Artifacts in Nanoecotoxicology

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Artifacts

Definition - an incorrect result from a confounded assay or misinterpretation of the results

This definition includes misattribution of the mechanism of toxicity such as the effect being attributed to ENMs when actually the toxicity is from an unidentified chemical in the ENM dispersion or from ENM dissolution into ions.

One important distinction to make is between artifacts and experimental uncertainty. Every step in an experimental procedure has uncertainty which cannot be avoided. In some cases, identification of and corrections for artifacts has been shown to completely change the perceived toxicity of an ENM.

Environmental Science & Technology

Critical Review

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Identification and Avoidance of Potential Artifacts and Misinterpretations in Nanomaterial Ecotoxicity Measurements

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Artifacts can potentially occur at each step of nanoecotoxicology testing

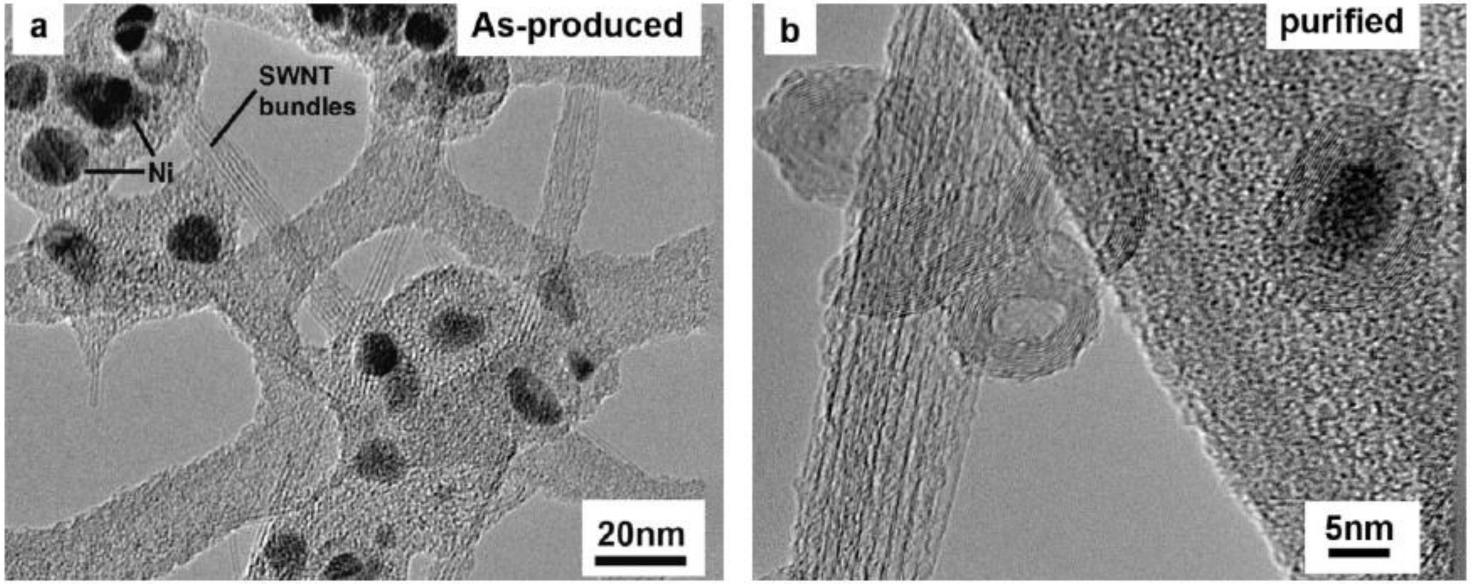
1. Procurement of NPs (impurities, incorrect sizes)
2. Storage (dissolution, release of coatings)
3. Dispersion (ROS from ultrasonication)
4. Measurement of toxic endpoints (interaction with test reagents)
5. Characterization in tissues (misidentification using TEM)

Procurement of NPs

	Vendor Information		Independently Measured	
	Outer diameter (nm)	Length (µm)	Outer diameter (nm)	Length (µm)
MWCNT-a	30 to 50	0.5 to 2.0	27.9 (8.6)	0.386 (0.264)
MWCNT-b	30 to 50	10 to 20	35.8 (10.6)	0.565 (0.364)
MWCNT-c	< 8	0.5 to 2.0	9.5 (2.4)	0.236 (0.126)
MWCNT-d	< 8	10 to 30	11.2 (3.4)	0.255 (0.143)

O'Carroll, D. M.; Liu, X.; Mattison, N. T.; Petersen, E. J., *J. Coll. Interf. Sci*. **2013,** *390*, 96-104.

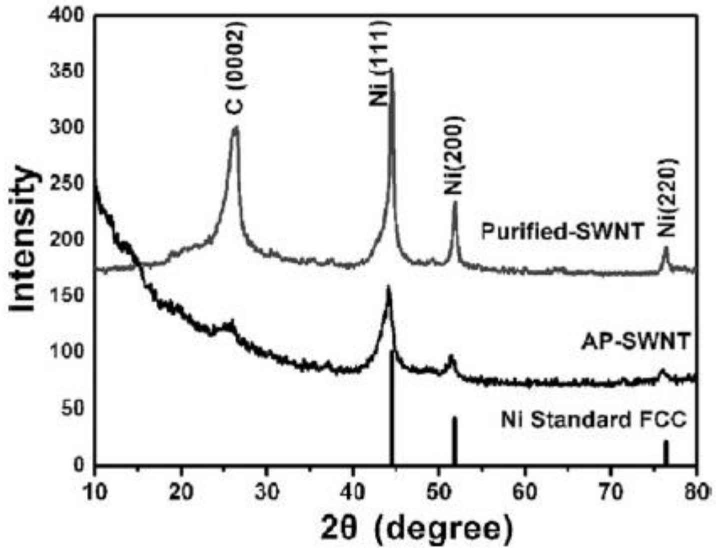
Procurement of NPs



c

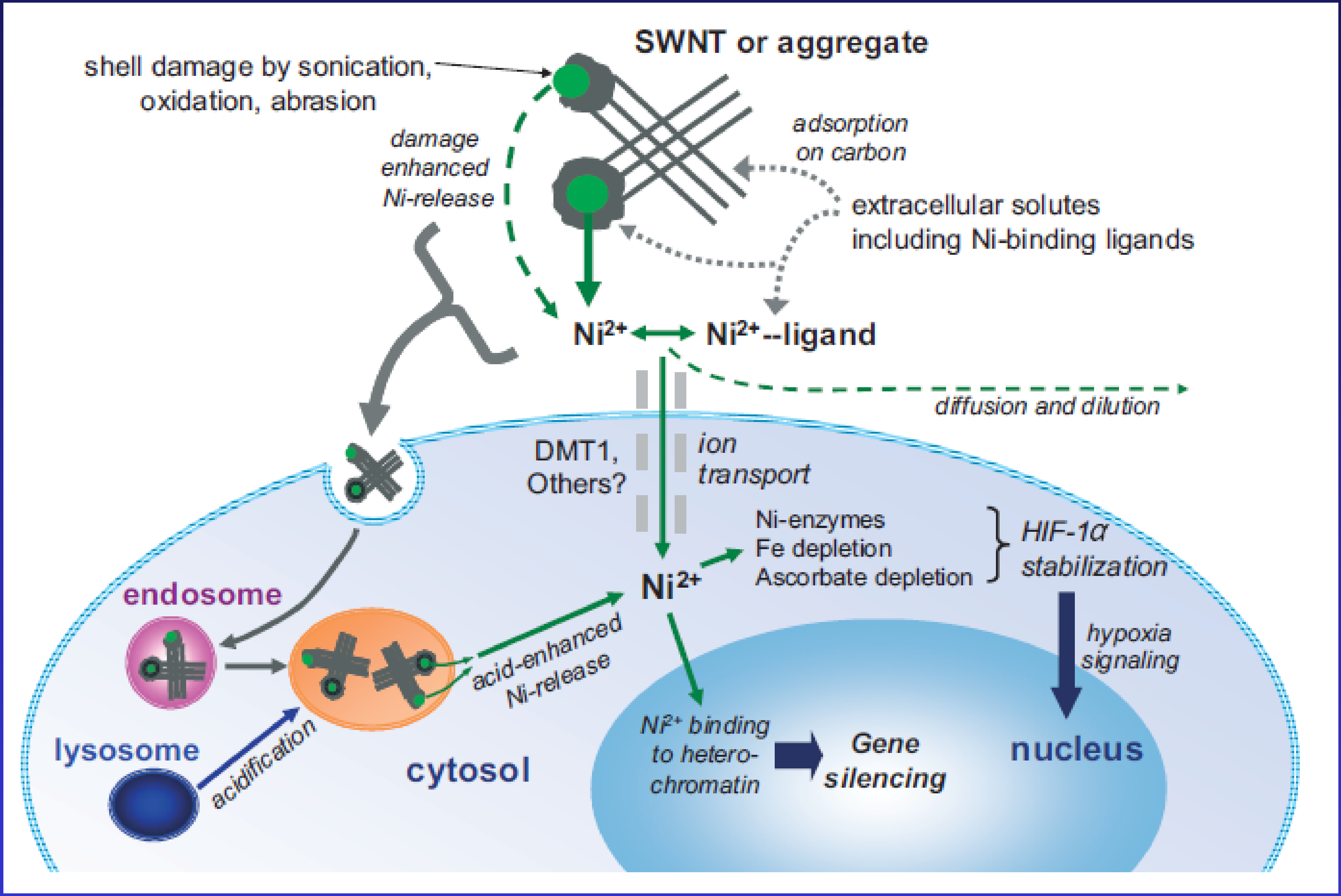
Vendor	SWNT product description	Catalyst (wt%)
A	As-produced (AP)	Ni 19.4% Y 5.49%
	Purified	Ni 14.3% Y 2.09%
B	As-produced	Ni 3.15% Co 9.21%
C	As-produced	Ni 22.8% Y 4.79%
D	As-produced	Ni 24.1% Y 4.17%
	High Purity	Co 3.30% Mo 1.27%

