Institut de recherche en biotechnologie
Biotechnology Research Institute

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NRC- Institute for National Measurement Standards
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Ecotoxicology of Nanoparticles: Issues and Approaches

Presented by
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Outline of presentation

- Nanoparticles and the Environment
- Ecotoxicological Risk Assessment:
  - Approaches and measurement tools
  - Use of standardized toxicity test methods
- Ecotoxicology of NPs using single species toxicity tests
- Bioaccumulation of single NPs and mixtures
- Issues and Priorities
- Recommendations and Closing remarks

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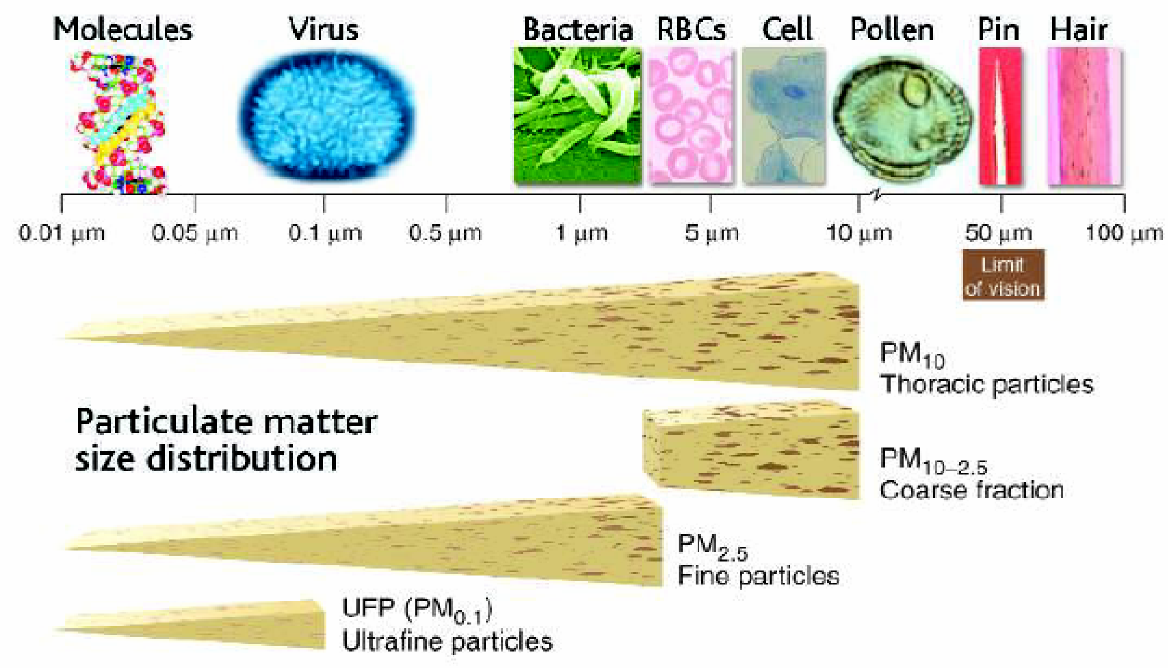
Nanotechnology and Nano(eco)toxicology

- **Nanotechnology uses matter sized "*at dimensions of roughly 1 to 100 nanometers*." (US- National Nanotechnology Initiative)**



- **Nano(eco)toxicology: Hazardous effects of Engineered NPs on ecological receptors**
- **Bioavailable NPs**
- **Nanotoxicity $\propto$ Size, shape, surface area, etc.**

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Sources of exposure to ultrafine particles and nanoparticles

Table 1. UFPs/NPs (< 100 nm), natural and anthropogenic sources.

Natural	Anthropogenic	
	Unintentional	Intentional (NPs)
Gas-to-particle conversions	Internal combustion engines	Controlled size and shape, designed for functionality
Forest fires	Power plants	
Volcanoes (hot lava)	Incinerators	Metals, semiconductors, metal oxides, carbon, polymers
Viruses	Jet engines	
Biogenic magnetite: magnetotactic bacteria protoctists, mollusks, arthropods, fish, birds human brain, meteorite (?)	Metal fumes (smelting, welding, etc.)	Nanospheres, -wires, -needles, -tubes, -shells, -rings, -platelets
	Polymer fumes	
	Other fumes	Untreated, coated (nanotechnology applied to many products: cosmetics, medical, fabrics, electronics, optics, displays, etc.)
Ferritin (12.5 nm)	Heated surfaces	
Microparticles (< 100 nm; activated cells)	Frying, broiling, grilling	
	Electric motors	

- Oberdörster et al. (2005)

***** Emerging area of toxicology and ecotoxicology *****

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There are more than 300 nanoproducts already on the market

Textiles, tennis rackets, paints, etc

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Nanotechnology applications in the environmental sector

- New production methods that are more environmentally friendly and consume less resources
- New, environmentally friendly materials
- More efficient methods for the production and consumption of energy
- New methods for the preparation of water, soil decontamination, and new environmental sensors.

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Nanotechnology and Nano(eco)toxicology

- **Need quantitative data on ecotoxicology of NPs (dose-response relationships).**
- **Ecotoxicological risk assessments should account for:**

  - toxic effects of the NP on an ecological receptor

  - the probability of exposure of that NP to ecological receptor,

  - and possible release of NP into the environment.

