# Toxicity Evaluation of $HfO_2$ Nanoparticles

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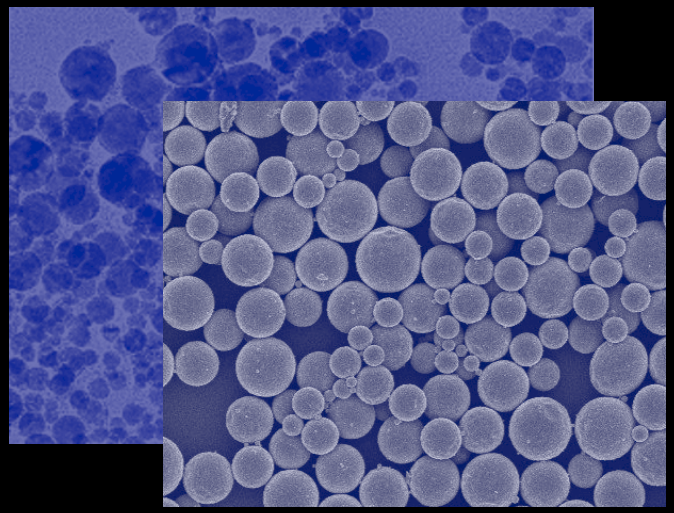

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NESAC/BIO
An NIH Resource Center

U W E B

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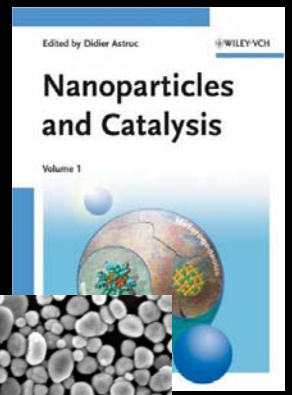
# Engineered Nanomaterials (ENM)

- Engineered structures with at least one dimension at 100 nm or less
- Increasing industrial / commercial applications, e.g.
  - Catalysis
  - Medicine
  - Environmental technology
  - Cosmetics
  - Semiconductors
  - Microelectronics




http://www.slashgear.com

- Nanotechnology projected to become a 1 trillion US $ market by 2015.

# Nanoparticles in Semiconductor Manufacturing

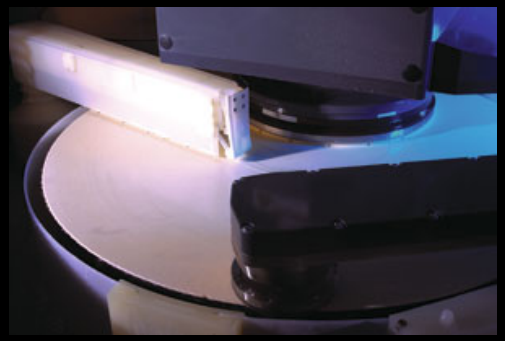
**CMP slurries**

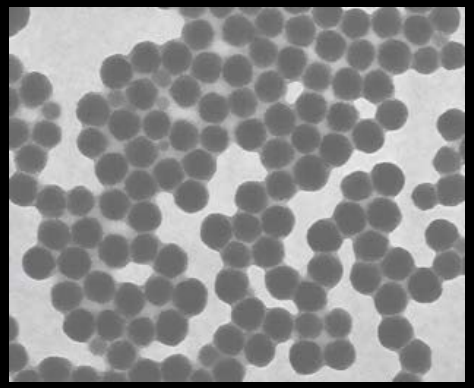
- $SiO_2$
- $Al_2O_3$
- $CeO_2$

**$HfO_2$** (immersion photolithography)

**Quantum dots**

**Carbon nanotubes**



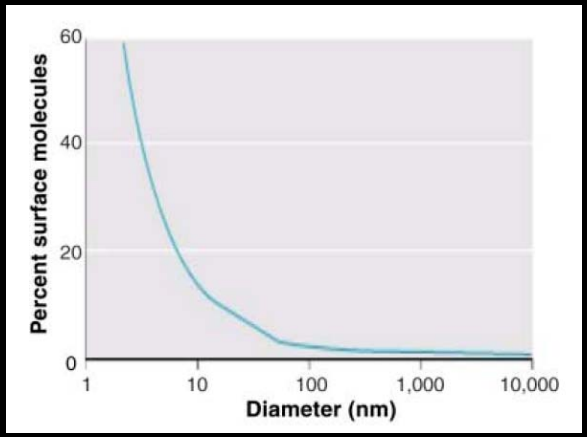


**Colloidal silica (10-130 nm)**

(Source: www.bjgrish.com)

# Nanoparticles - ESH Concerns

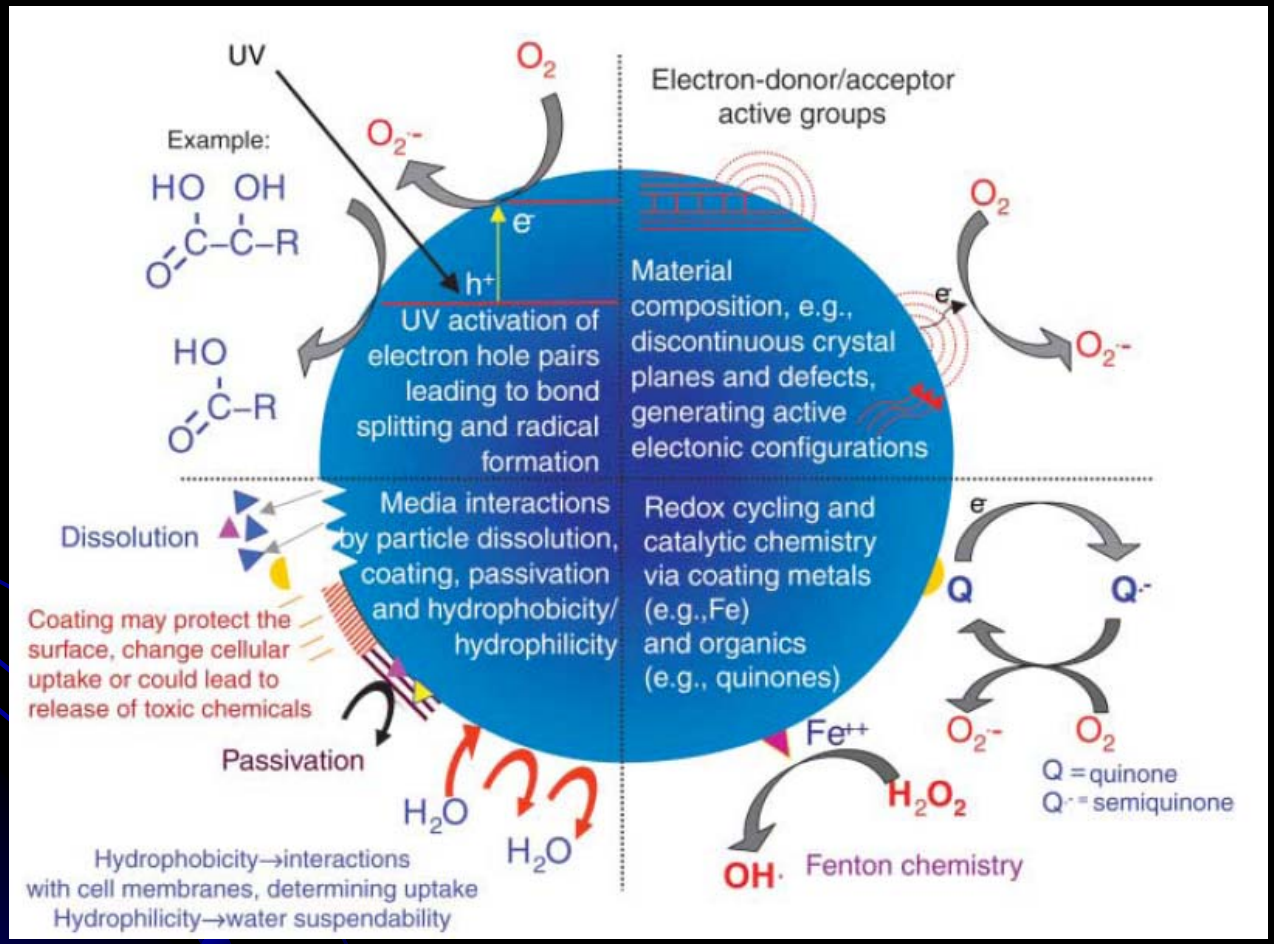
- Concern about the adverse effects of nanomaterials on biological systems
  - ENM: unusual properties due to their small size
  - Increasing evidence that some ENM cause toxicity




Nel et al. Science, 2006, 311:622-627

- Poor understanding of “nanotoxicity”
- Uncertainty about the real-life hazards of engineered nano-materials
- Need for principles and procedures to ensure the safety of nanotechnology for workers, consumers, and the environment.

# Engineered Nanomaterials – Possible Interactions w. Biological Tissue




Nel et al. Science, 2006, 311:622-627

# Objectives

- Evaluate the toxicity of hafnium oxide, $HfO_2$, nanoparticles (NP).
- Physico-chemical characterization of the $HfO_2$ samples

# Samples: Particles Tested

- **Reference micron-sized $HfO_2$ particles**

  Reported particle size: < 44 µm

- **Batch 1 nano-sized $HfO_2$ particles: "Batch-1 NP"**

  Reported average particle size: approx. 20 nm

- **Batch 2 nano-sized $HfO_2$ particles: "Batch-2 NP"**

  Reported average particle size: approx. 1-2 nm

- **Batch 3 nano-sized $HfO_2$ particles: "Batch-3 NP"**

  Reported average particle size: approx. 100 nm

# Particle Size Distribution (PSD) & Zeta Potential

Particle size distribution (PSD)

- Electron microscopy
- Dynamic light scattering (Malvern Zeta Sizer Nano ZS)
- Laser diffraction (micron-sized $HfO_2$)

Zeta potential

- Electrophoretic mobility (Malvern Zeta Sizer Nano ZS)

Preparation of dispersions

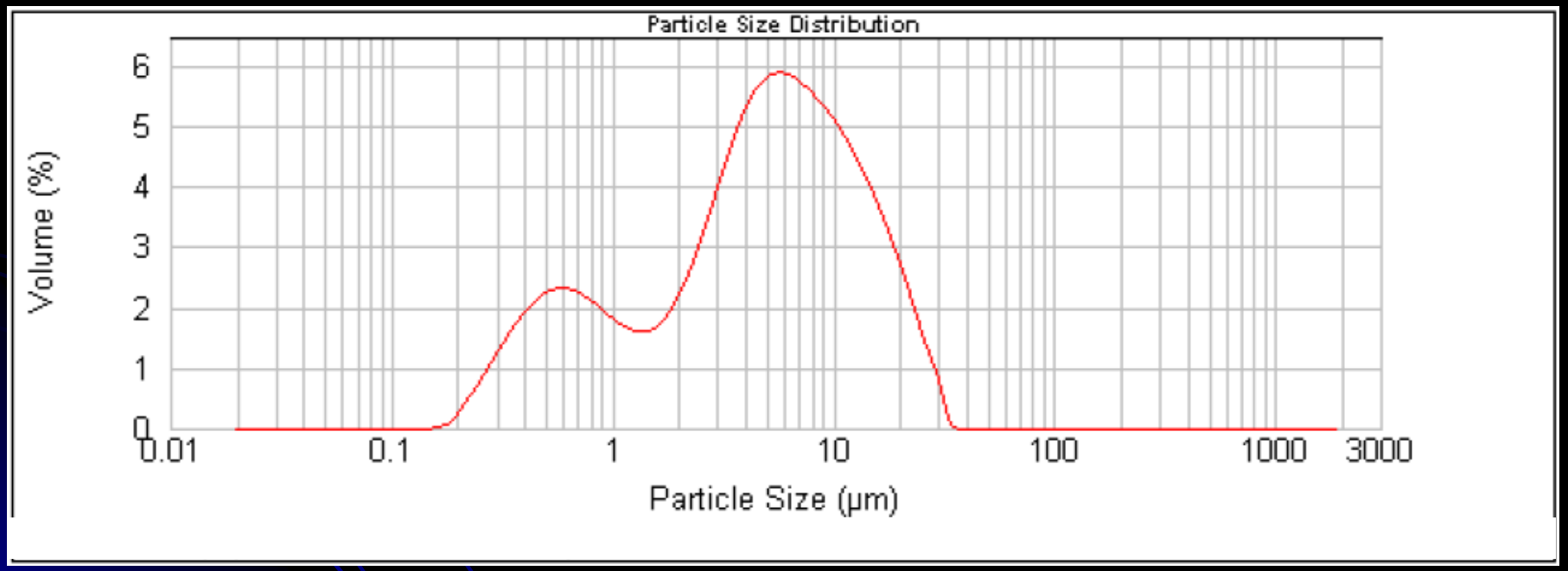
- $HfO_2$ dispersed in 100 mg/L Acetic Acid (pH 4)
- Ultrasonic treatment (130 W, 20 KHz, 60% amplitude) for 5 min