d



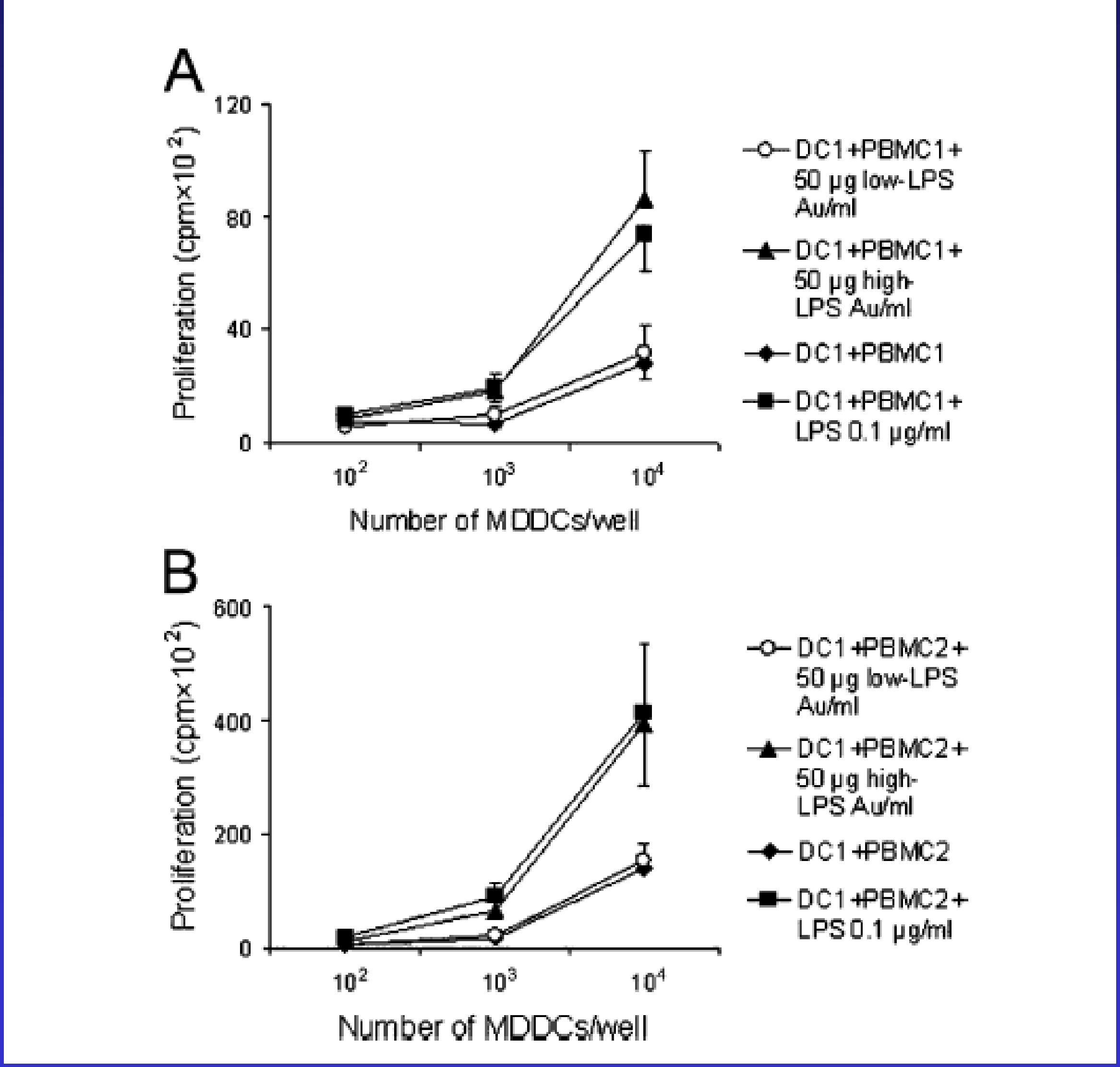
Liu et al., Advanced Materials, 19, 2007, 2790-2796.

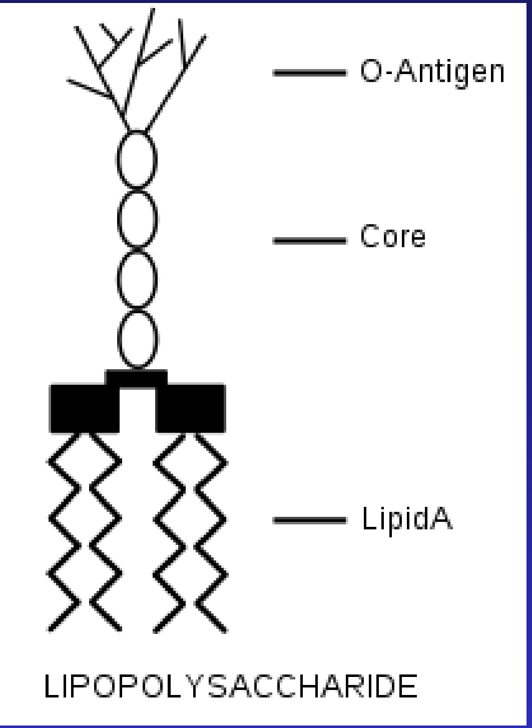
Procurement of NPs



Liu et al., Advanced Materials, 19, 2007, 2790-2796.

Procurement of NPs: Lipopolysaccharides (LPS, endotoxin)

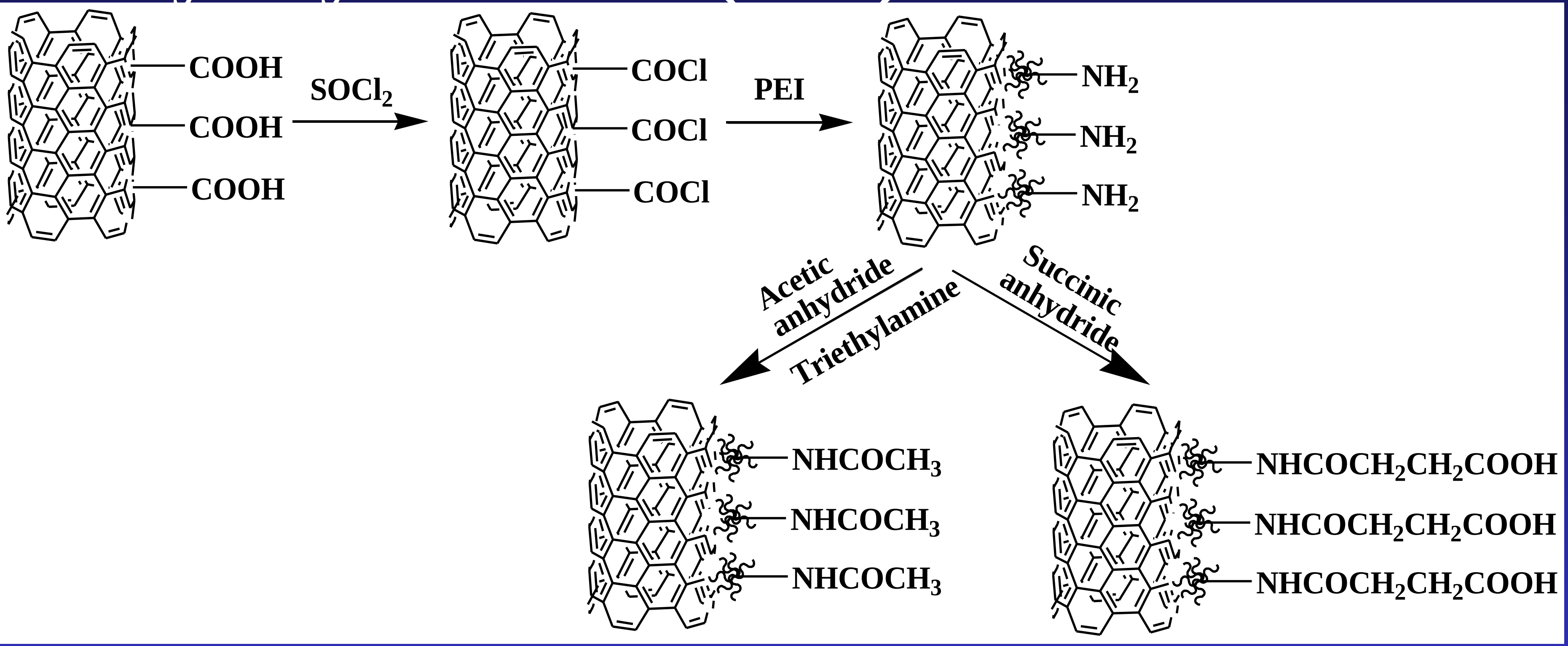




Found in membrane of Gram-negative bacteria

Vallhov et al., Nano Letters, 6(8), 2006, 1682-1686.

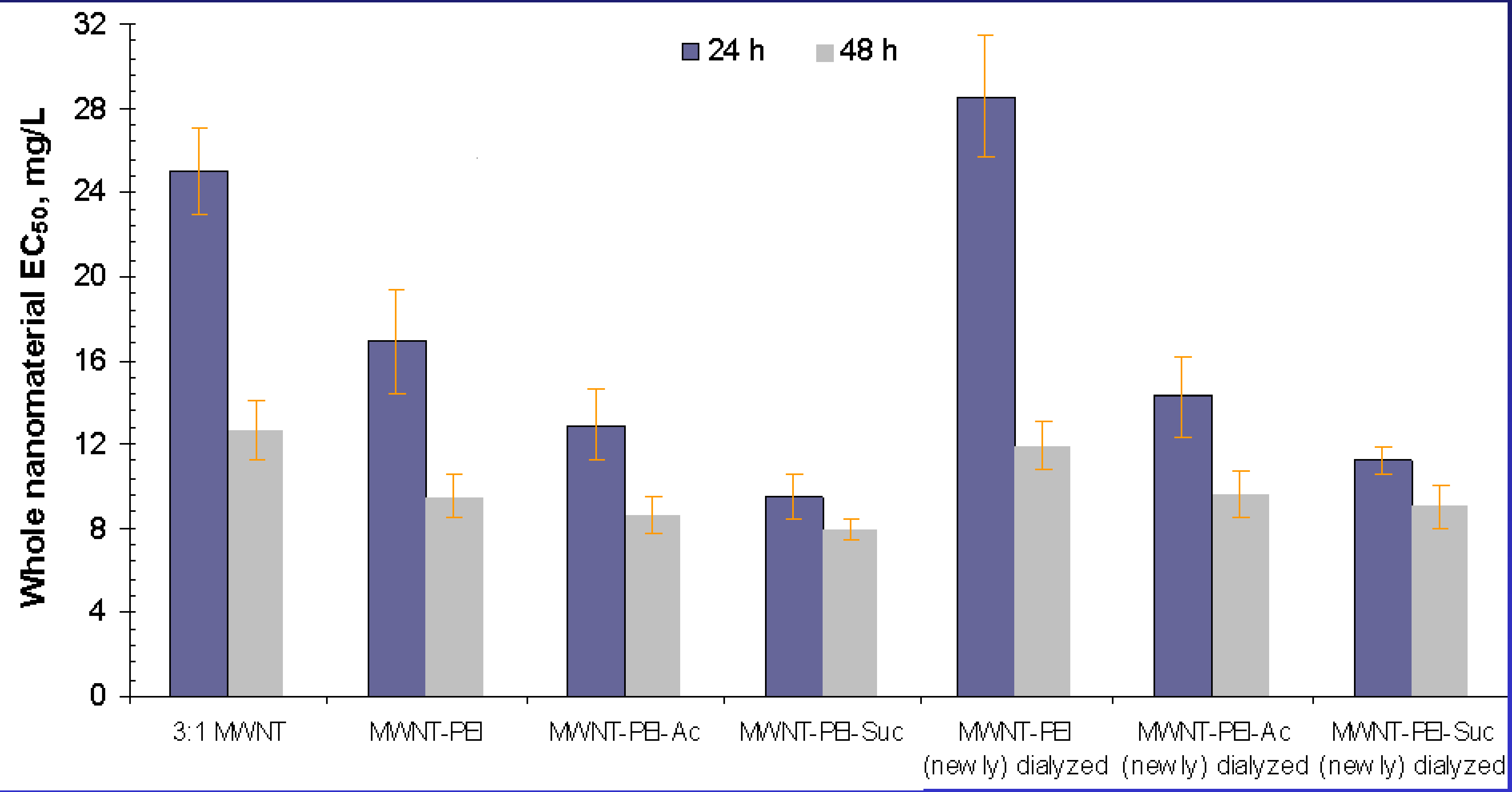
Polyethyleneimine (PEI) Modified MWNTs



Materials	MWCNTs	CNT/PEI	CNT/PEI.Ac	CNT/PEI.SAH
Electrophoretic Mobility (10^{-8} m^2 V^{-1} s^{-1})	-1.71 ± 0.09	1.32 ± 0.09	0.03 ± 0.03	-0.78 ± 0.04