Ecotox Risk ∝ Toxicity x Exposure

- Need for standardized methods

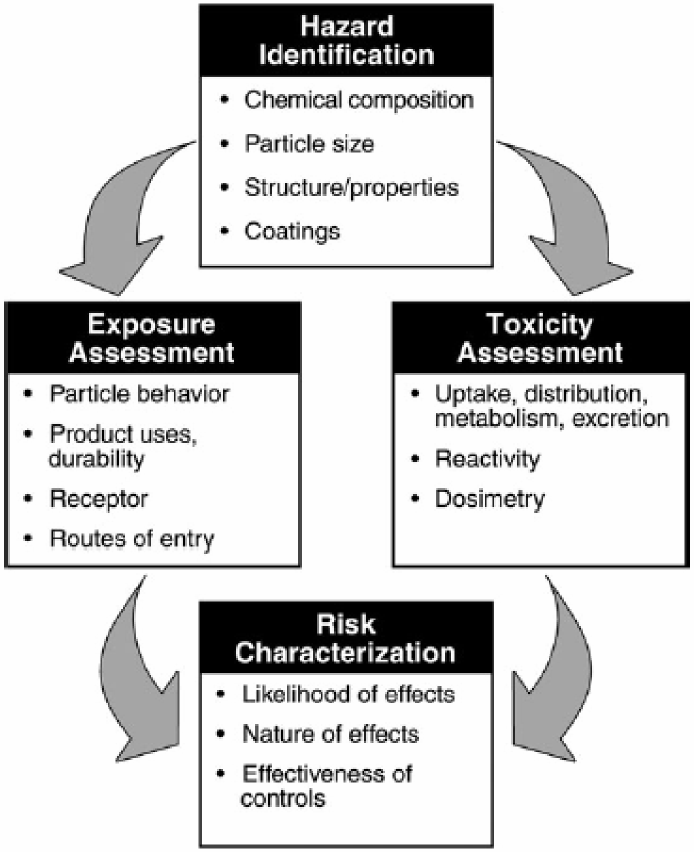

FIG. 4. Risk assessment framework for nanomaterials.

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Nano(eco)toxicology and Life Cycle Impact Assessment

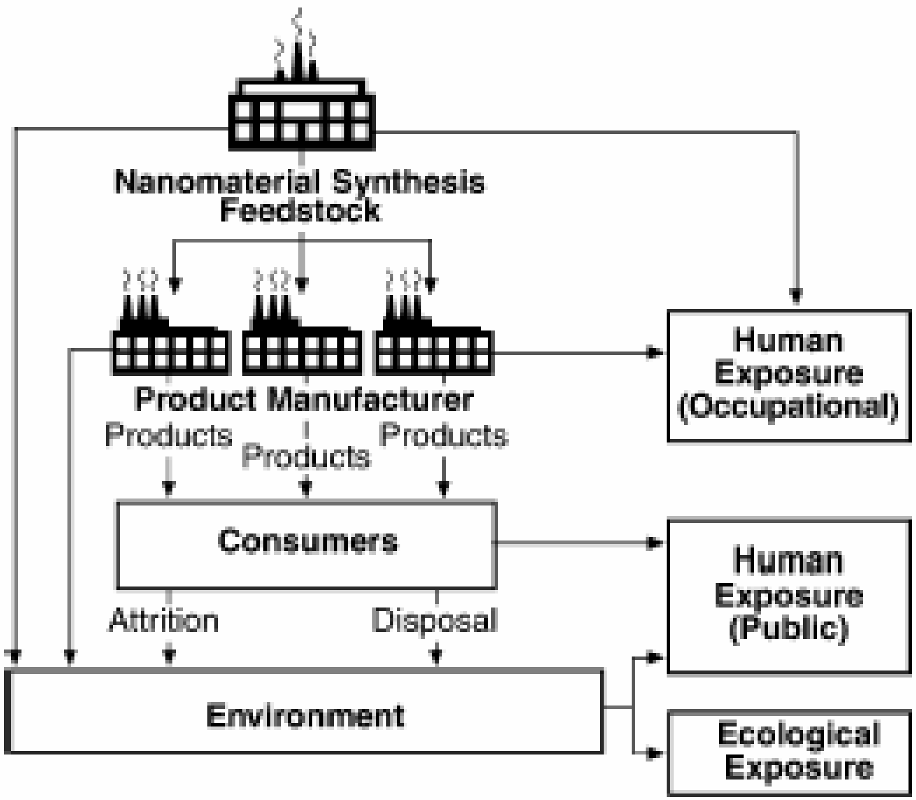

FIG. 5. Potential for release and exposure to nanoscale substances.

Tsuji et al. 2005

Water, Soil, and Air

LCIA Environmental concerns (e.g., ISO 14042):

- ***Direct Effects*: Toxic effects on ecological receptors.**
- ***Indirect Effects*: Bioaccumulation and biomagnification of NPs in the food chain. Other impacts (eutrophication, GHG).**
- **Contamination of water and soil from release and improper disposal of NPs and related products**

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Establishing a Chain of Ecotoxicological Evidence for Potential Toxicity and Bioaccumulation

TOXICOLOGICAL
(Response of Individuals)

Acute Toxicity

Altered Physiology

Reduced Growth

Reproductive Effects

Biomarkers (exposure, toxicity) for Better ERA

?