# Particle Size Distribution

## Reference micron-sized particles

### PSD - Laser Diffraction

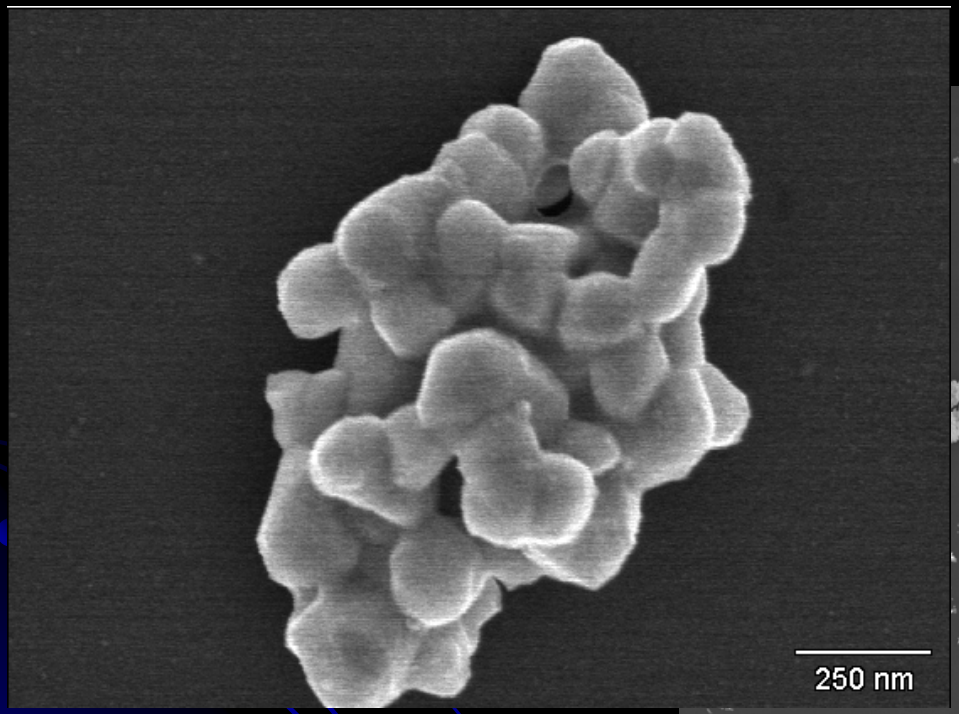
Alfa Aesar #35666

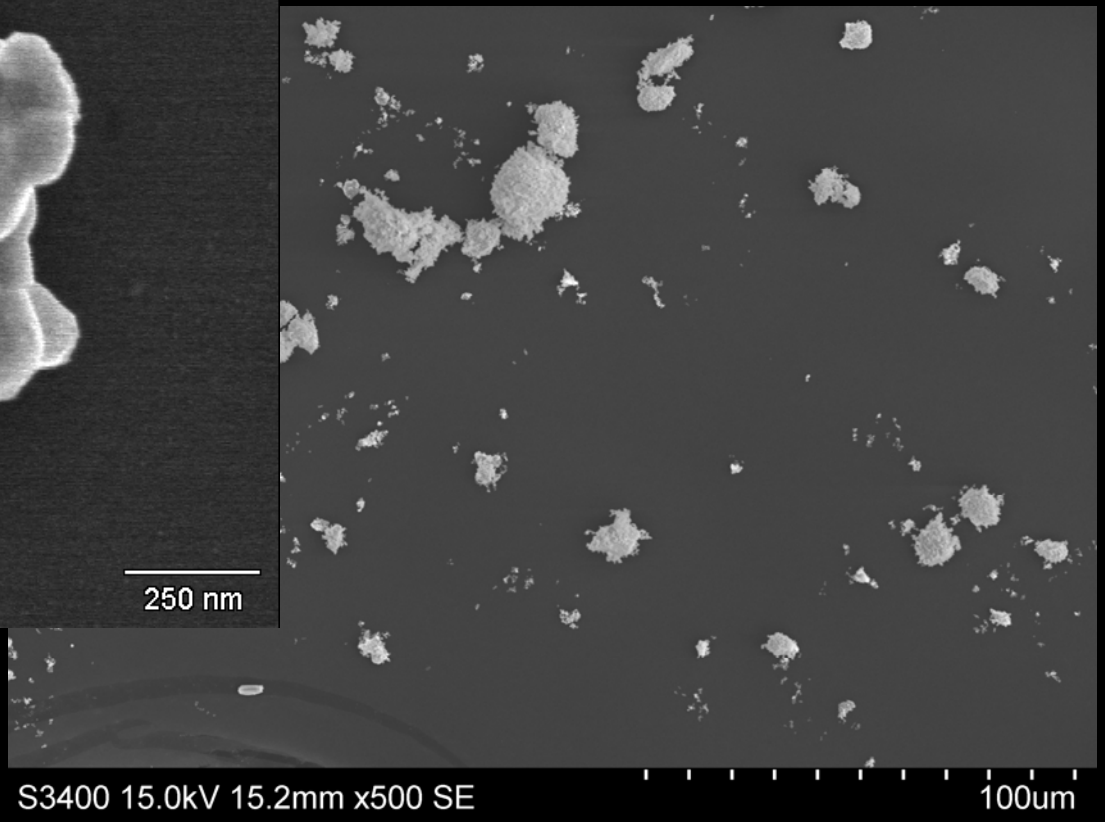



0.2 to 30 microns

# SEM

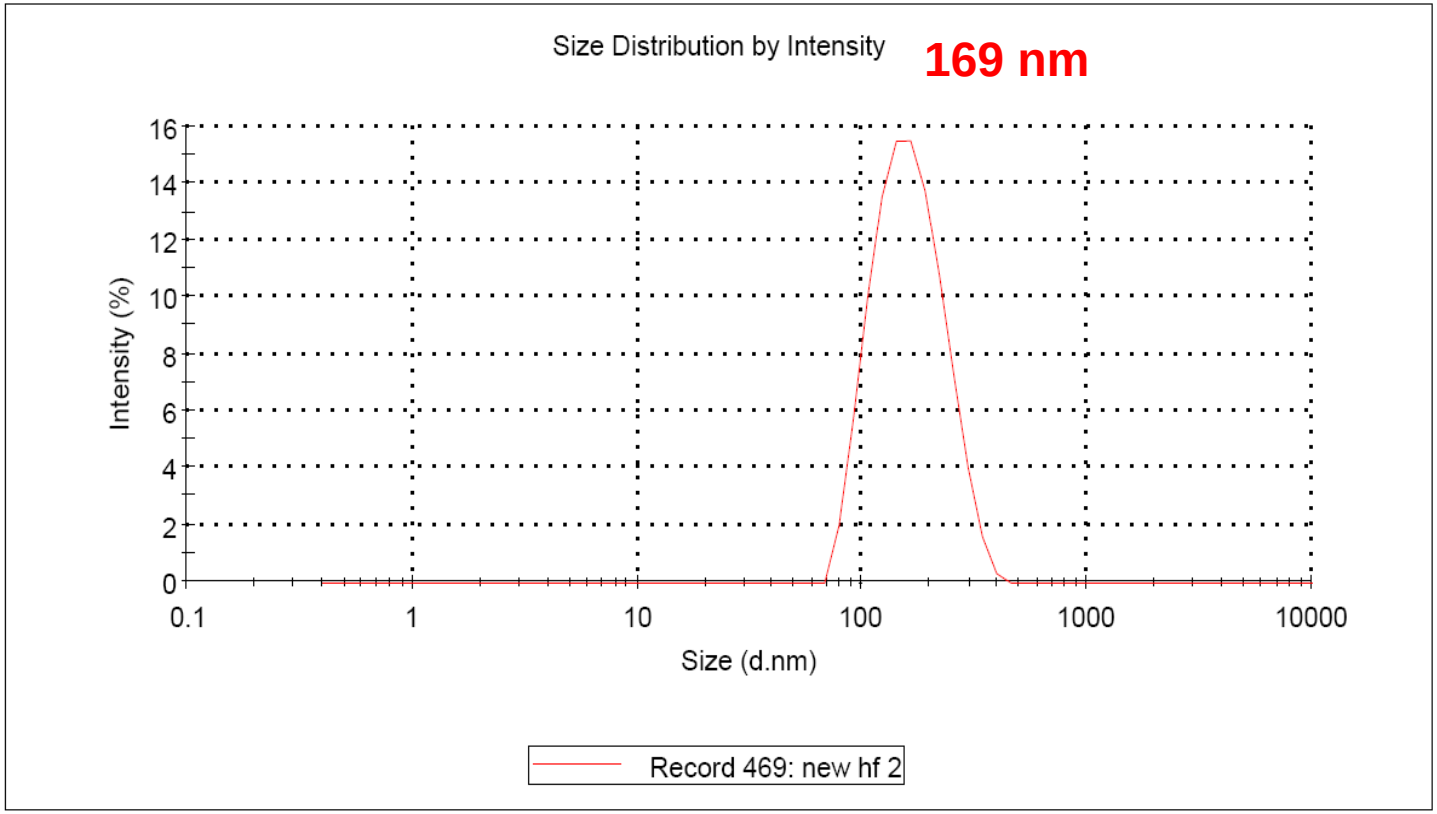
## $HfO_2$ “micron sized”