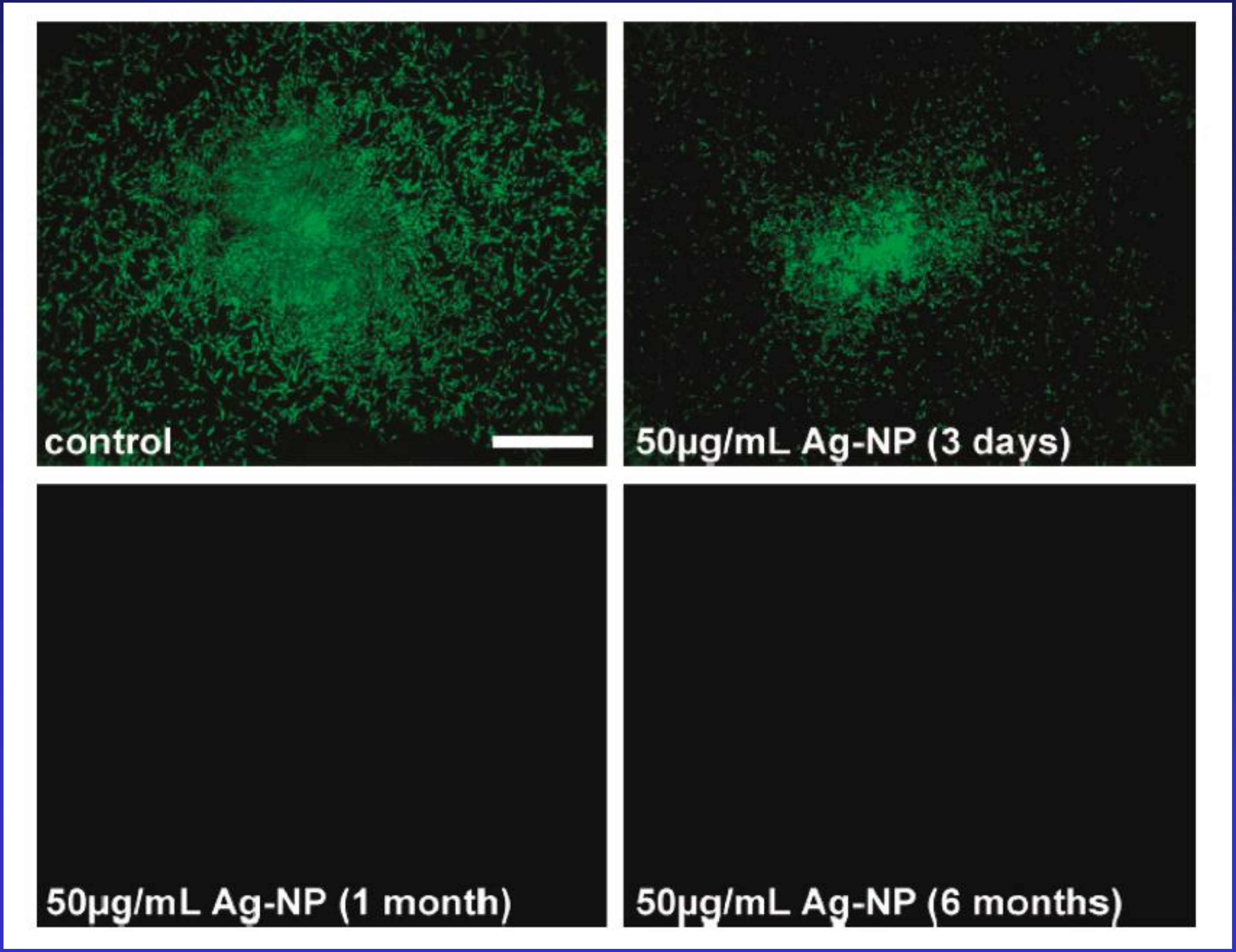
Shen, M., Wang, S.H., Shi X., Huang Q., Petersen, E.J., Pinto R.A., Baker, J.R., Jr., and Weber, W. J., Jr. 2009. *Journal of Physical Chemistry C.* **2009**, 113, (8), 3150-3156.

Storage artifact: Toxicity of PEI MWNTs to *Daphnia Magna*



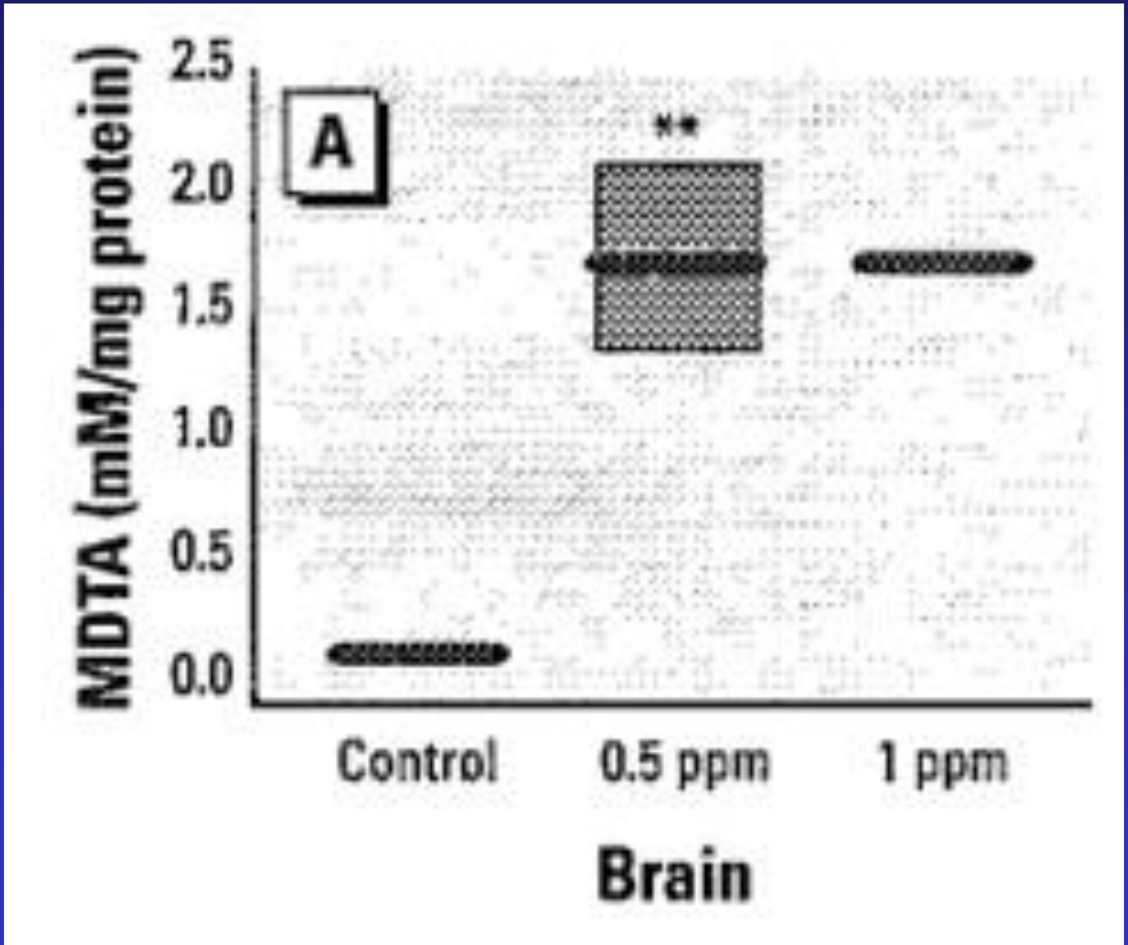
Petersen, E.J., Pinto R.A., Mai, D. J., Landrum, P. F., and Weber, W. J., Jr. 2011. *Environ. Sci. Technol.* **2011**, 45, (3), 1133-1138.

Storage effects: AgNPs and human mesenchymal stem cells



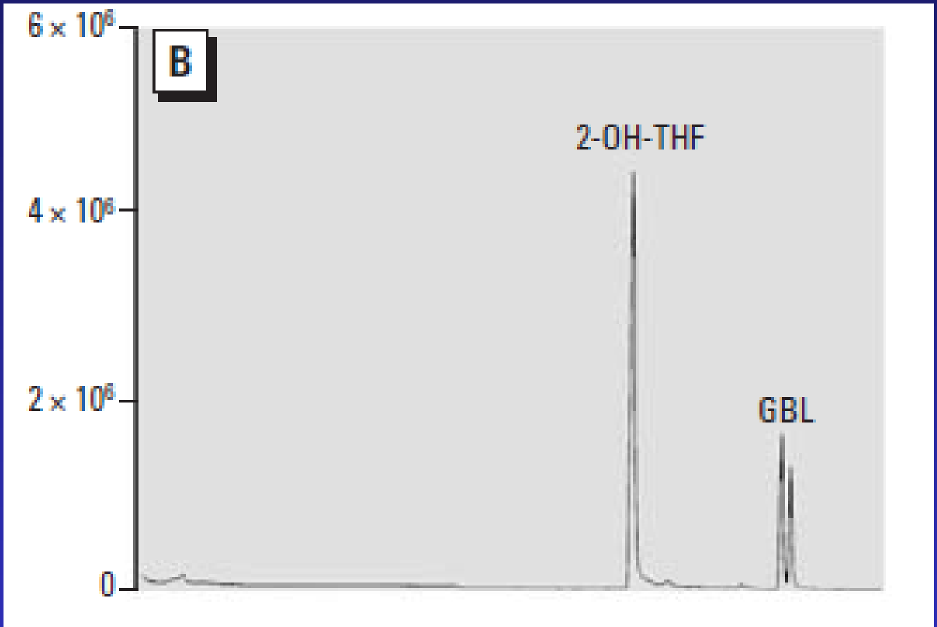
Kittler et al., *Chem. Mater.* 2010, 22, 4548-4554

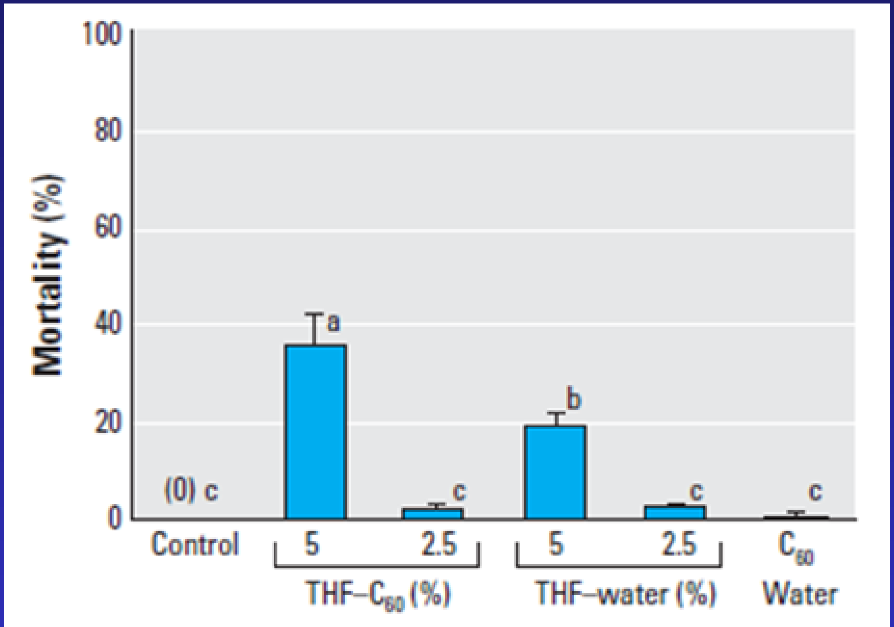
Artifacts: THF dispersion of fullerenes



Oberdorster et al., *Environ Health Persp.* 2004, 112(10), pages 1058-1062.