SHORT-TERM

LONG-TERM

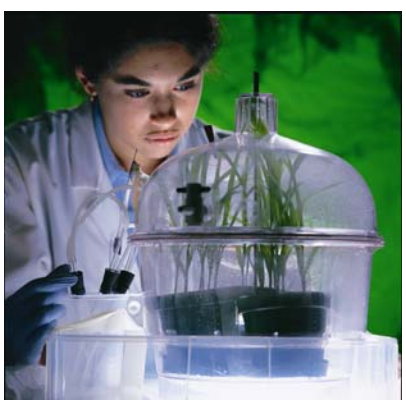
Adapted from Gunderson et al. 1997

Symbiotic Dissociation

Population Decline

Community Disruption

ECOLOGICAL
(Community & Population Responses)



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Ecotoxicological Hazard Assessment- Screening for Bioavailable Toxicants using Ecotoxicity Tests

Soil Sample

Direct Contact Bioassays →

Microbial toxicity
- Community function tests

Plant Bioassays
- Seed germination
- Growth

Invertebrate Bioassays
- *Eisenia andrei*
- *Enchytraeus albidus*

Aqueous and Organic Extracts (Groundwater) ↓

Plant Bioassays
- Lettuce root elongation
- Green algae (*S. capricornutum*)

Bacterial toxicity
- Microtox (*Vibrio fischeri*)

Mutagenicity
- SOS Chromotest (*E.coli*)
- Fluctuation Test
- Mammalian Cell Cultures
- Phytogenotoxicity

→

- Screen for and identify bioavailable toxicants
- Potential receptors
- Process Monitoring Aids

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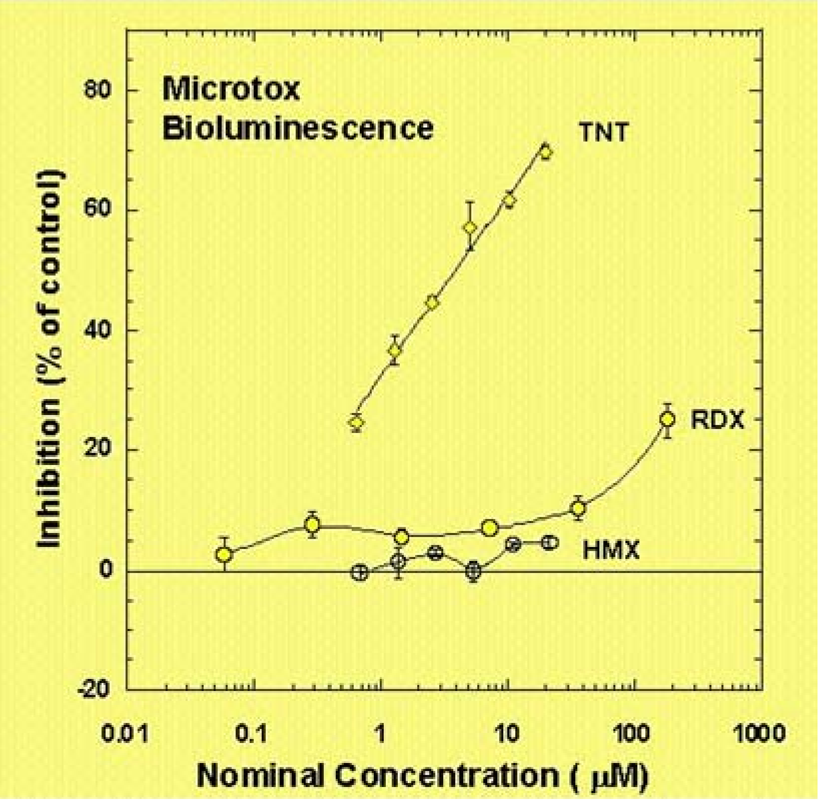
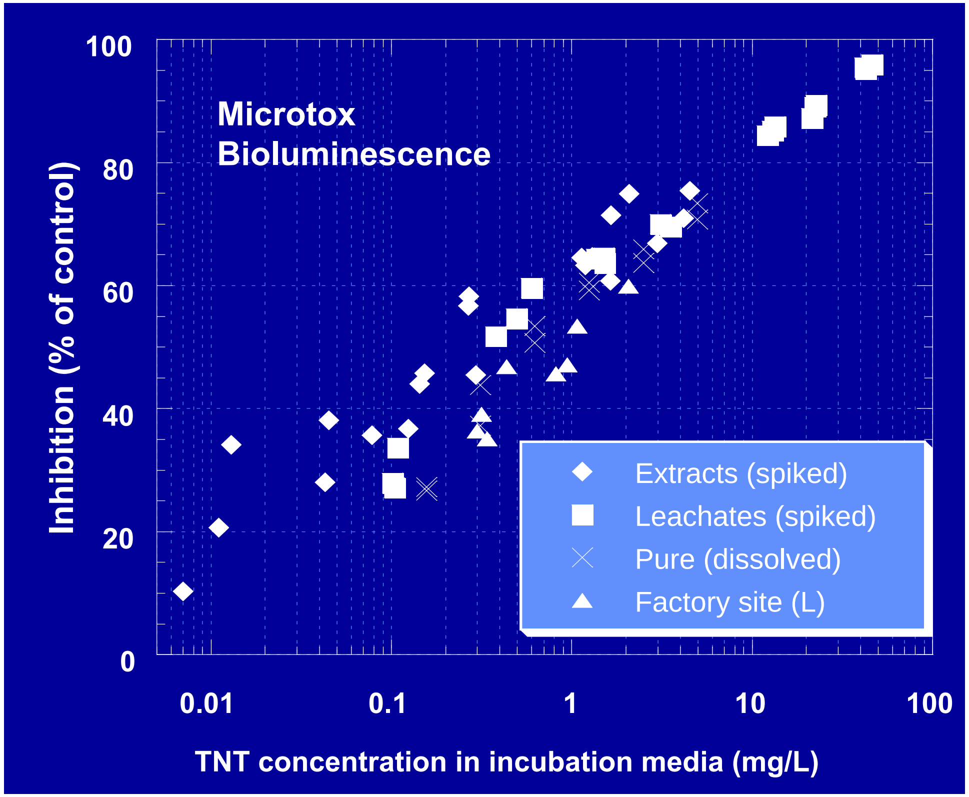
From Laboratory to Field: Toxicity to <u>Vibrio fischeri</u> using field soils having mixed contaminants