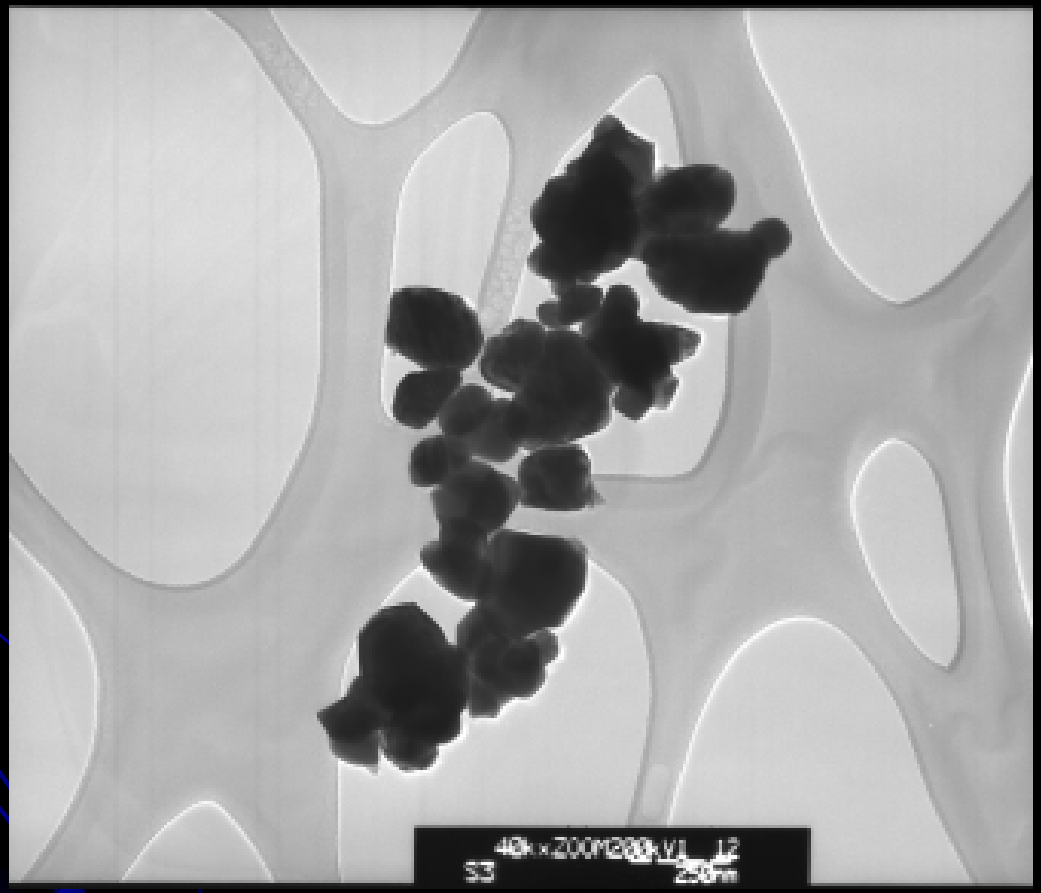
# Particle Size Distribution

## $HfO_2$ Batch 3 “100 nm”



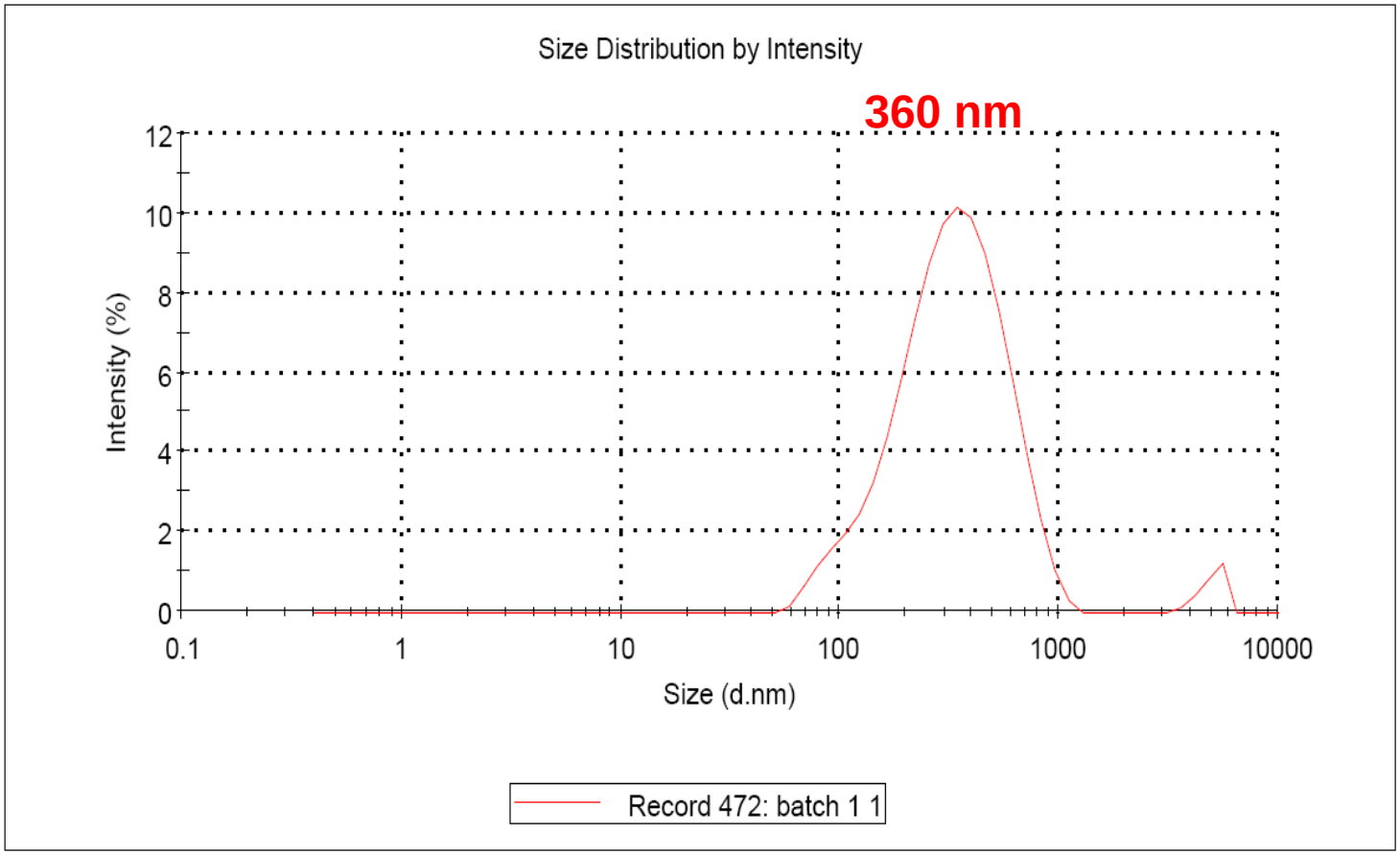
# TEM

## $HfO_2$ Batch 3 “100 nm”



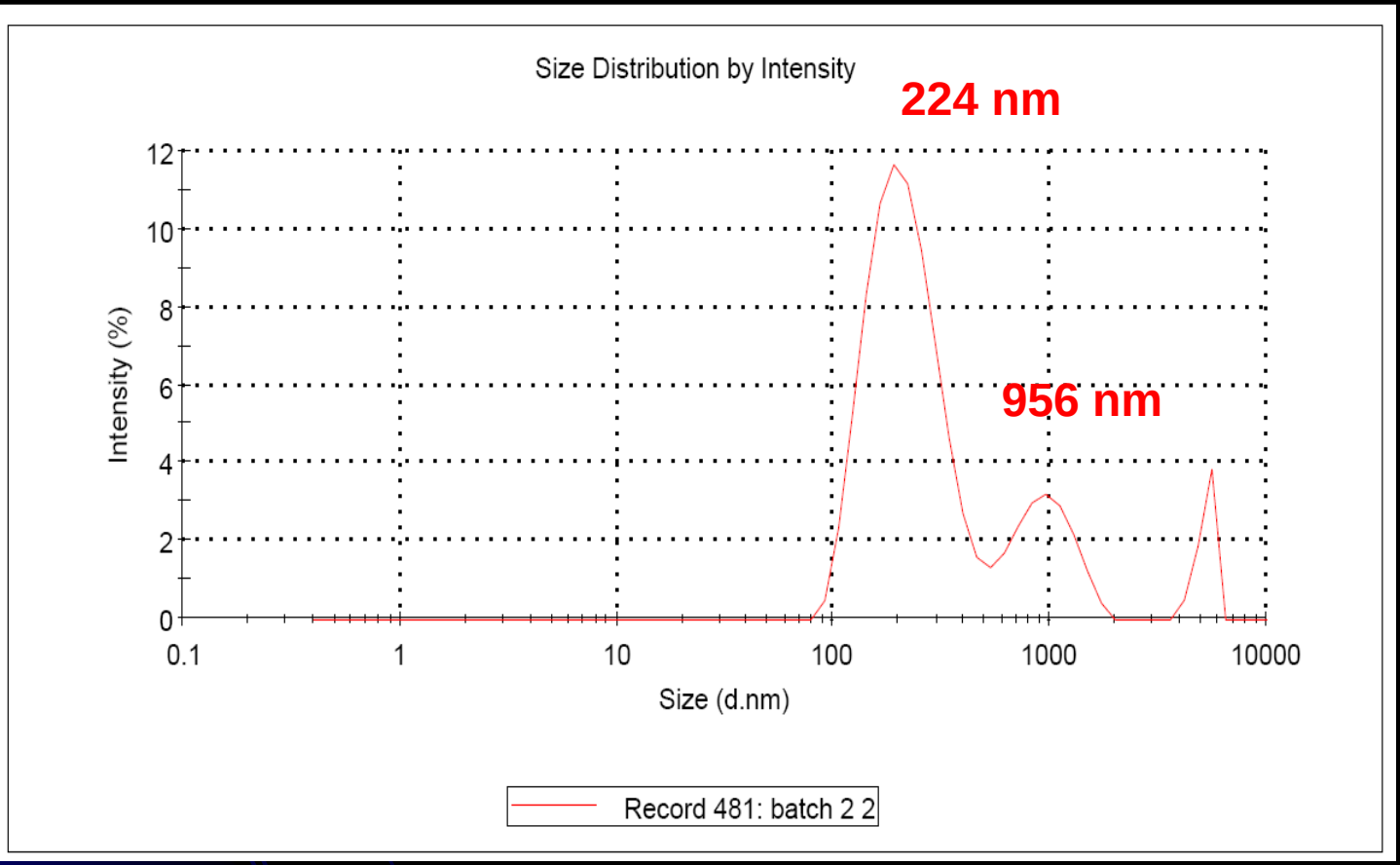
# Particle Size Distribution

## Batch 1- $HfO_2$ ("20 nm")



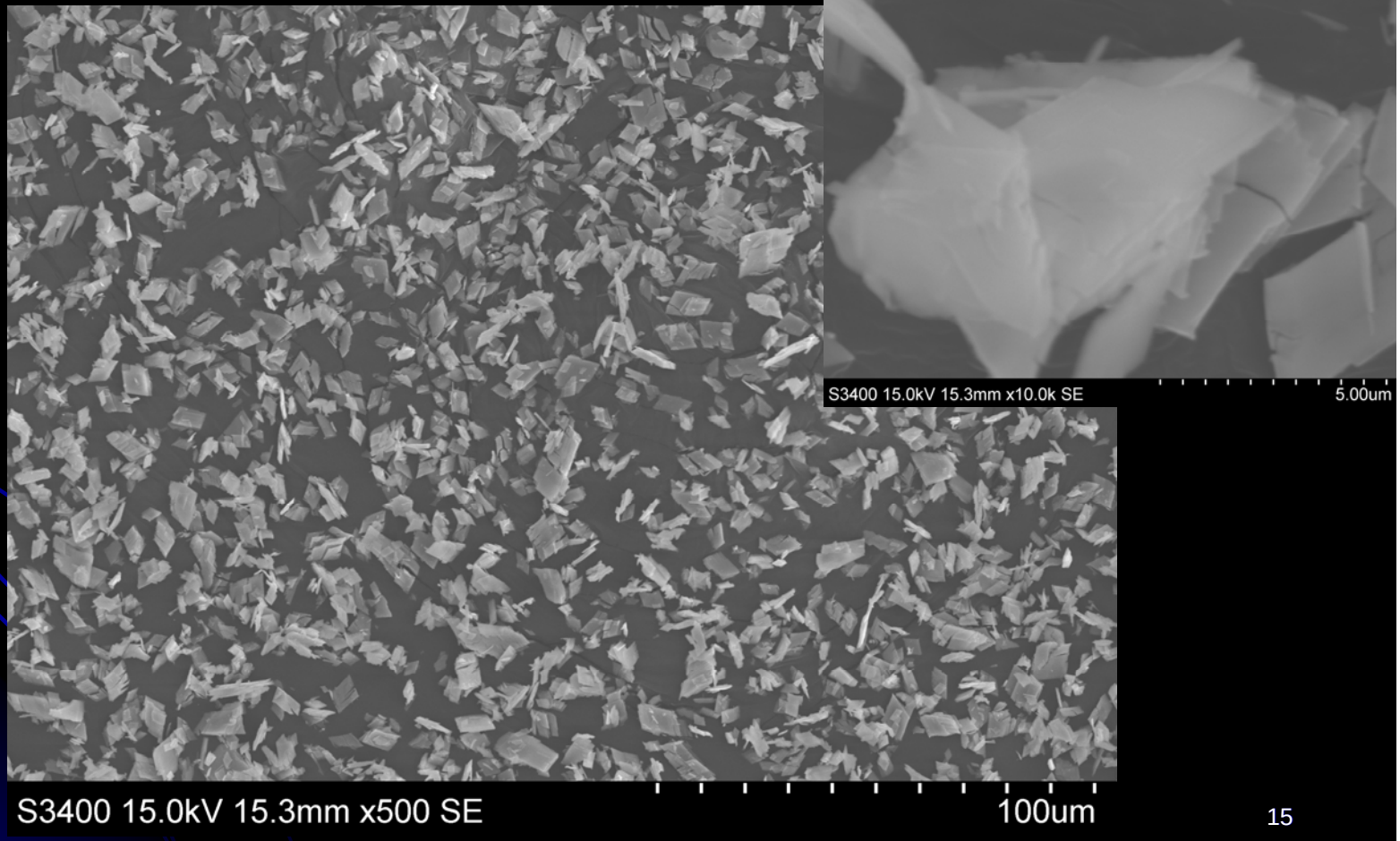
# Particle Size Distribution

## $HfO_2$ batch 2 ("2 nm")



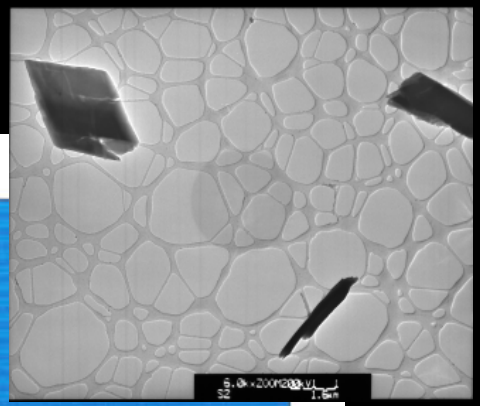
# SEM

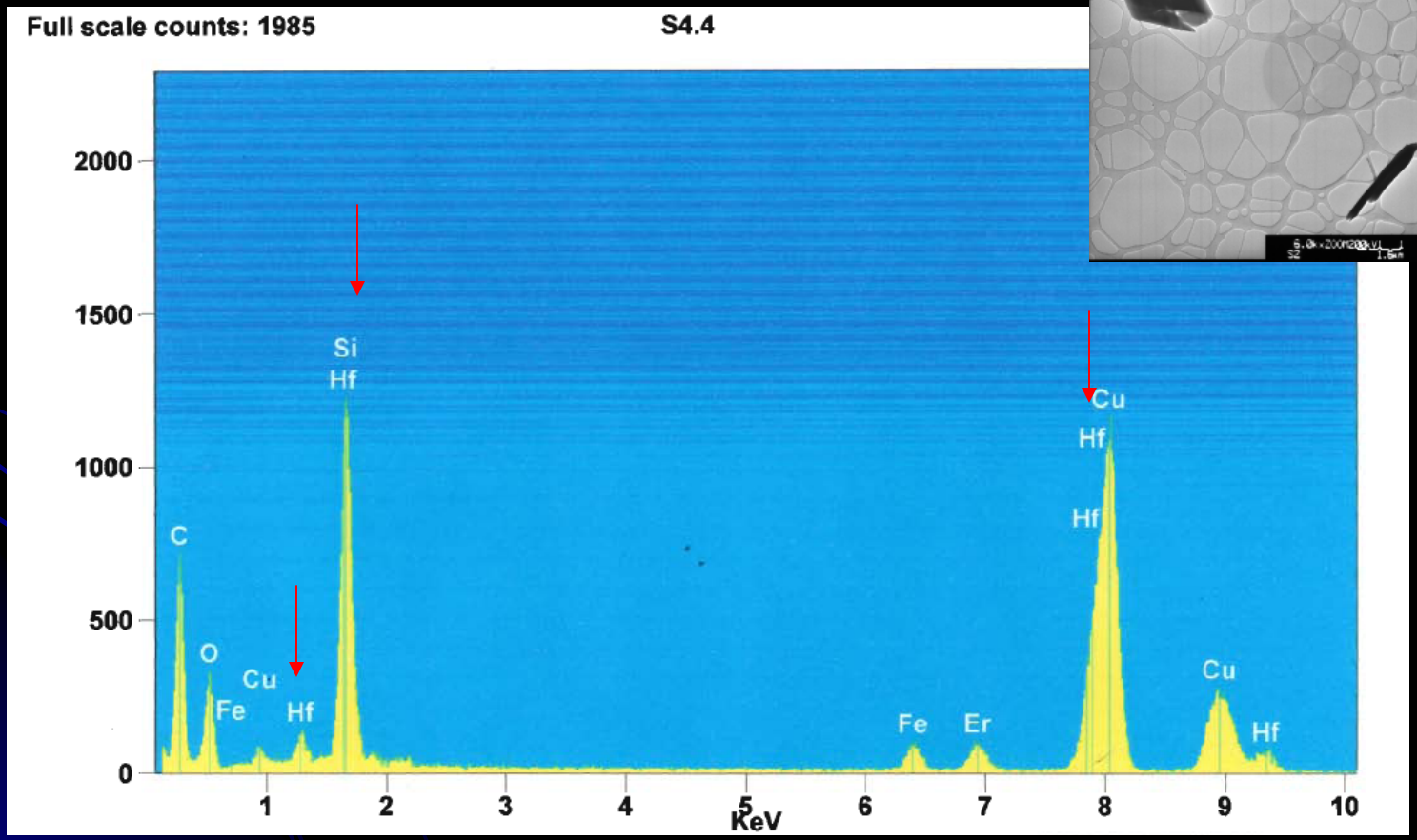
## $HfO_2$ batch 2 ("2 nm")



# Element Microanalysis (EDS)

## $HfO_2$ - Batch 2 ("2 nm")





# Particle Size Distribution (PSD) & Zeta Potential

| | Ref. Micro-sized | Batch 1 | Batch 2 | Batch 3 |
|---|---|---|---|---|
| **Average size (nm)** | | | | |
| - Expected | **< 44,000** | **20** | **2** | **100** |
| - DLS/Diffraction | 500 / **6,000** | **360** | **224** / 952 | **169** |
| - TEM/SEM (range) | 300-1,000 | ND* | 2,000-8,000 | 150-260 |