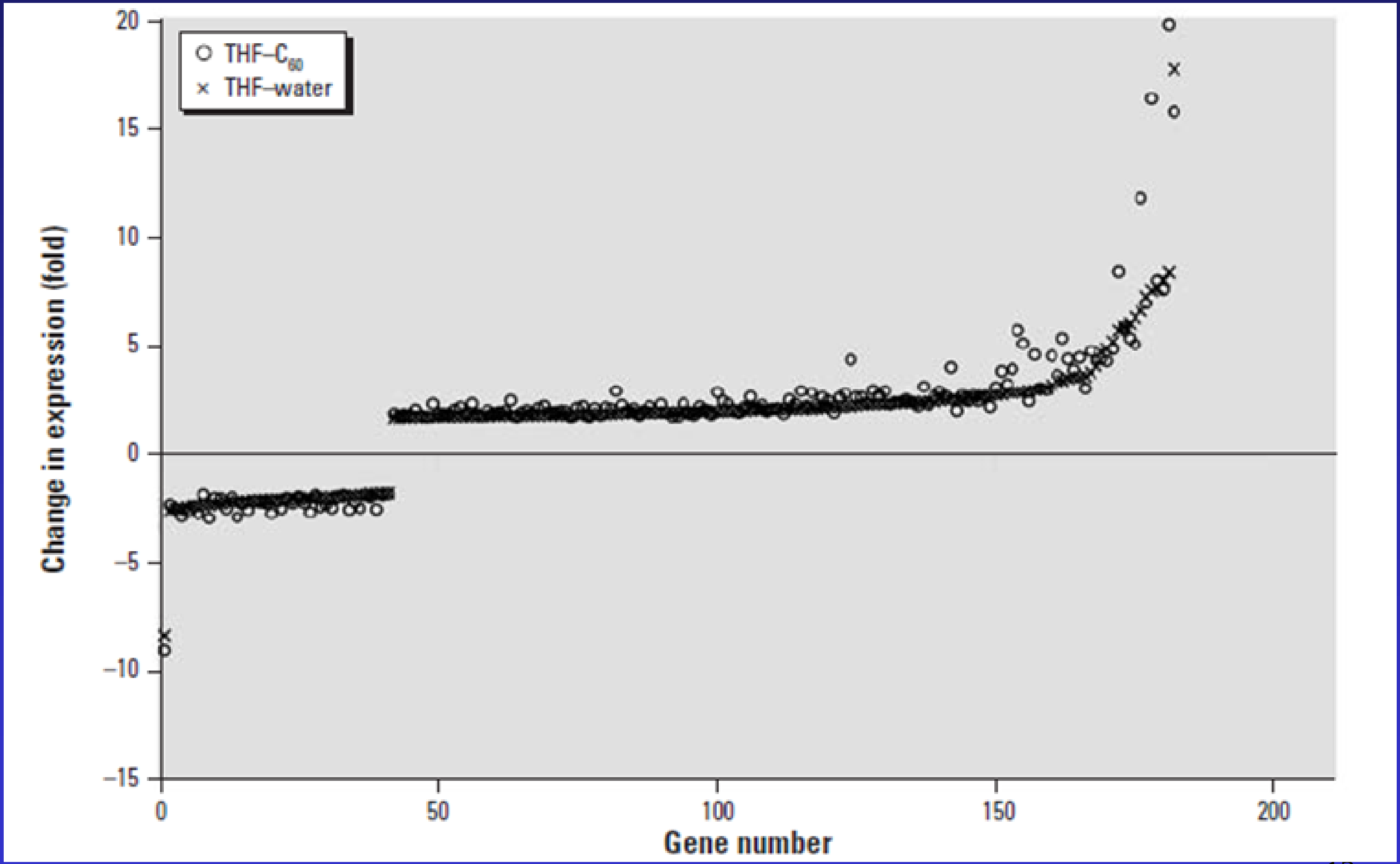
Artifacts: THF dispersion of fullerenes





Henry et al., *Environ Health Persp.* 2007, 115(7), pages 1059-1065.

Dispersion effects: THF dispersion of fullerenes



Henry et al., *Environ Health Persp.* 2007, 115(7), pages 1059-1065.

Dispersion effects: Sonication

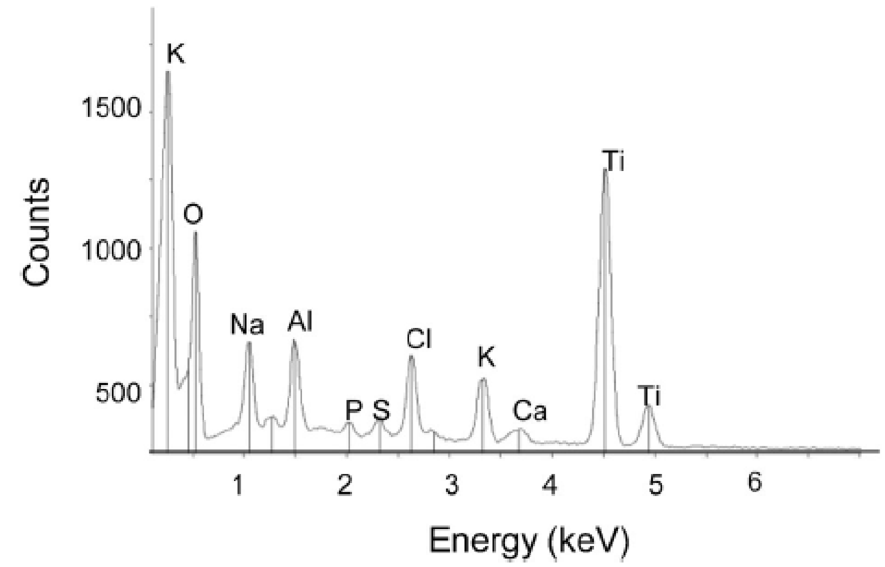


Fig. 1. Silicon drift detector energy dispersive X-ray spectrometry spectra of plant tissue exposed to sonicated TiO_2-nanoparticle suspensions (15 kV beam, 50 Pa, spot size 3, magnification 18,000 ×). The NSS software detected the presence of Al and other metallic elements in a 2-µm-sized Ti-agglomerate (elements other than Al attributable to KCl solution in which Ti is suspended and biologically relevant compounds from tissue).

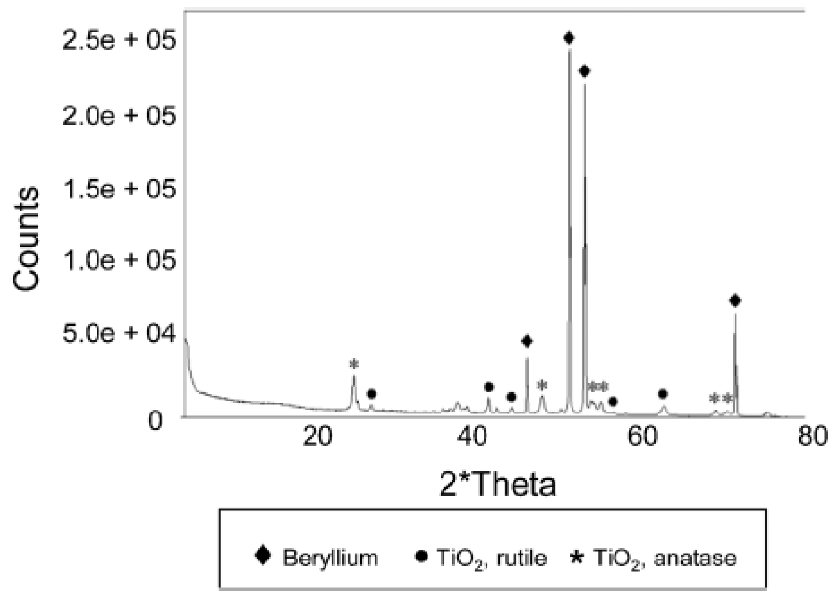


Fig. 2. X-ray diffraction phase pattern of dry TiO_2-nanoparticle powder in Be sample holder; Cu Kα, 20 mA, 2 deg/min scan speed, 5 to 80 deg range, 2Θ measurement axis. The PDXL software estimated 89.9% anatase, 10.1% rutile TiO_2.

Betts et al., Environ Toxicol Chem, 2013, 889-893.

Dispersion effects: Sonication

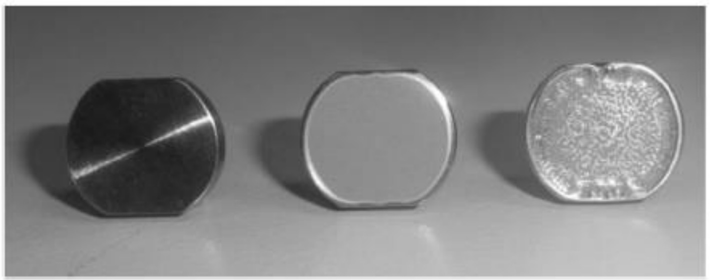


Fig. 3. Images of Ti alloy sonicator probe (1.27 cm, grade 5 Ti alloy) under varying conditions: (left) new, unused probe; (middle) probe after single use, matted finish; (right) aged probe with obvious pitting on surface.