Pure Compounds



Soil leachates and extracts from a contaminated site



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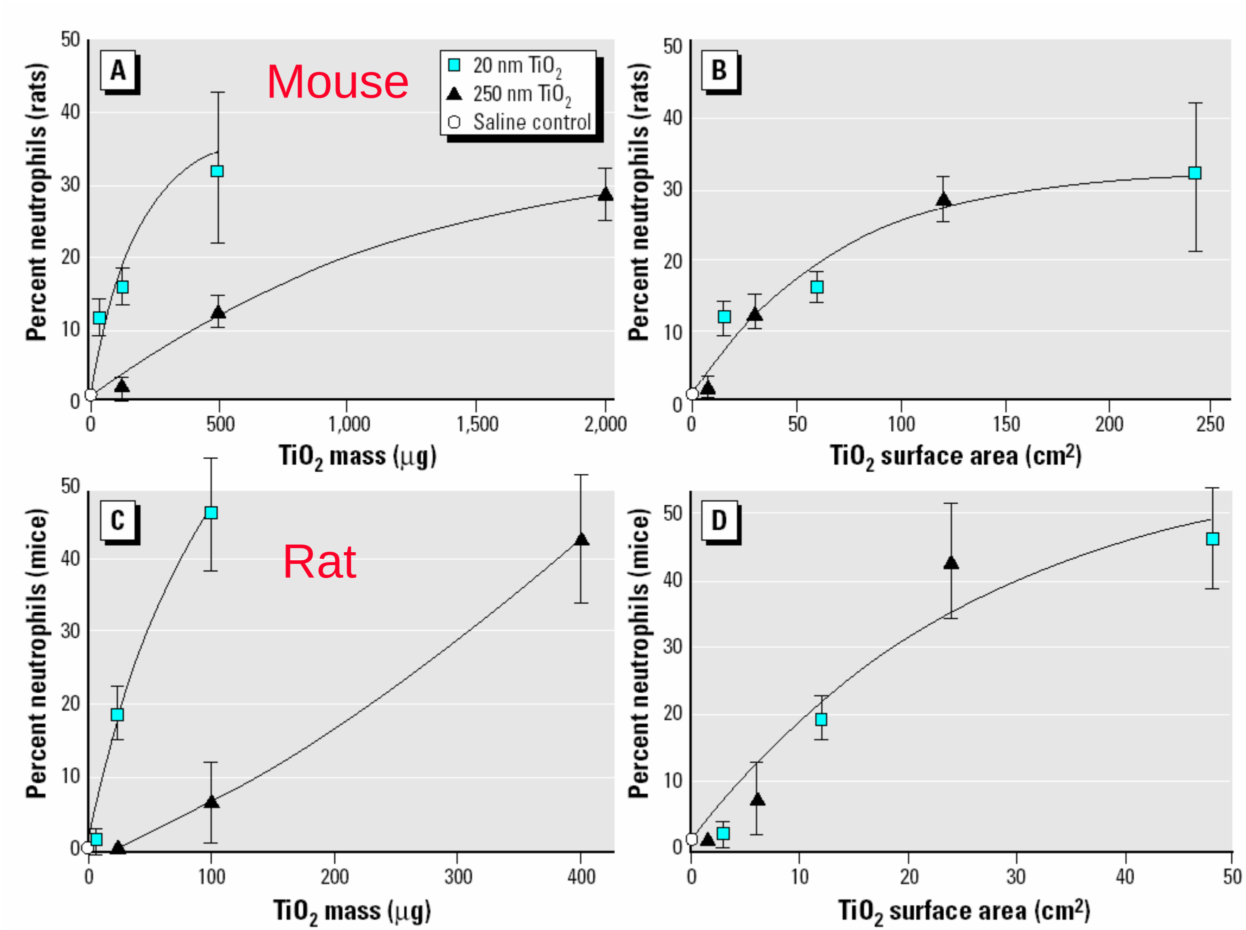
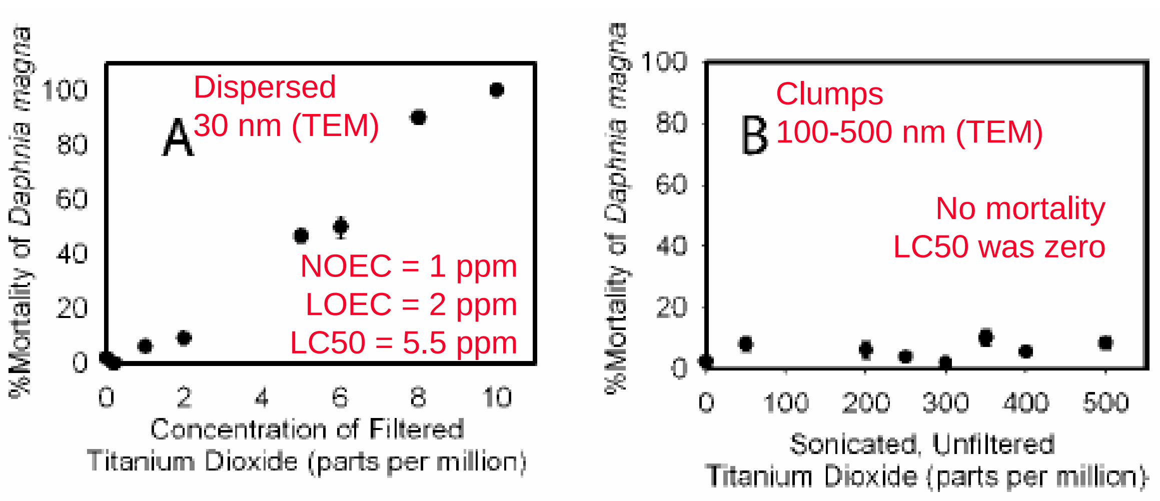
Dose-metric: Mass vs. Surface area
Effect of TiO_2 particle size on toxicity