| - Filtration/ICP (range) | | | 78% **20-200** | |
| **Zeta** | 52 | 44 | 66 | 64 |

* Agglomerates: 1,000-5,000 nm

**Average particle size of some $HfO_2$ appears to be different than reported by supplier!!**

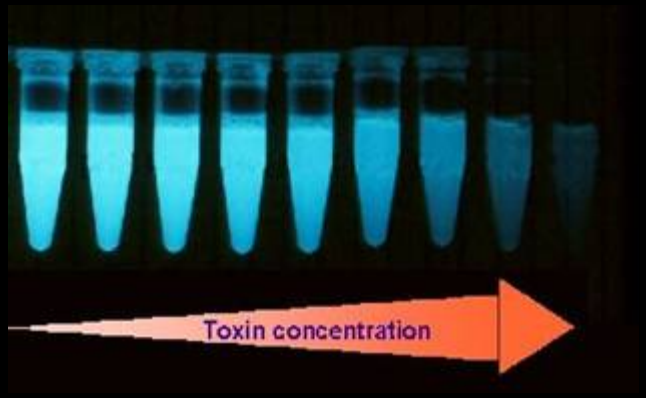
# Toxicity Evaluation

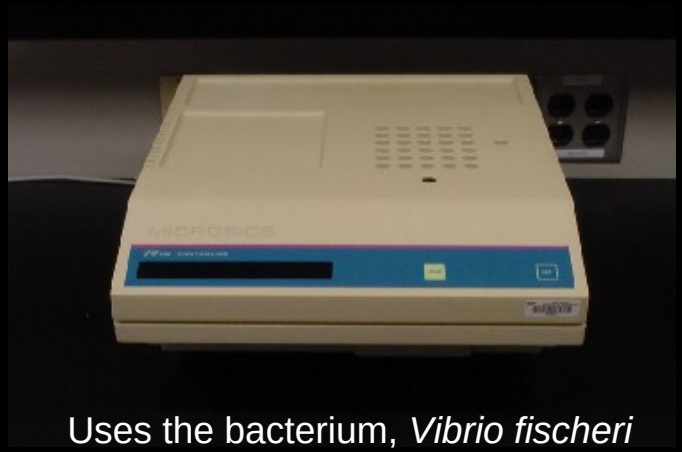
## Evaluate the potential toxicity of $HfO_2$ nanoparticles

- **Microtox** (bacterium, *Vibrio fischeri*)
- **Methanogenic Toxicity** (anaerobic microbial consortium)
- **Mitochondrion Toxicity Test or MTT** (ureter epithelium cells)
- **Live-Dead Assay** (skin epithelium cells)

# Microbial Toxicity Tests

**Microtox Test:** Monitoring of bioluminiscence *vs*. toxicant concentration



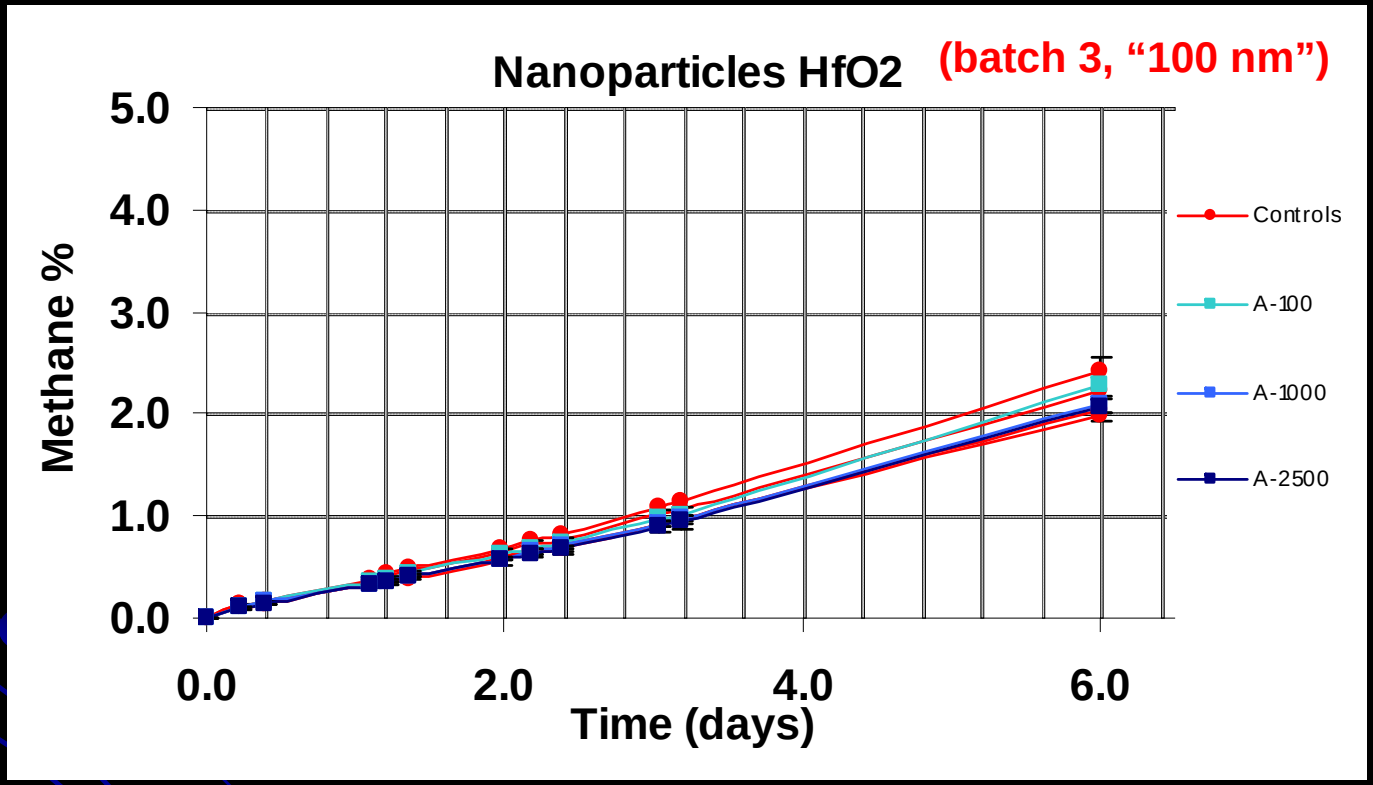



Uses the bacterium, *Vibrio fischeri*

**Methanogenic Toxicity Tests:**

Monitoring of $CH_4$ production rate at different toxicant concentrations.