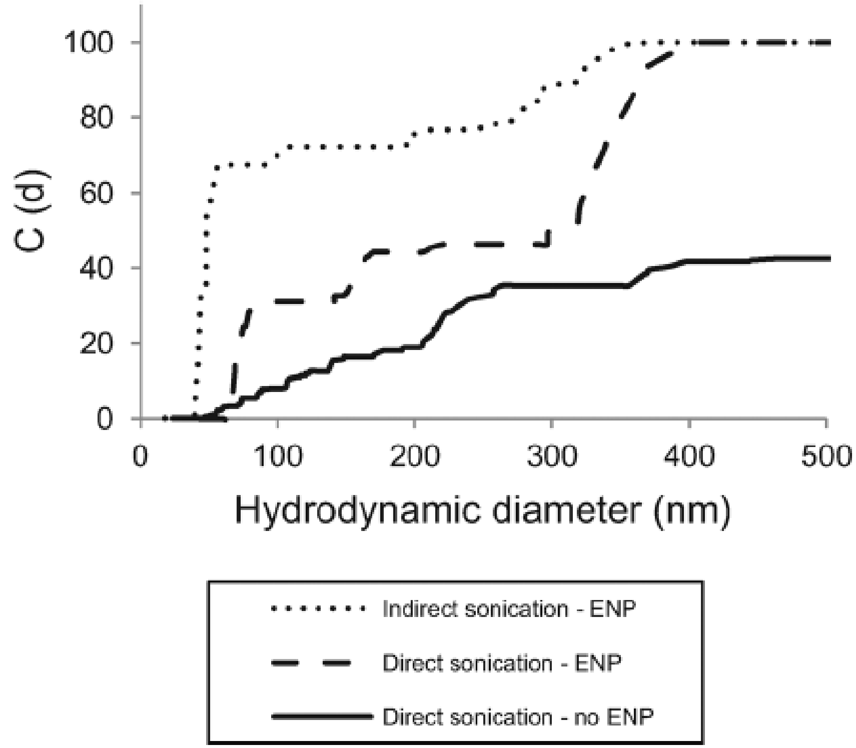
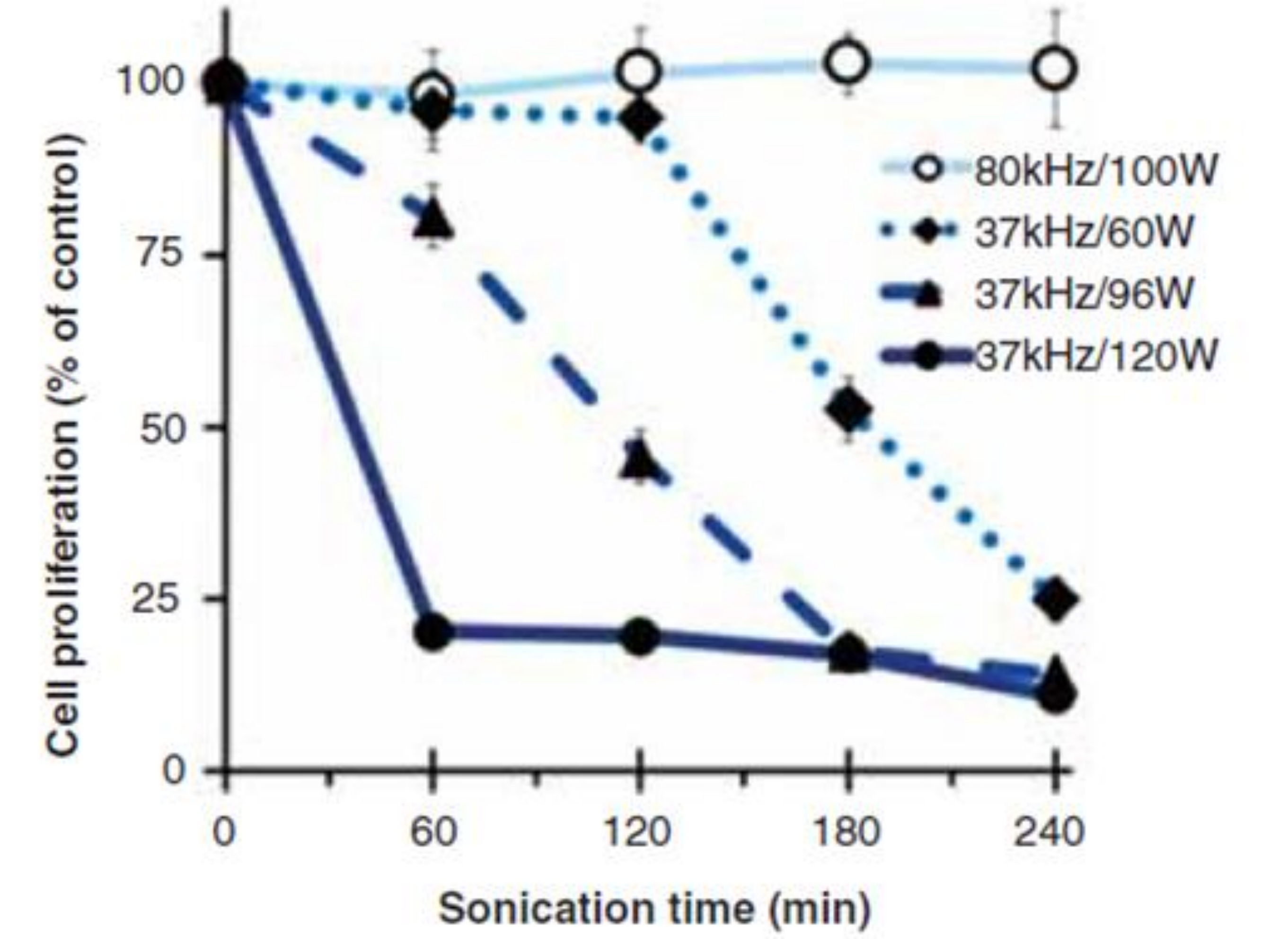


Fig. 4. Cumulative distribution function, C(d), of suspensions with respect to particle size distribution: 50 mg TiO_2/L with indirect sonication ($n = 3$), 50 mg TiO_2/L sonicated with a Ti-alloy probe ($n = 3$), and three consecutive sonications of KCl with a Ti-alloy probe (no ENP, $n = 9$). ENP = engineered nanoparticle.

Betts et al., Environ Toxicol Chem, 2013, 889-893.

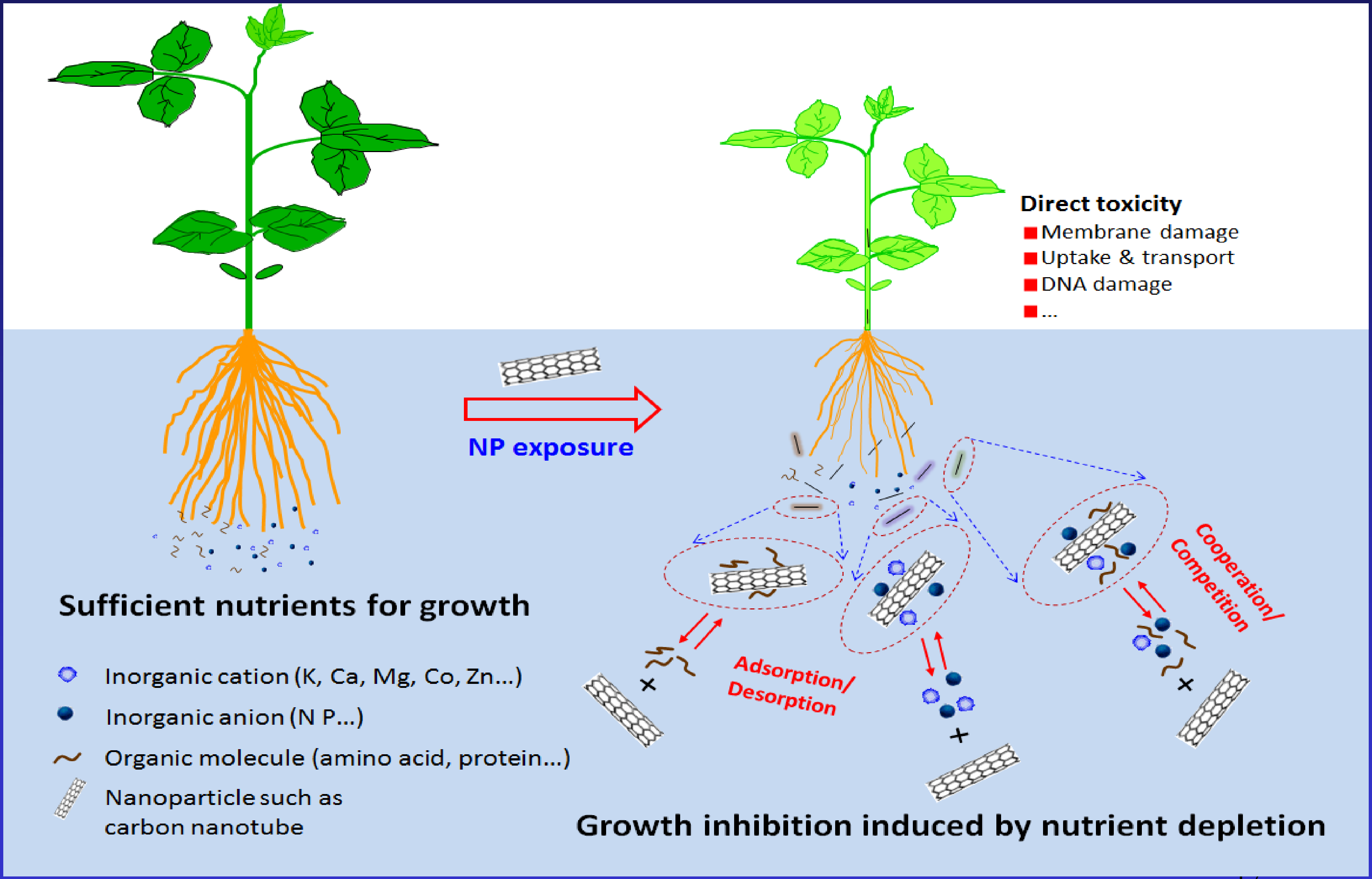
Dispersion effects: Sonication of Pluronic Surfactants Leads to Cytotoxicity



Sonication changed the cytotoxicity of the Pluronic surfactant

Wang et al., Nanotoxicology, 2013, 7(7), 1272-1281.

Indirect Toxic Effects



Petersen et al., 2014, Environ. Sci. Technol. 48 (8), 4226-4246.