- Oberdörster et al. (2005)

Sample preparation influences TiO_2 toxicity: 48-h <u>Daphnia magna</u> lethality test (USEPA Method 2024) (Lovern and Klaper, 2006)

- **Freshwater Daphnia spp.,**
- **Filter-feeder, food chain**
- **48-h acute mortality test (USEPA Method 2024)**
- **Titanium dioxide in deionized water <u>+</u> THF**
- **Passed through 0.22 µm nylaflo filter**
- **Concentrations: UV spectroscopy (325-350 nm)**



•Lovern and Klaper (2006) ETC <u>25</u>: 1132-1137

Uptake in Daphnia, Stone 2006

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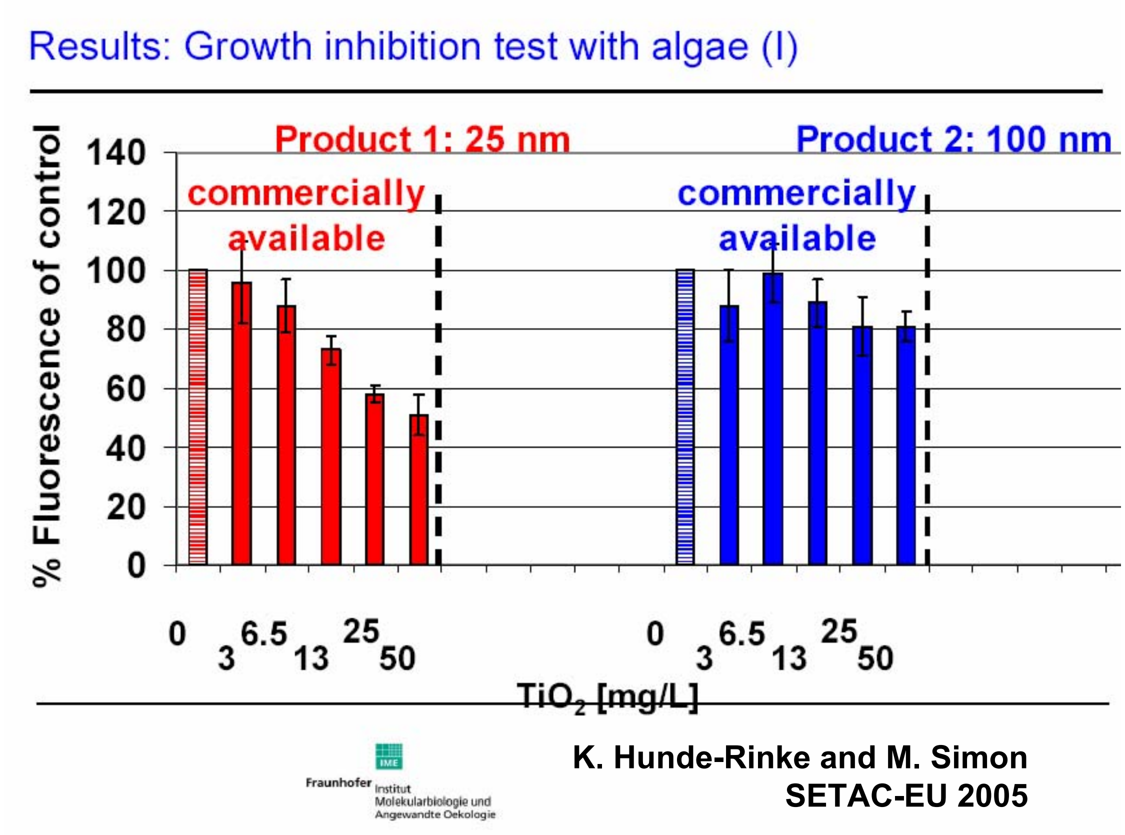
Titanium Dioxide (25 nm vs 100 nm) inhibits algal growth (OECD 201)