# Methanogenic Toxicity

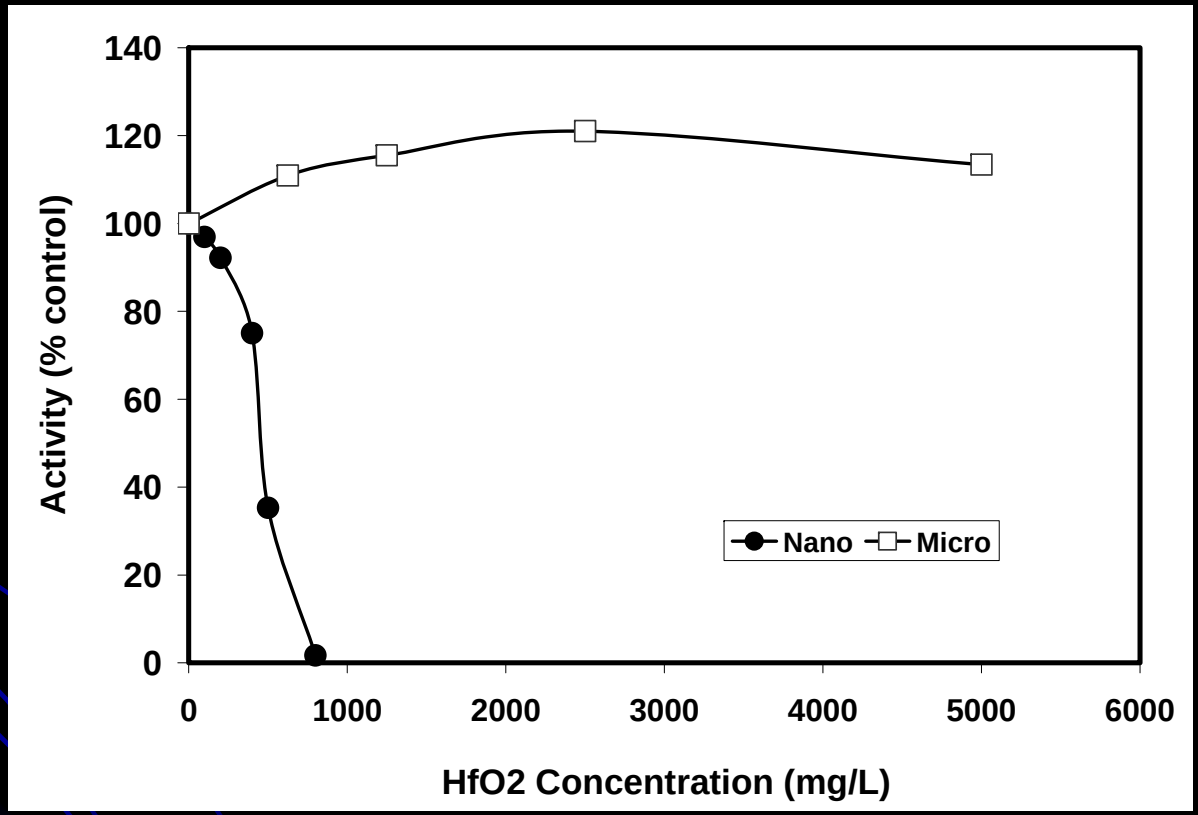



- % Activity = 100 $\frac{\text{Activity of Treatment}}{\text{Activity of Control ( toxicant free)}}$
- % Inhibition = 100 - % Activity

# Microtox Results

## $HfO_2$ micron-sized *vs*. $HfO_2$ nano Batch 1 "20 nm"

Toxicity

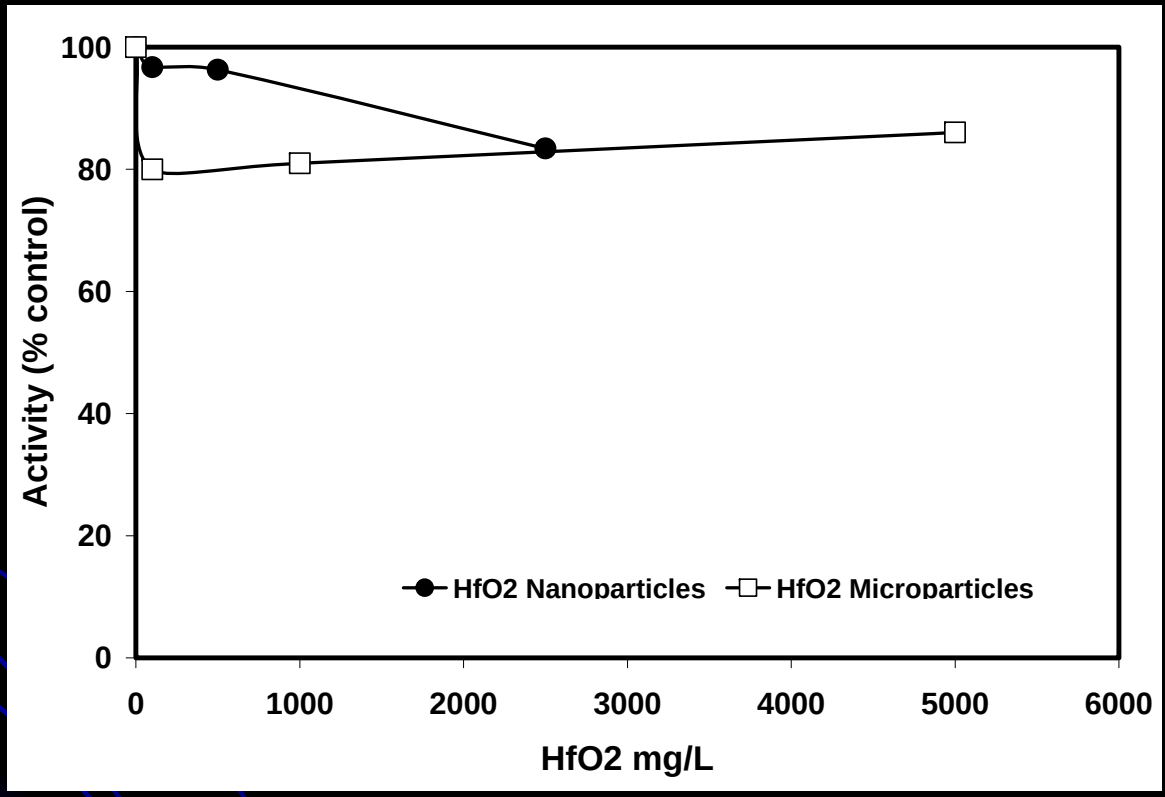



$HfO_2$ Batch 1 toxic to the bioluminescent bacterium used in the Microtox assay

# Methanogenic Toxicity

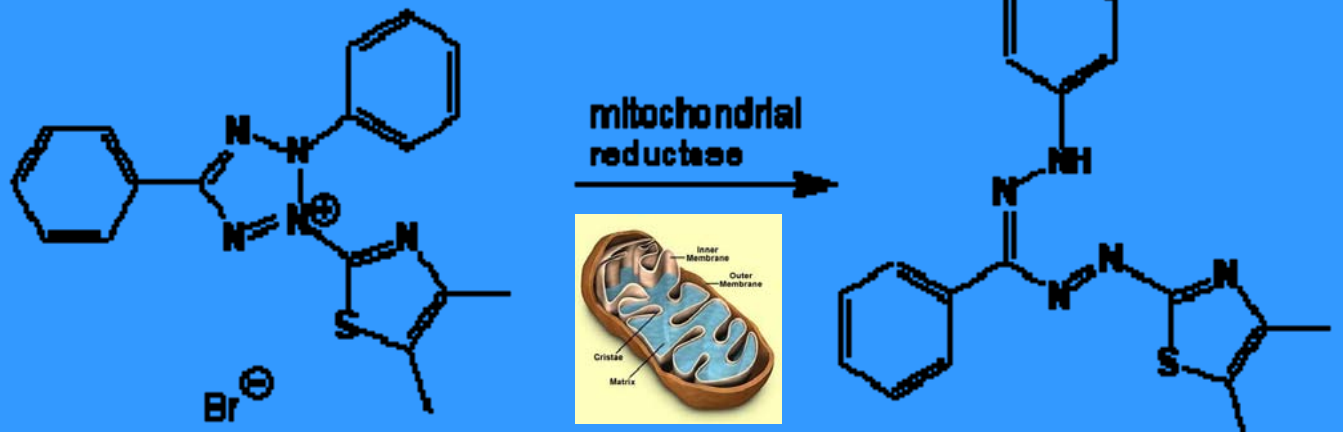
**$HfO_2$ micron-sized *vs*. $HfO_2$ nano Batch 1 "20 nm"**

Toxicity




Nano-$HfO_2$ Batch 1 not toxic to methanogens in wastewater treatment sludge

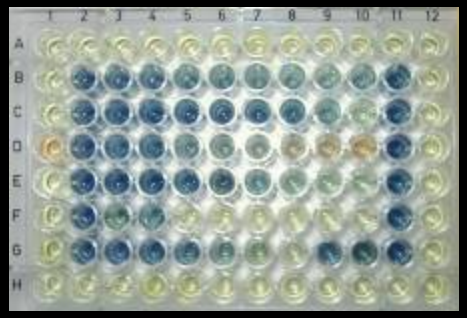
# Mitochondrial Toxicity Test (MTT)