Direct Interactions with Assay

NANO LETTERS

2006
Vol. 6, No. 6
1261–1268

Oops They Did It Again! Carbon Nanotubes Hoax Scientists in Viability Assays

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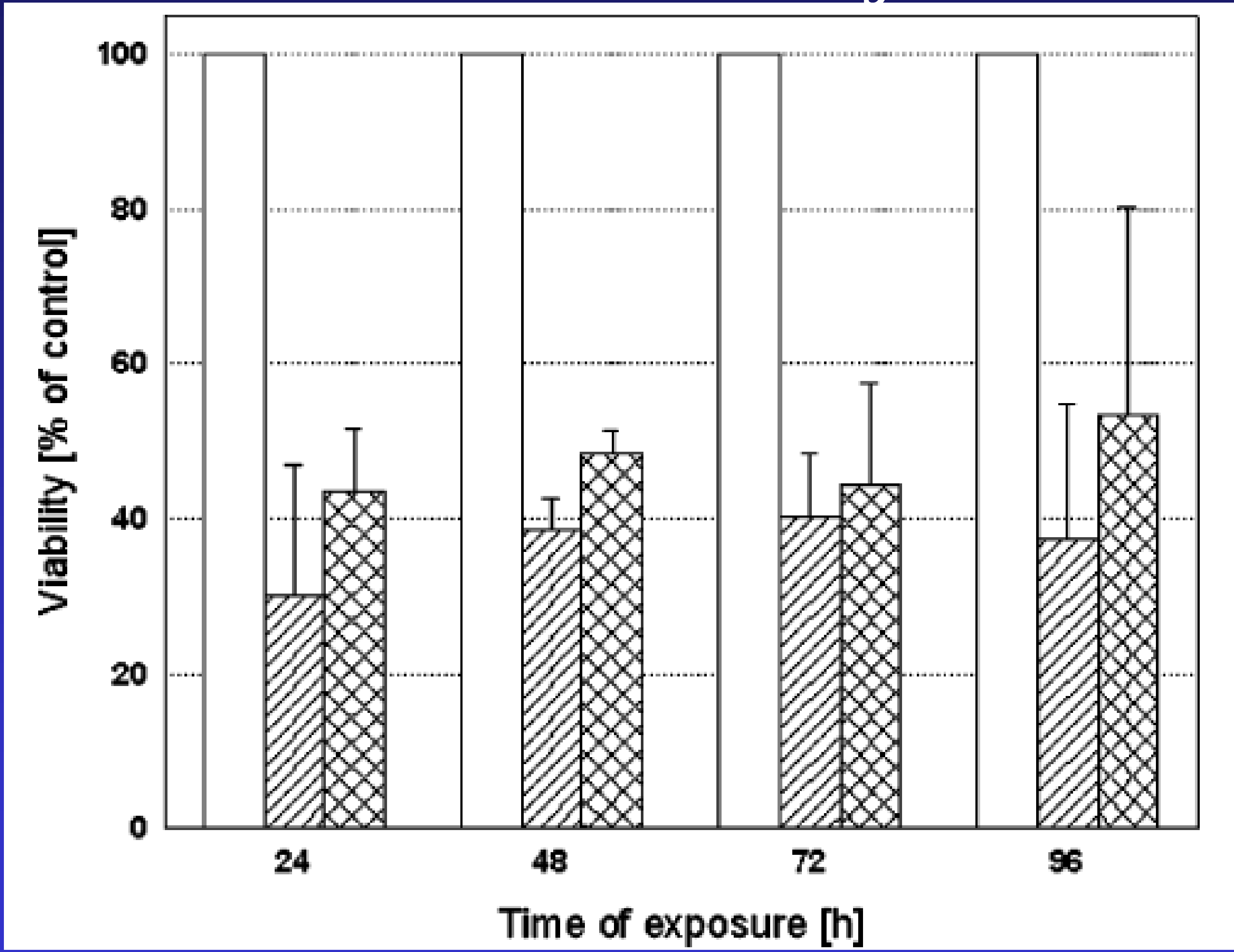
Forschungszentrum Karlsruhe, Institute of Toxicology and Genetics, Department of Molecular and Environmental Toxicology, P.O. Box 3640, D-76021 Karlsruhe, Germany