- **Size-dependent inhibition of algal growth by TiO_2**

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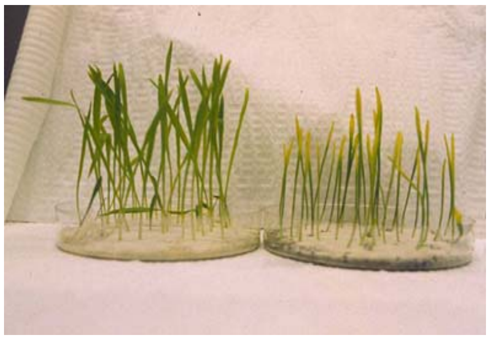
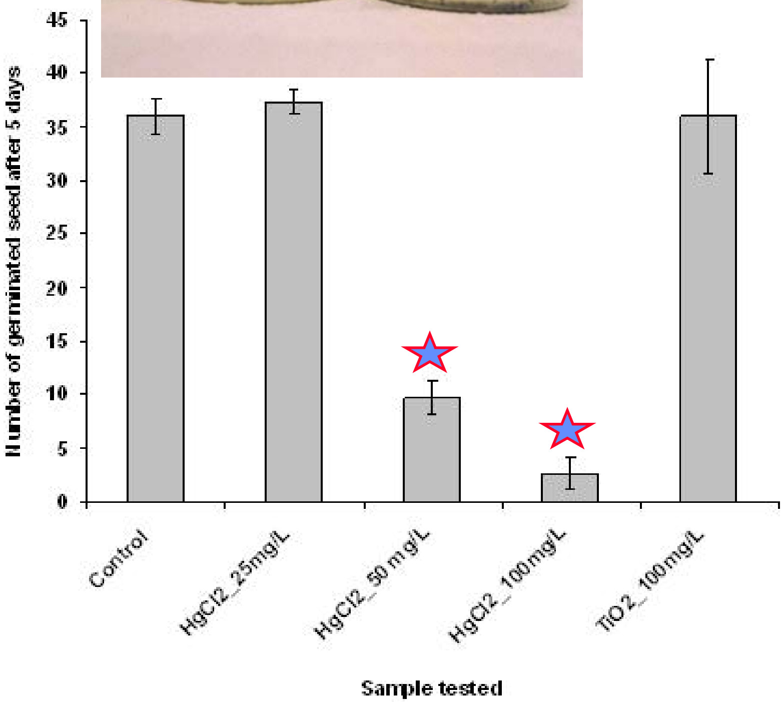
Effect of TiO_2 on plants using terrestrial toxicity tests (unpublished data)

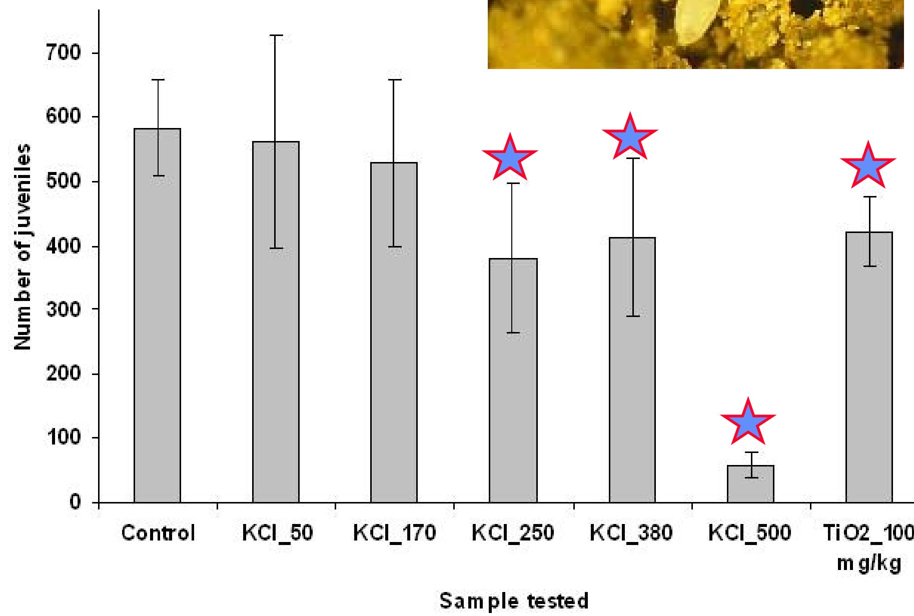


Plant seed germination (ASTM 1999, USEPA 2000)



- **No apparent phytotoxicity by TiO_2**

<u>Folsomia candida</u> - Survival and Reproduction test (ISO 11267:1998; Env Canada, 2005)



- **Slight decrease in reproduction by TiO_2**

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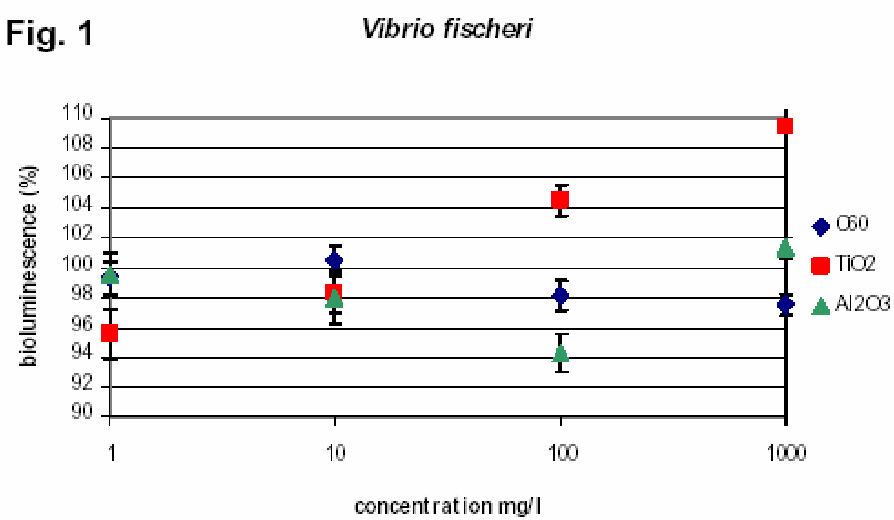
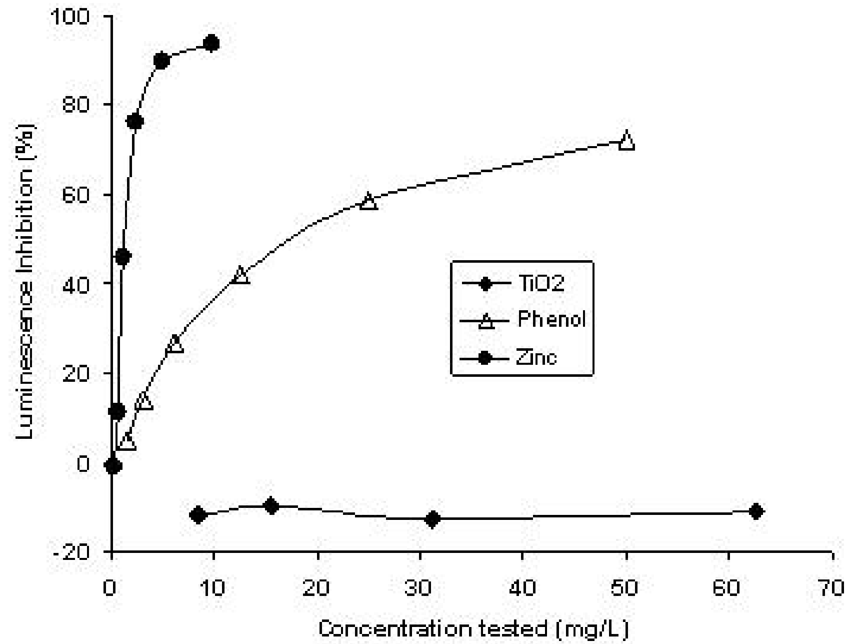
Effect of TiO_2 on Microtox test (Vibrio fischeri) (ISO 11348)