**Yellow** MTT (3-(4,5-Dimethylthiazol-2yl)-2,5-diphenyltretazolium bromide

**Purple** Formazan

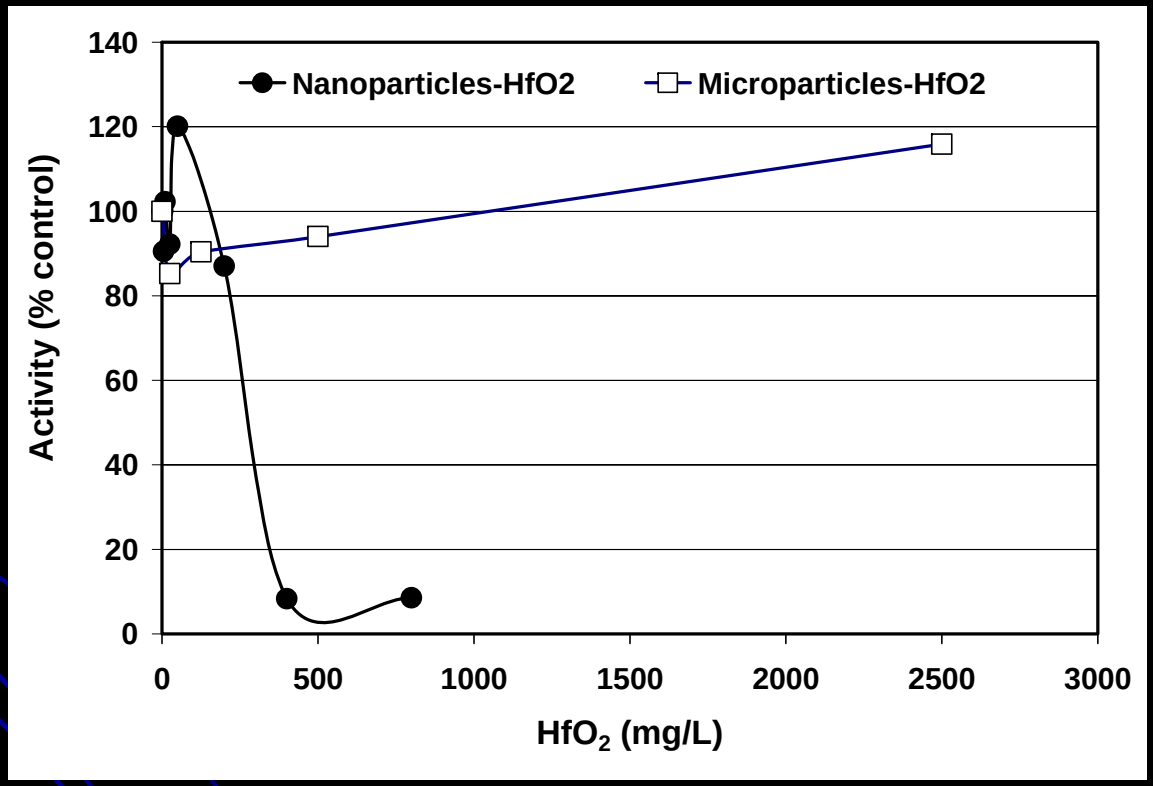
Live cells are blue colored



# MTT Results

## $HfO_2$ micron-sized *vs*. $HfO_2$ nano Batch 1 "20 nm"

Toxicity




$HfO_2$ Batch 1 nanoparticles toxic to mitochondria in eukariotic (human) cells

# Microbial & Mitochondrial Assays

| | 50% Inhibiting Concentrations | | |
|---|---|---|---|
| | Methanogenic | Microtox | MTT |
| Ref. micron | NT* | NT* | NT** |
| Batch 1 | NT** | 463 | 294 |
| Batch 2 | NT** | 330 | NT** |
| Batch 3 | NT** | 3,000 | 180 |

NT*= Not toxic at conc < 5,000 mg/L

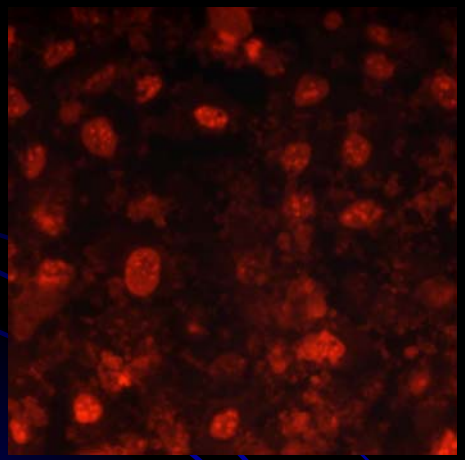
NT**= Not toxic at conc < 2,500 mg/L

- Micron-sized $HfO_2$ shows low toxicity
- Toxicity of nano-sized $HfO_2$ varies depending on 1) the batch, no correlation with particle size; 2) cell type.

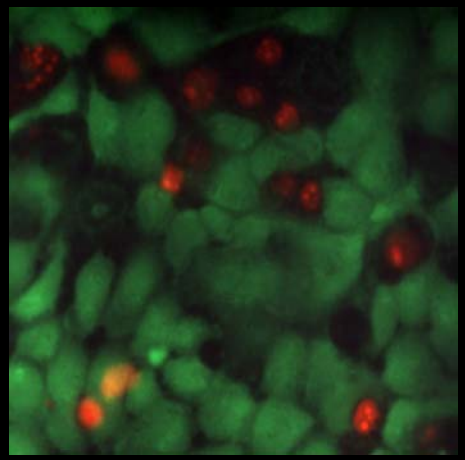
# Live - Dead Result
## (Skin epithelium cells, HaCat)

Nano-$HfO_2$ (Batch 1 “20 nm”)

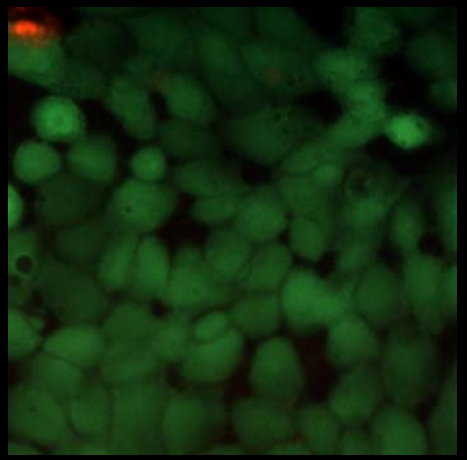
HaCat, 3000 ppm



HaCat, 300 ppm



HaCat, 3 ppm

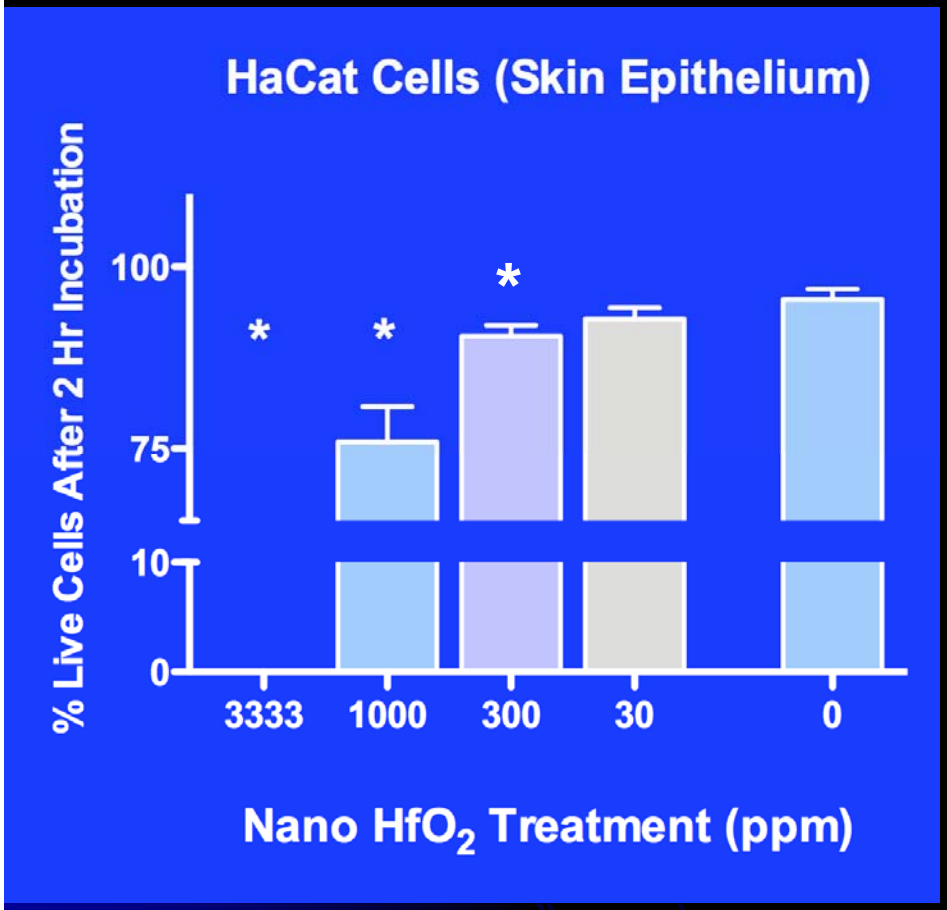


**Green**: Live cell

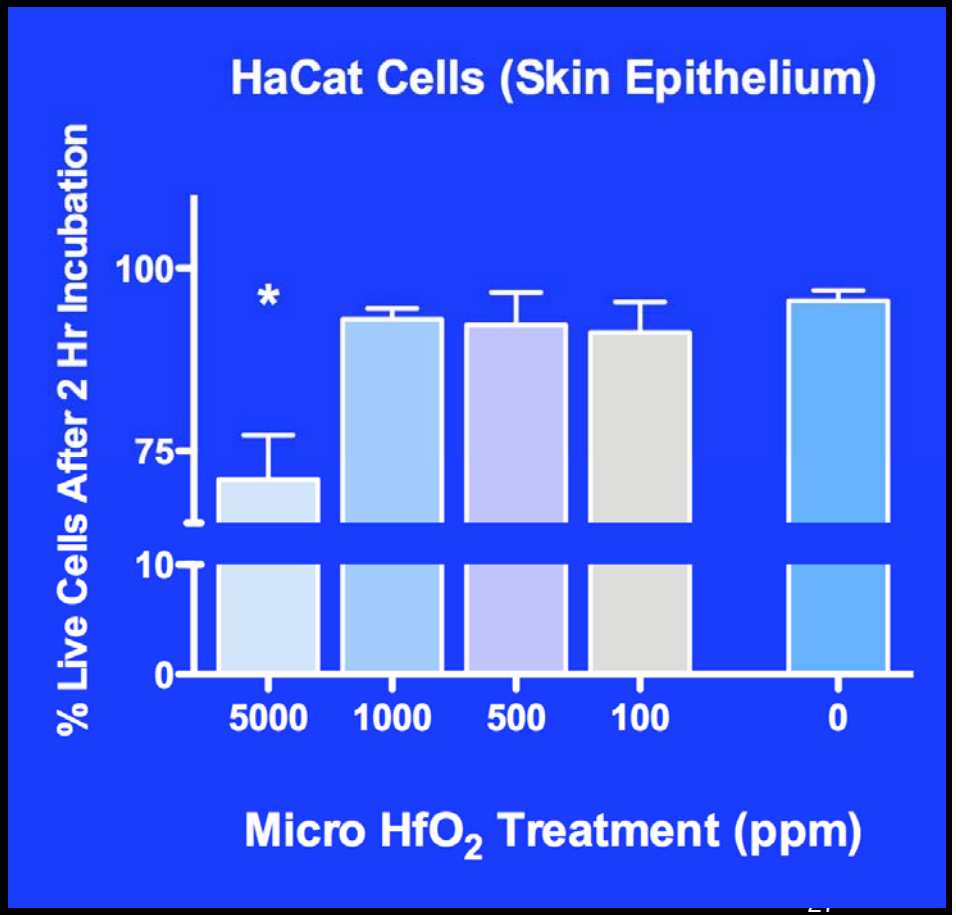
**Red**: Dead cell

# Live Dead Result
## (Skin epithelium cells, HaCat)

Nano-$HfO_2$ (batch 1, “20 nm”)