Received January 25, 2006; Revised Manuscript Received March 29, 2006

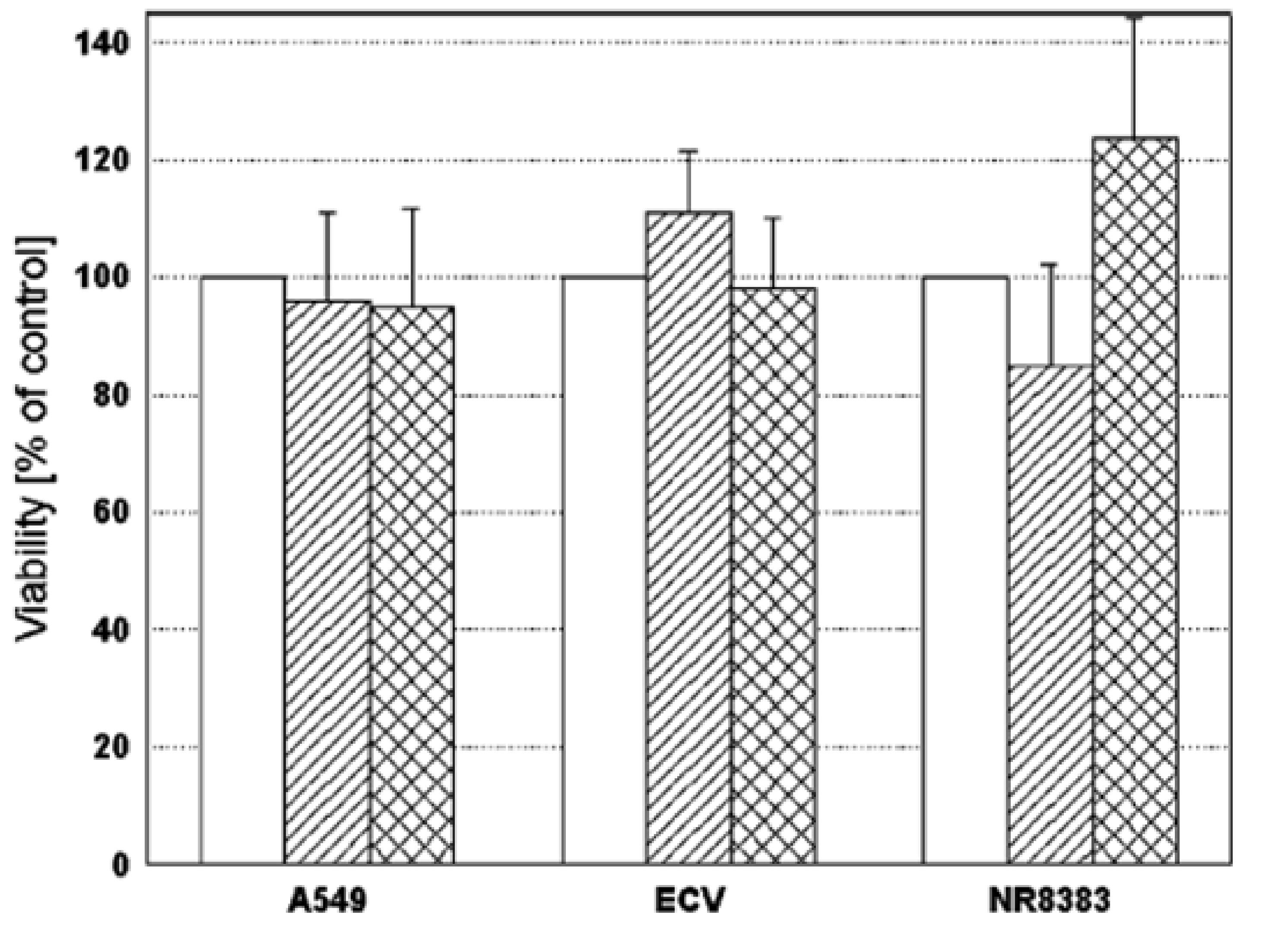
Worle-Knirsch et al, Nano Letters, 2006

Direct Interactions of SWCNTs with MTT Assay



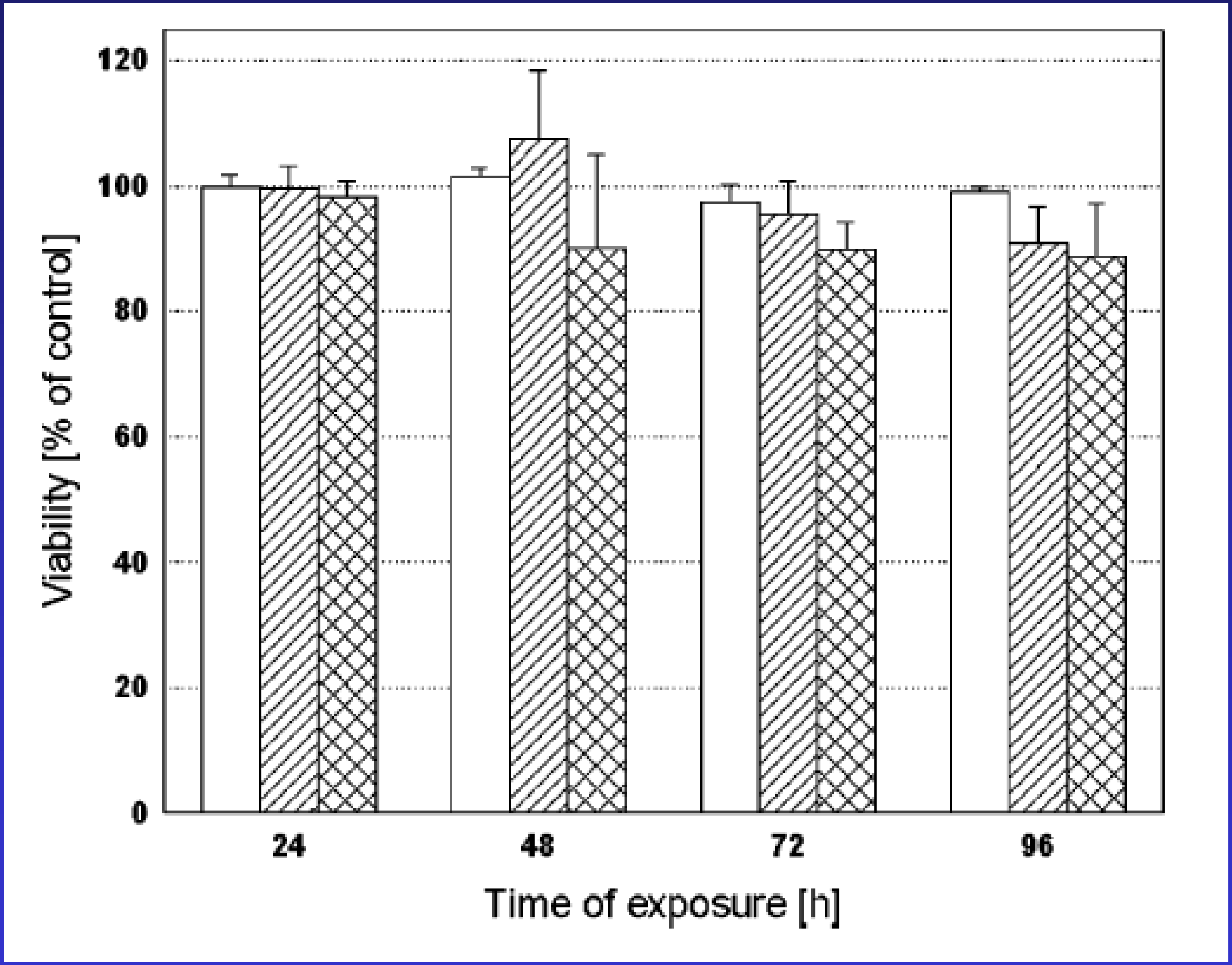
Worle-Knirsch et al, Nano Letters, 2006

Direct Interactions with Assay
WST-1 Assay



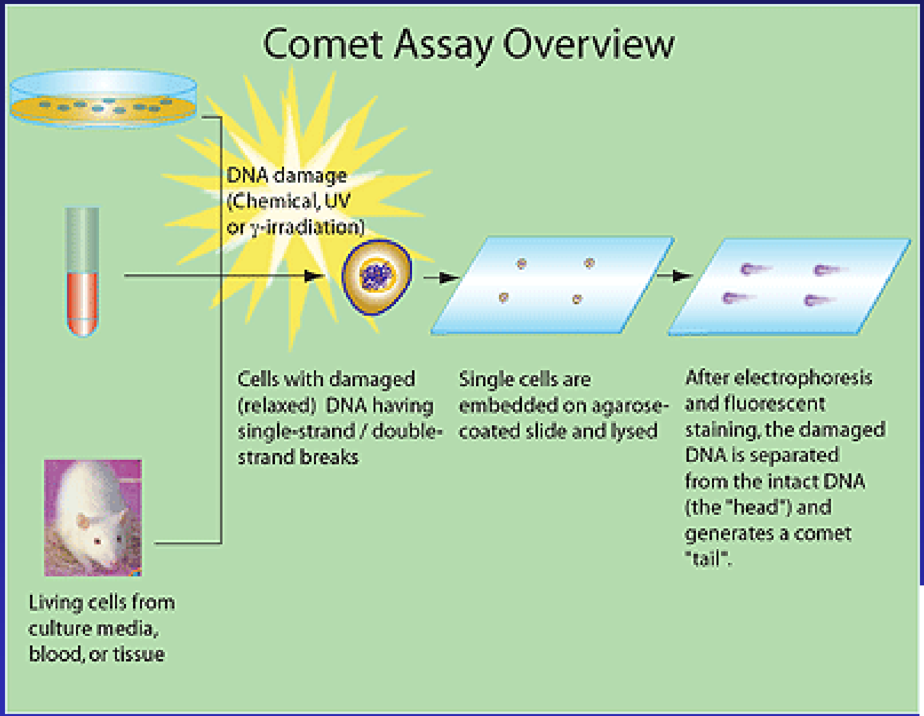
Worle-Knirsch et al, Nano Letters, 2006

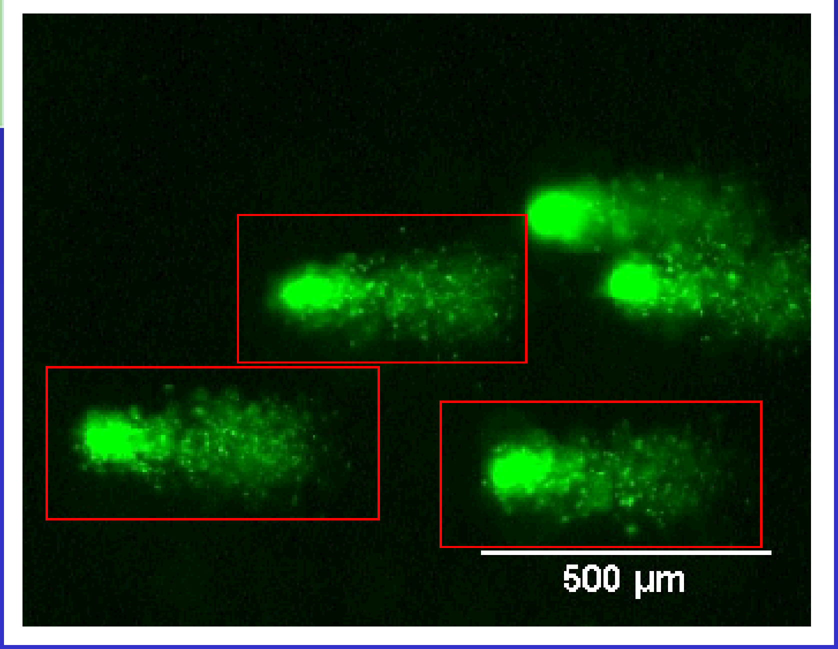
Direct Interactions with Assay
Lactate Dehydrogenase Assay



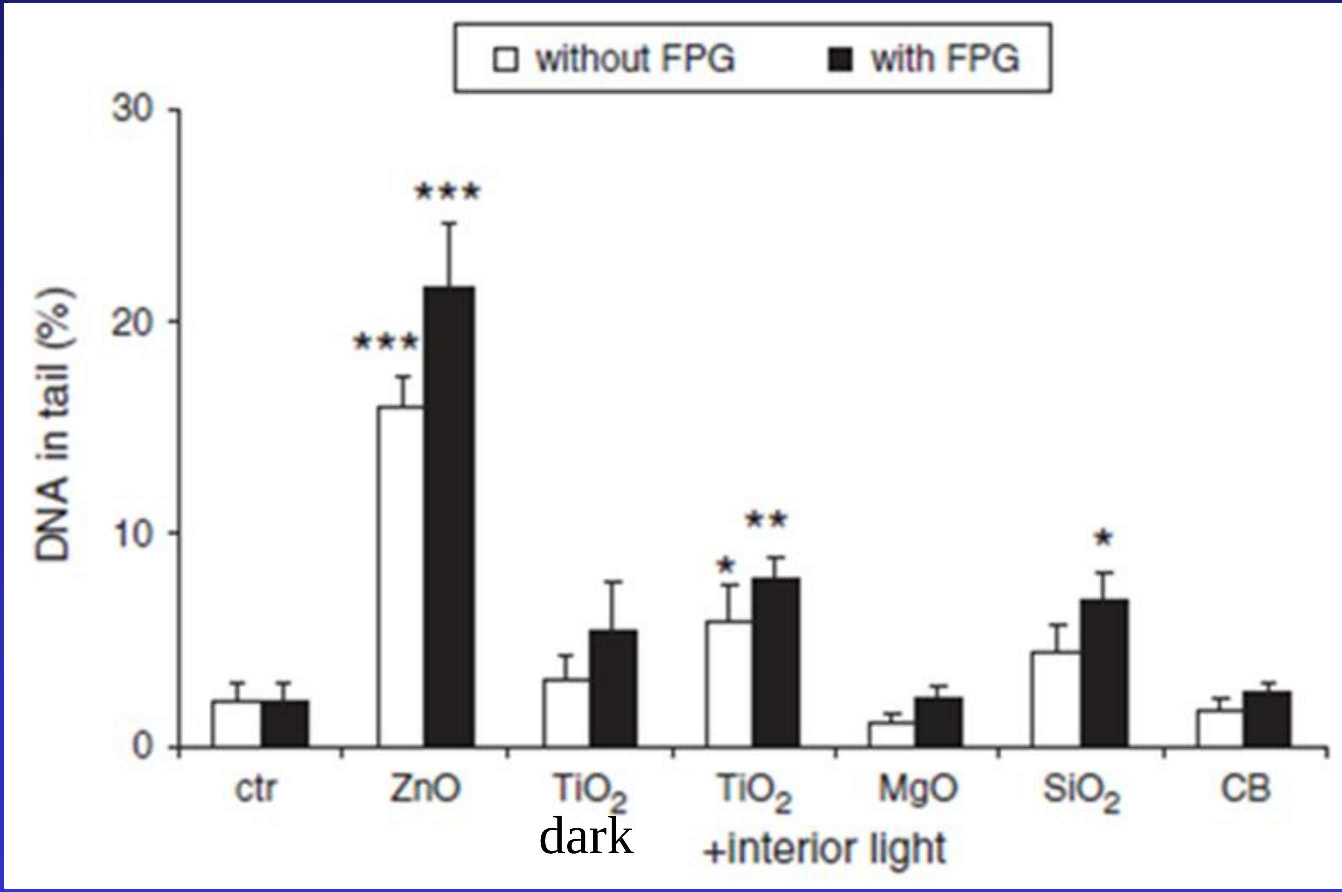
Worle-Knirsch et al, Nano Letters, 2006

Direct Interactions with Assay





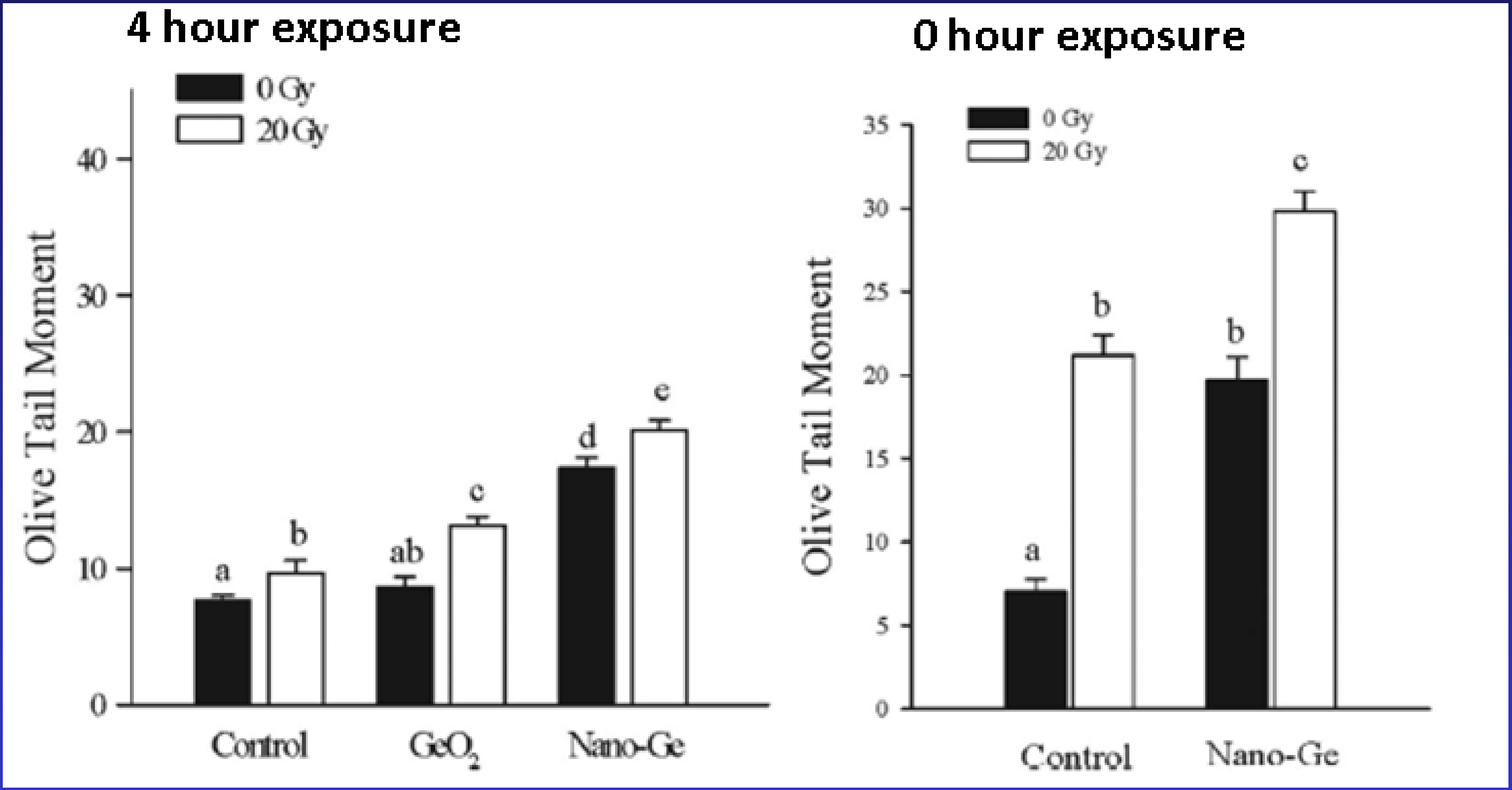
Comet Assay Measurement of TiO2 ENM DNA Damage



Different results observed for TiO2 samples analyzed in dark or with laboratory light

