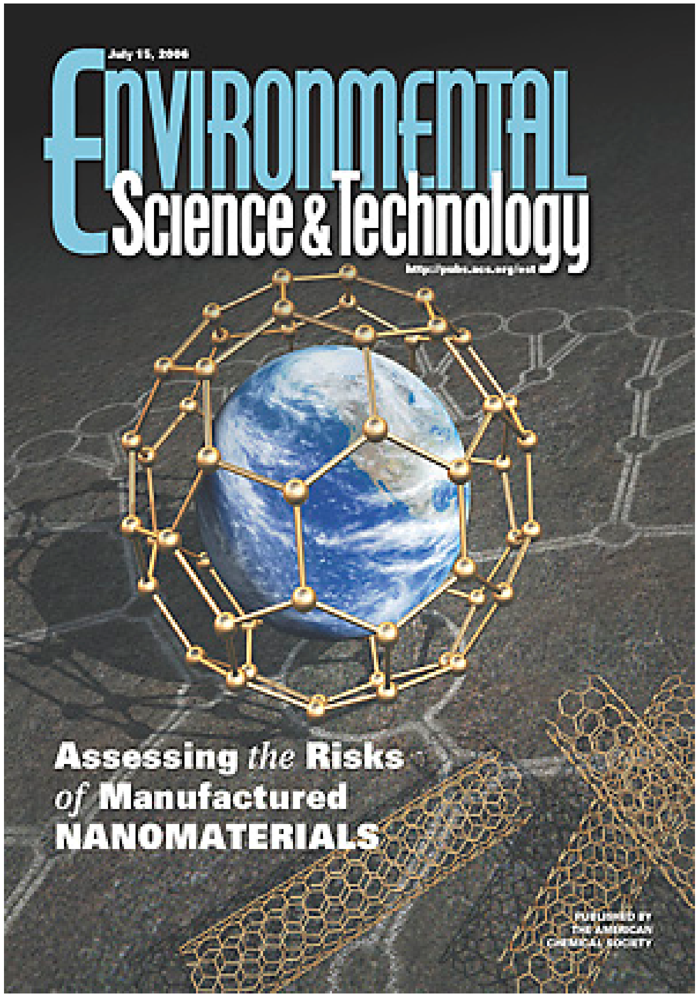

**$nC_{60}$ trapped by humic colloids**




Humic acid concentrations as low as 0.1 mg/L eliminated toxicity

# Conclusions

- $nC_{60}$ can be bactericidal (oxidative stress)
- Implications:
  Ecotoxicology- Biodiversity and food webs?
  Biogeochemical cycling?
  Mitigated by NOM, salts
- Applications:
  DBP-free disinfection, antifouling or anticorrosion coatings? Membranes?



# Any Questions?