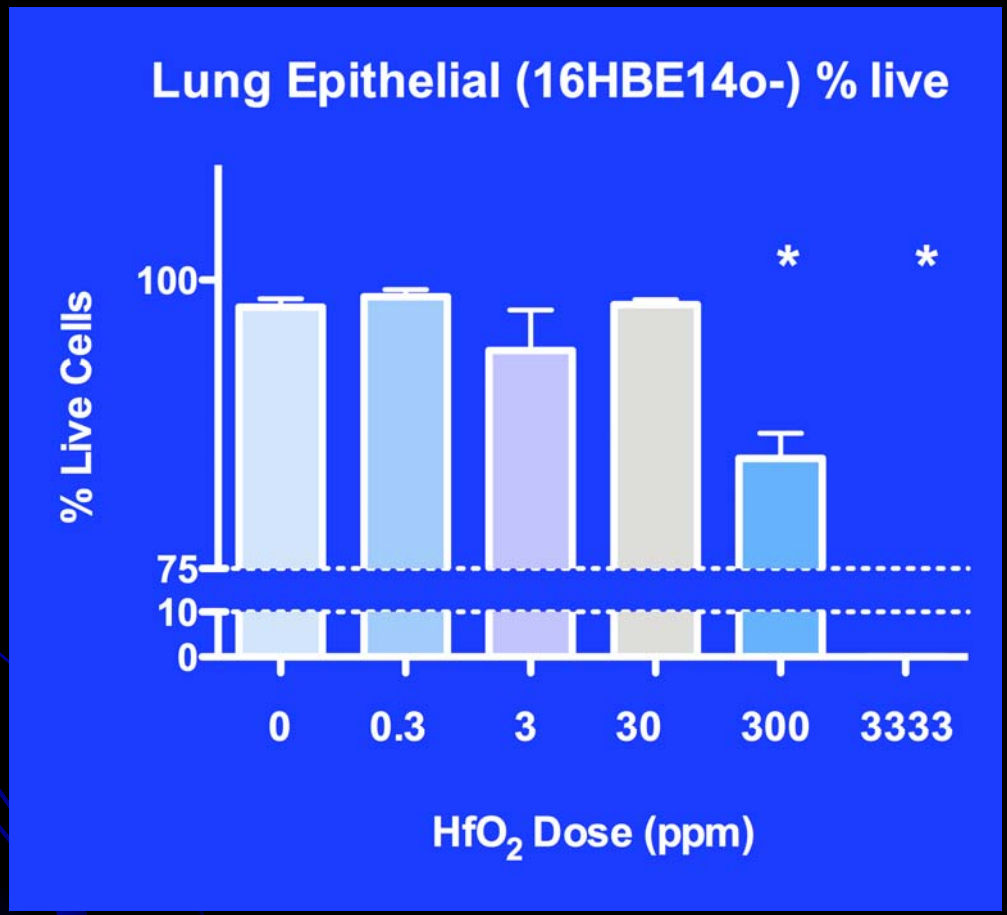



Micron-$HfO_2$



# Live Dead Result
## (Lung epithelium cells, 16HBE14o-)

Nano-$HfO_2$ (batch 1, “20 nm”)



# Dead-Live Assays

| | | Inhibitory Concentrations (mg/L) IC25 | IC100 |
|---|---|---|---|
| **Ref. micron** | skin cells | NT | NT |
| **Batch 1** | skin cells | 1,000 | 3,333 |
| | lung e. cells | > 300 | 3,333 |
| **Batch 2** | skin cells | NT* | NT* |
| **Batch 3** | skin cells | NA | NA |

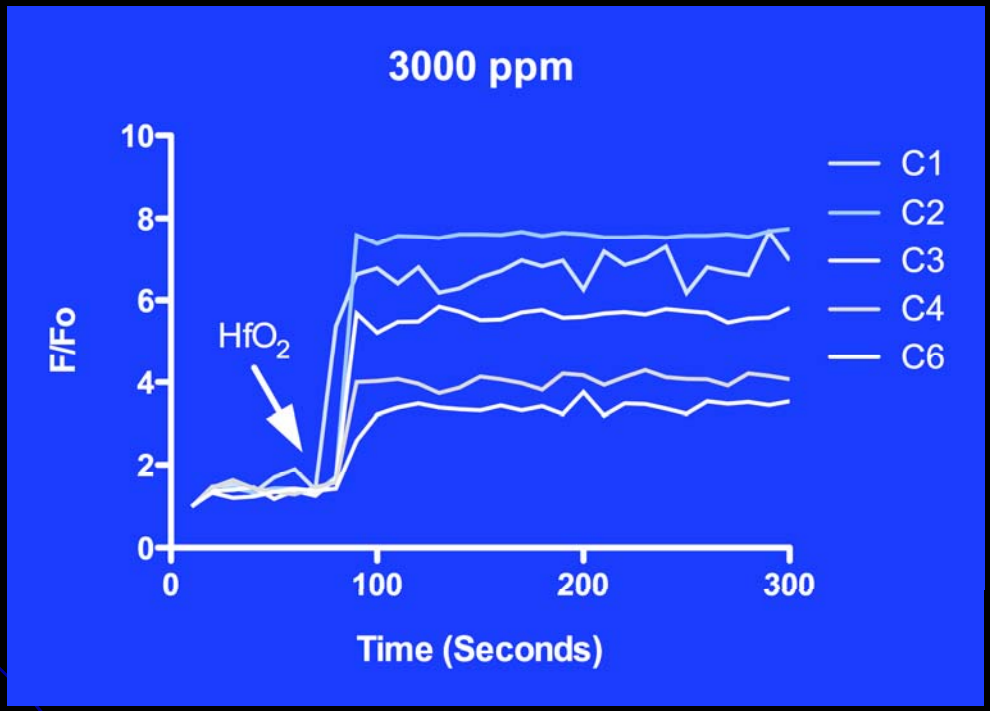
NT = not toxic (~ 20% inhibition at 5,000 mg/L)

NT* = not toxic (~ 10% inhibition at 2,000 mg/L)

- Micron-sized $HfO_2$ shows low toxicity
- Toxicity of nano-sized $HfO_2$ varies depending on the batch, no correlation with particle size

# ROS Dye (HaCat)

Nano-$HfO_2$ (Batch 1 “20 nm”)

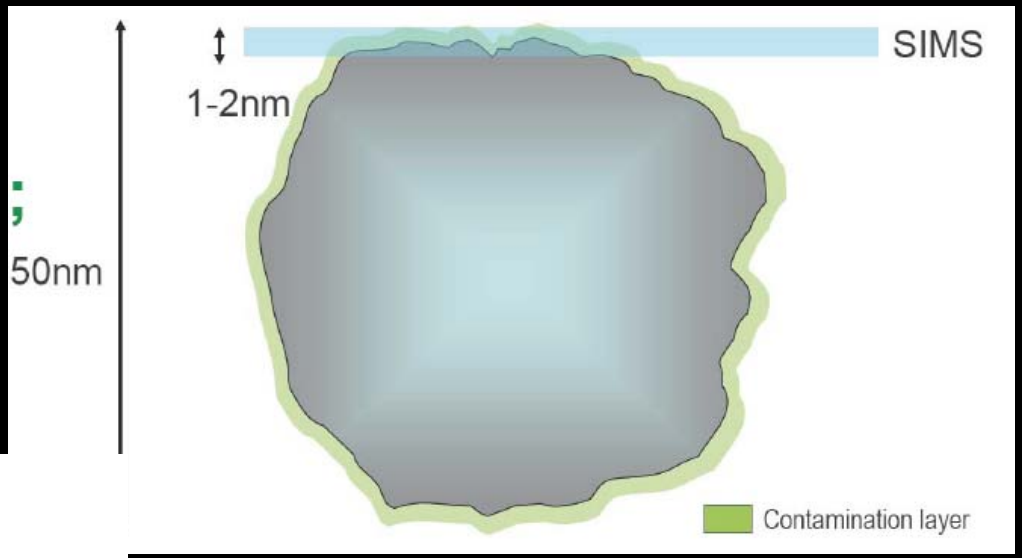


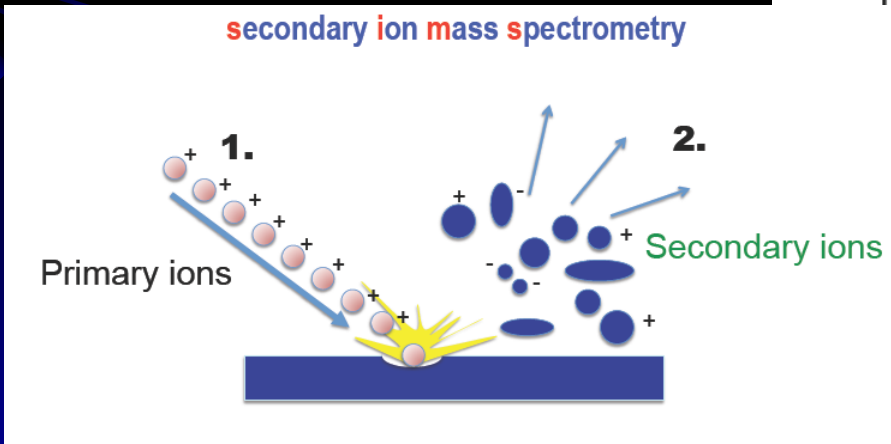

- $HfO_2$ nanoparticles (Batch 1) elicit the formation of reactive oxygen species (ROS) in lung epithelium cells (Hacat)

# Surface Chemistry –
## Secondary Ion Mass Spectrometry (SIMS)

**SIMS looks at a 1 nm surface zone**

**Very high sensitivity!!**

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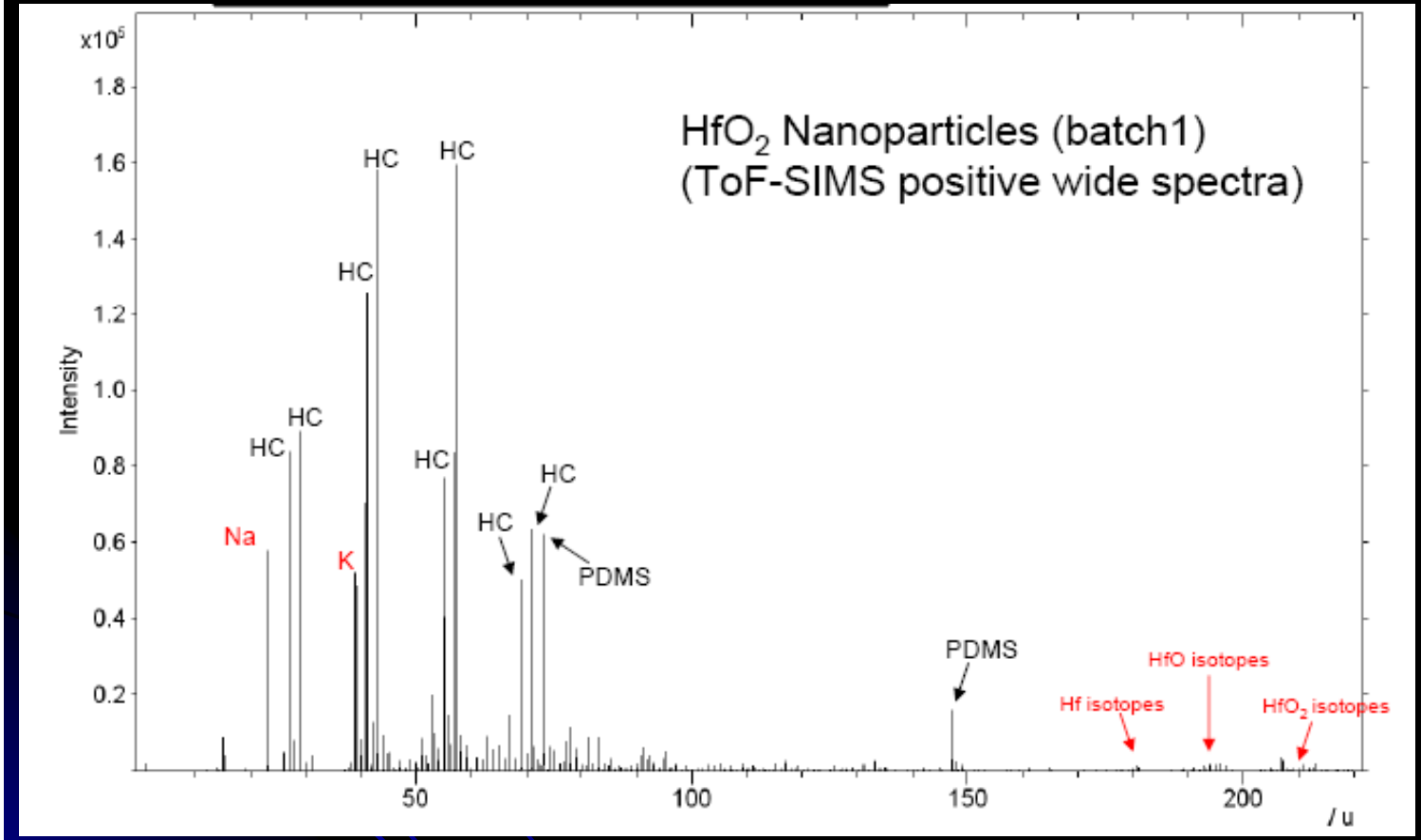
# Surface Characterization by ToF SIMS