Fig. 1



Schaefer and Scott-Fordsmand, SETAC-EU 2005



- **NPs dispersed in water using sonication (15 min)**
- **No significant effects by TiO_2 (<100 nm) on *V. Fischeri***

- **TiO2 - sonicated 15 min**
- **Size to be confirmed**
- **No effect of TiO_2 on Microtox test**

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Effect of nC60 on Largemouth Bass

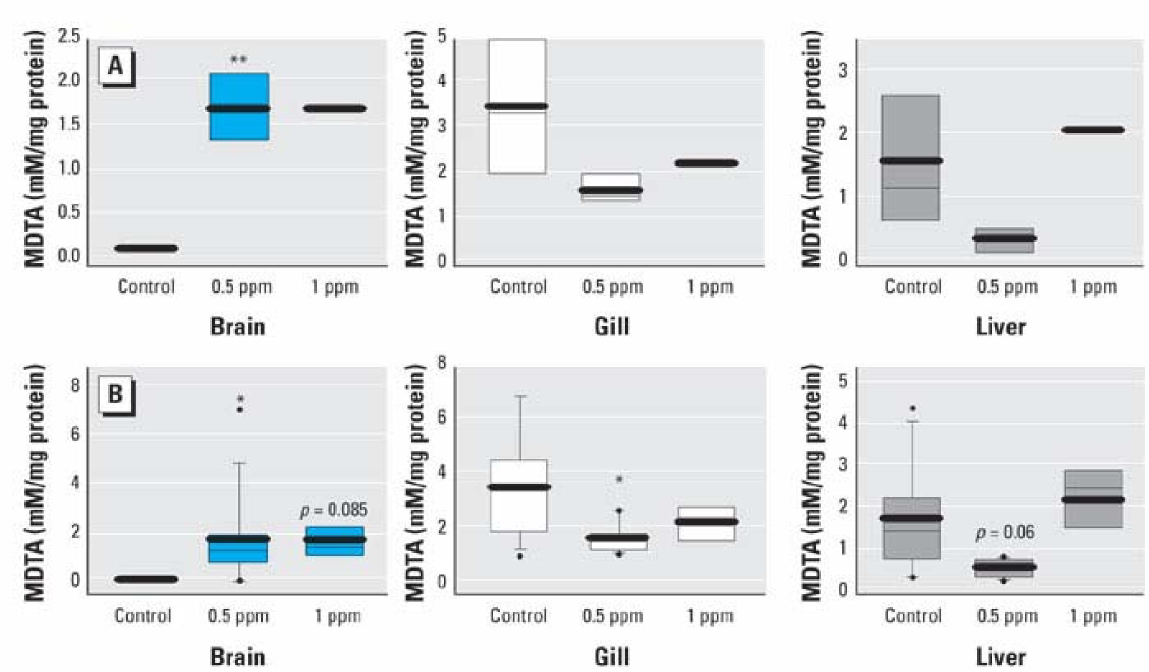

Figure 1. Lipid peroxidation of brain, gill, and liver of largemouth bass after 48 hr of exposure to 0.5 or 1 ppm nC_{60}. MDTA, 1,1,3,3-tetraethoxy propane. (*A*) Aquarium averages. (*B*) Data using individual fish; in the brain, 0.5 ppm nC_{60} caused a significant 17-fold increase in lipid peroxidation, whereas in gill and liver there is a trend for reduction of lipid peroxidation at 0.5 ppm nC_{60}. Heavy black bands represent the means; thinner lines indicate medians; boxes represent 25th and 75th percentiles; error bars indicate minimum and maximum; and circles represent outliers.
*$p < 0.05$. **$p < 0.01$.