HC = hydrocarbon peak

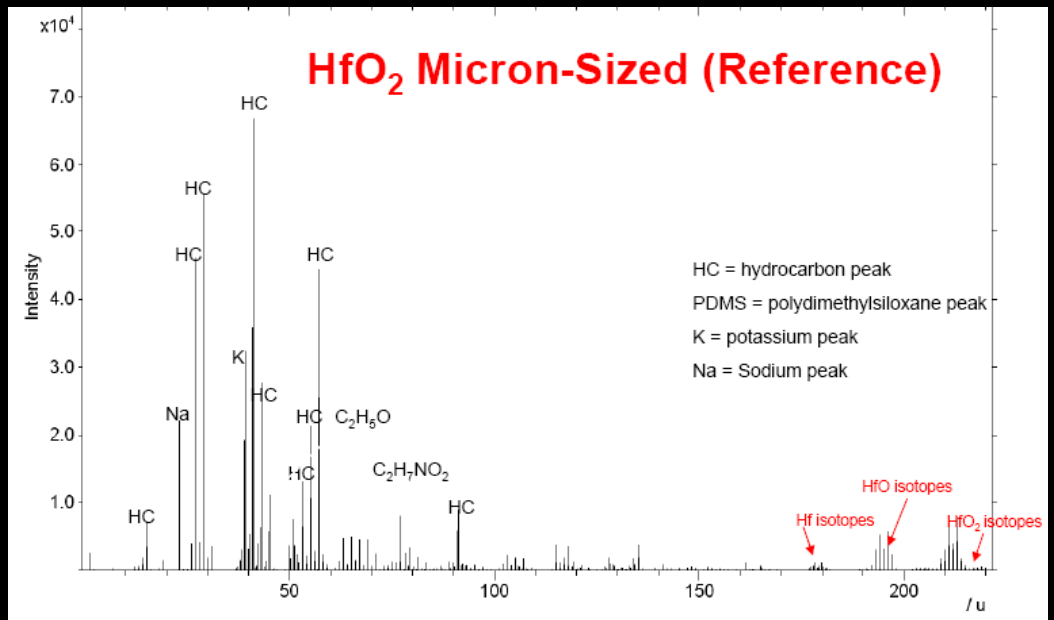
PDMS = polydimethylsiloxane peak

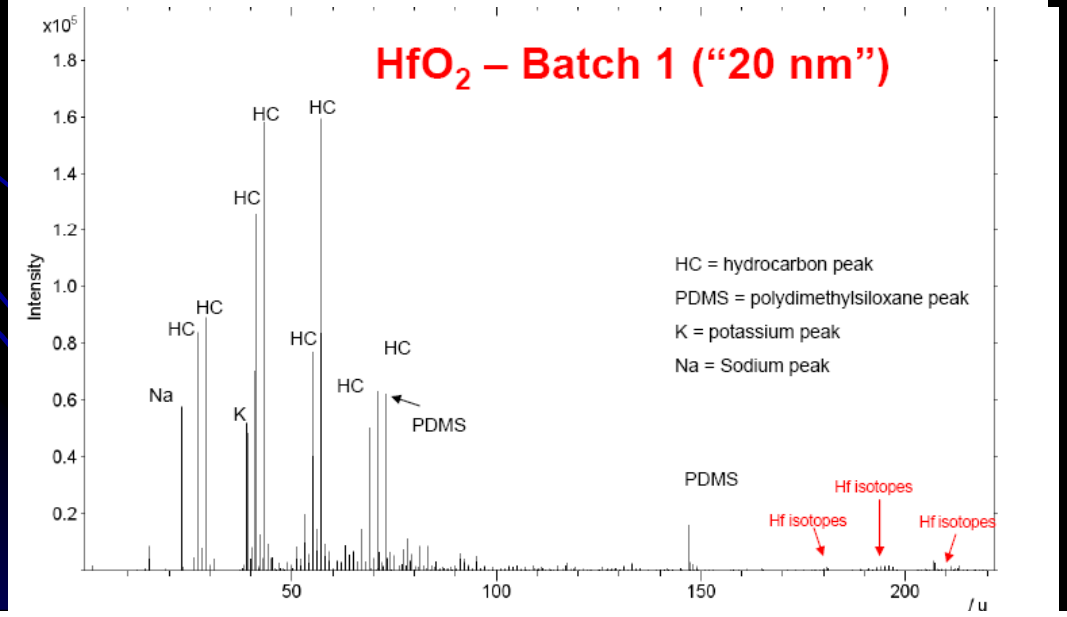
K = potassium peak

Na = Sodium peak

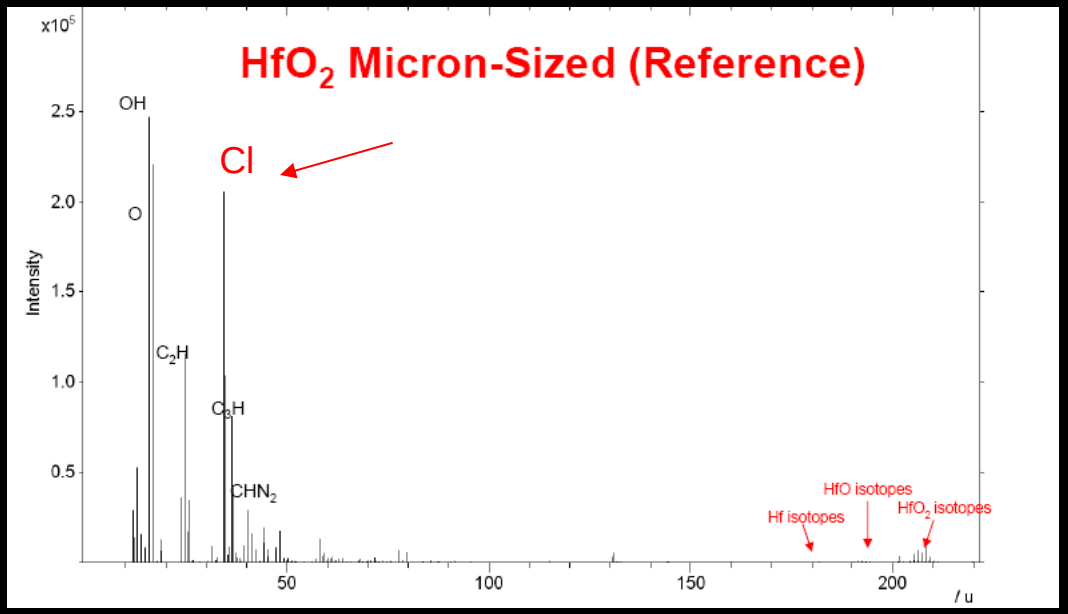
- Various organic/inorganic contaminants detected on the surface of $HfO_2$ NPs
- The nature of the impurities varied depending on the source of the NPs

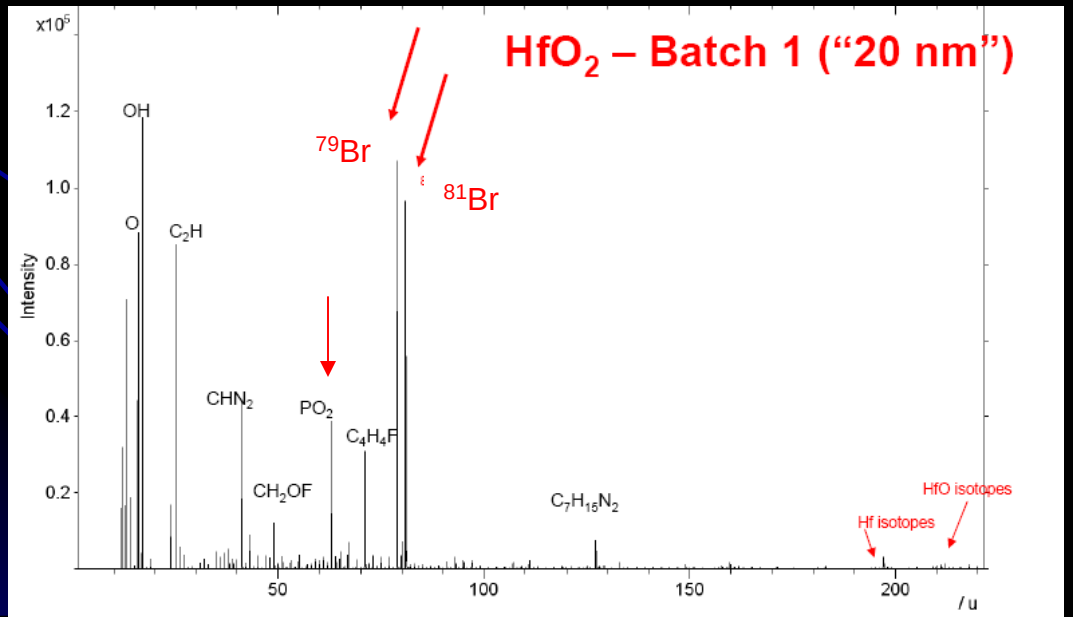
# Surface Characterization by ToF SIMS (positive spectra)



# Surface Characterization by ToF SIMS (negative spectra)



# Surface Characterization Summary/ Preliminary Conclusions

SIMS Analysis

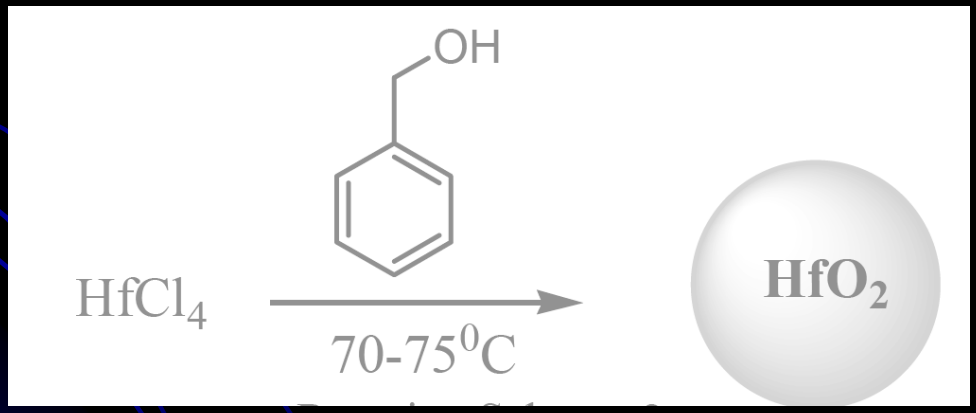
| Impurity | Ref Micro | NP1 20 nm | NP2 1-2 nm |
|---|---|---|---|
| Light Organics (<100 MW) | + | + | + |
| Heavy Organics (>100 MW) | | | + |
| Silicon | + | | + |
| Chlorine | + | + | |
| Bromine | | + | |
| Rare Earth Metals | + | + | + |

- The nature of the impurities varied depending on the source of the NPs

Ratner et al.

# Synthesis of $HfO_2$ Nanoparticles


TOPO= Tri-n-octylphosphine oxide




Zimmerman et al. 2008. J Photopolym Sci Technol. 21(5):621-629

# Conclusions

- Reference micron-sized $HfO_2$ was not toxic in various assays with microbial and mammalian cells.
- The toxicity of $HfO_2$ nanoparticles varied depending on the batch and cell type used. $HfO_2$ “Batch 1” was moderately toxic in most bioassays.
- The differences observed in the toxicity of $HfO_2$ nanoparticles do not appear to be correlated with particle size, they are most likely related to their specific surface chemistry.