- ***Micropterus salmoides***
  - exposed to 0.5 ppm or 1 ppm of uncoated nC60 (4 nm) for 48 hr.
- **THF – vehicle (Rotovap); filtered nC60**

nC60 exposure caused:

- **slightly ↓ BW**
- **↑ lipid peroxidation in brains at 0.5 ppm**
- **no clear DRC**
- **slight ↓ GSH in gills**
- **↑ water clarity, possibly due to bactericidal activity.**

Eva Oberdörster, EHP 112 (July 2004)

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Bioaccumulation of NPs in fish: Environmental co-contaminants - Mixtures

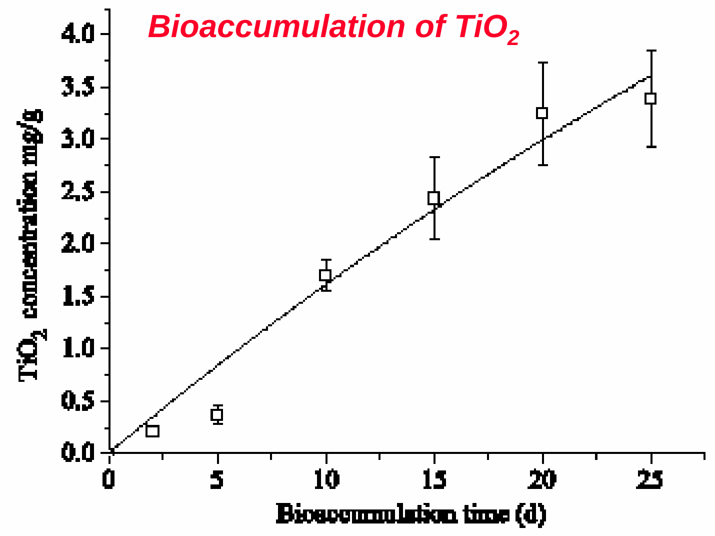
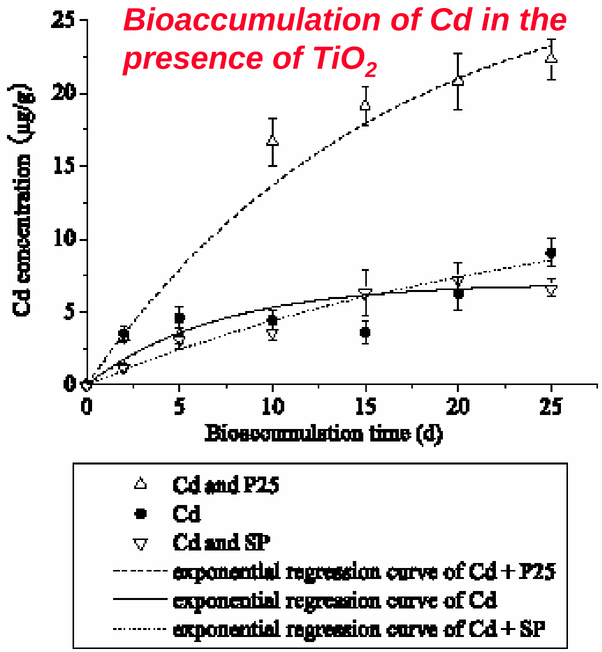


Fig. 4. The accumulation of TiO_2 nanoparticles in carp.

Zhang et al.(2007) Chemosphere 67: 160-166



BCFs for Cd and TiO_2 in different parts and whole body of carp at the 20th day

	Exposed media	Skin and scale	Muscle	Gills	Viscera	Whole body
Cd	Cd	4.11	0.31	33.6	213	64.4
	Cd and SP	5.41	1.72	58.3	364	88.9
	Cd and TiO_2	11.1	3.49	152	1679	606
TiO_2[a]	Cd and TiO_2	17.0	9.00	74.0	1065	325

[a] The initial TiO_2 concentration 10 mg/l was used to calculate BCF for TiO_2.

What is the metric for bioaccumulation? Form?

Nano(eco)toxicological Risk Assessment: Issues and Priorities

Ecotoxicological Hazard Assessment:

- Novel Toxicity? Ecological receptors?
- Ecotoxicological effects (acute, chronic)?
- Measurement endpoints? Dose?
- Related to which physico-chemical properties?
- Physico-chemical form after bioaccumulation?

- **Standardized ecotoxicity test protocols?**
- **Reference nanotoxicants?**
- **Adequate?**
- **Dose-metric?**

Exposure Assessment:

- Environmental stability (persistency)?
- Rapid Mobility?
- Chemical and physical form in the environment?
- Exposure dose-metric? (Tissue residues?)

- **Environmental Dose-metric for NPs?**
- **Reference nano-materials?**
- **Adequate?**

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Closing remarks

<u>In summary,</u>

- ***Environmental health hazards related to NP exposure in air, water, and soil will challenge traditional ecotoxicological approaches.***

We should consider using…..

- ***Multi-disciplinary approach (toxicologists, material scientists, and engineers)***
- ***Integrated and holistic approach (LCIA) that links physico-chemical characteristics of NPs, effects in biological hierarchy, and environmental relevance.***

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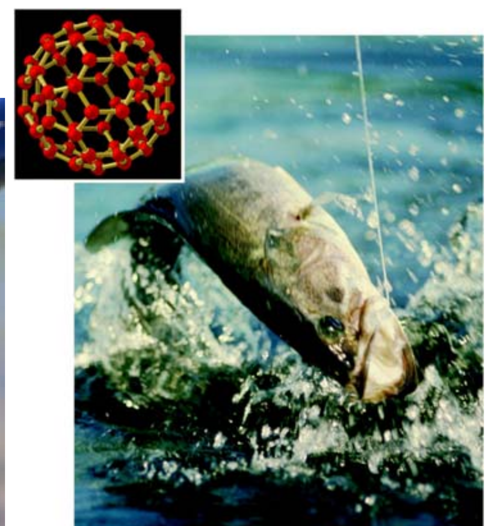
Thank you

- **BRI-Applied Ecotoxicology Group**
- **Collaborators at the Biotechnology Research Institute (BRI)**



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