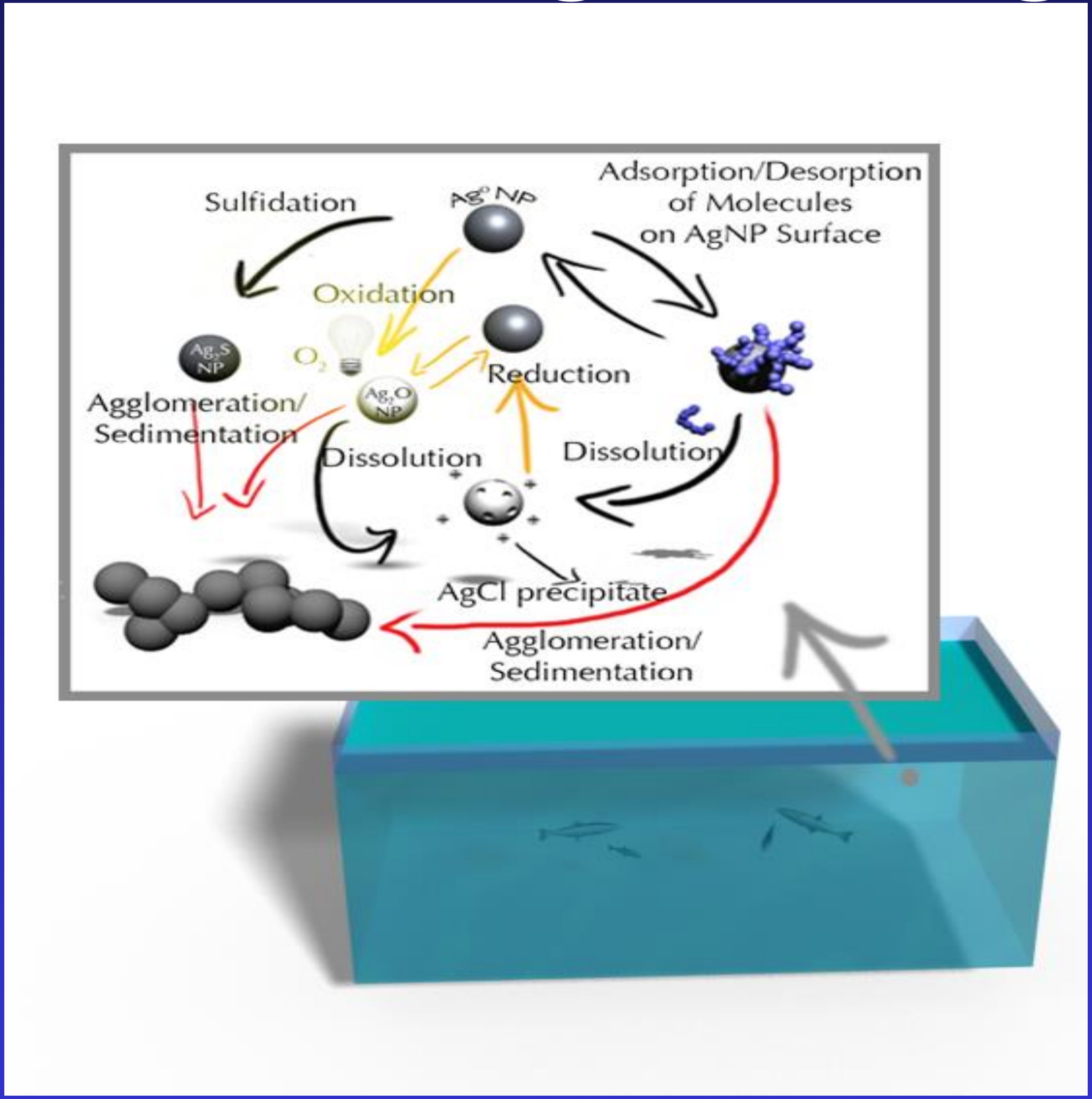
Gerloff et al., Nanotoxicology, 2009

Direct Interactions with Assay



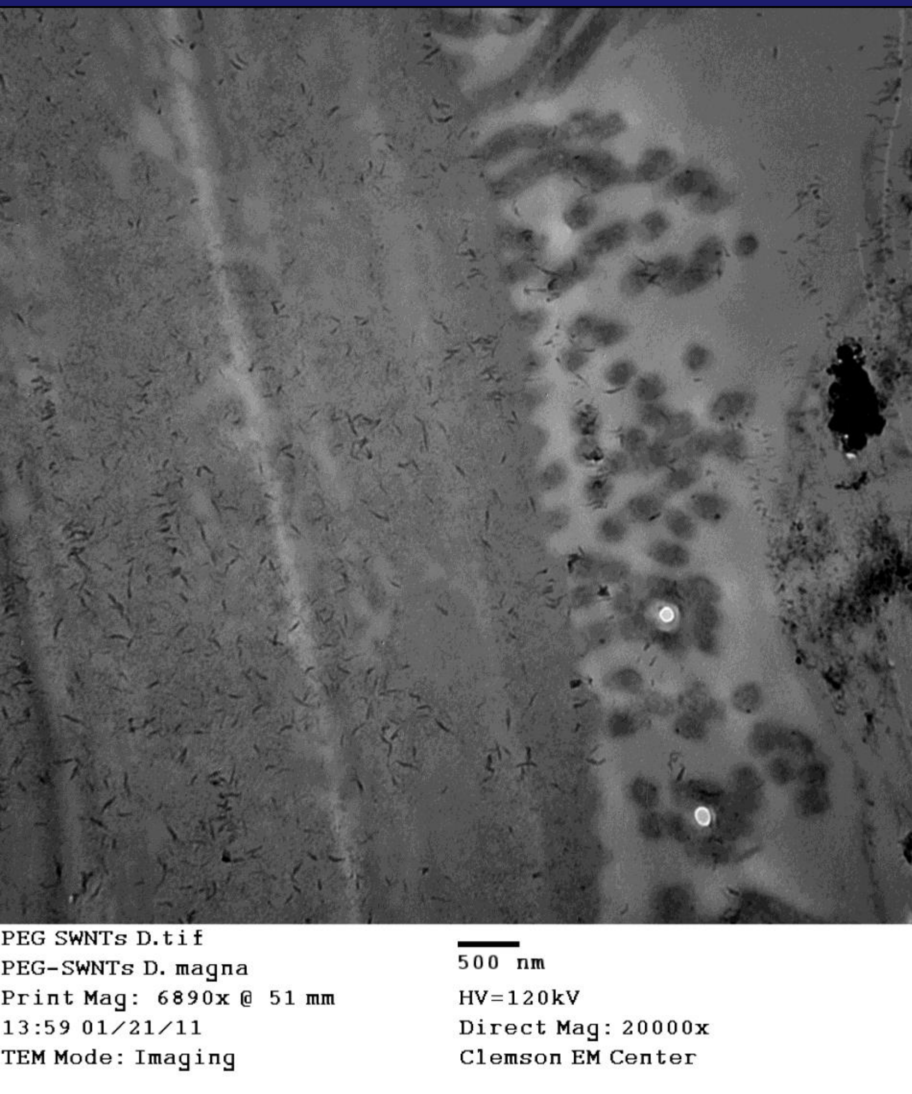
Lin et al., Int. J. Radiat. Biol., 85(3), 2009

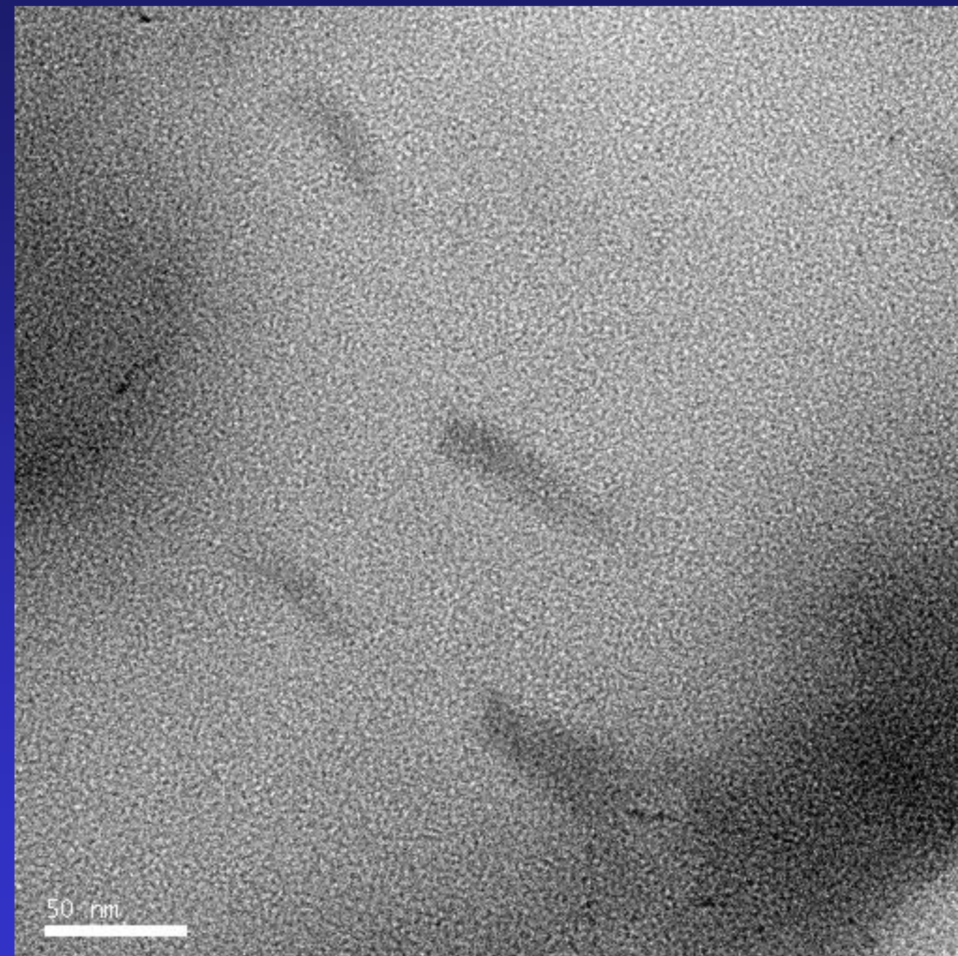
Dynamic Changes During Assay



Petersen et al., 2014, Environ. Sci. Technol. 48 (8), 4226-4246.

Characterization artifact: Single-walled carbon nanotubes in *Daphnia magna*





Potential Control Experiments

Potential control experiments	Purpose(s)
0 h control	Test if ENMs causes a toxicological effect (e.g., DNA damage) during processing steps after conclusion of exposure period
	Test if ENMs would interact with test reagents or biomolecules and cause a false negative or false positive result
Coating control	Test if coating has toxicological or stimulatory effects on organisms or cells
Direct interference control (production of a signal similar to measurand)	Assess if ENMs produce a signal (e.g., absorbance, fluorescence) that could impact the analytical method
Dispersant control	Test if dispersant has toxicological or stimulatory effects on organisms or cells

Potential Control Experiments

Potential control experiments	Purpose(s)
Dissolved ion control	Allows for comparison of endpoints between ENM and constituent dissolved ions
	Assess if NP formation could occur from ions in test media or in organism or cells
Endotoxin inhibition/enhancement control	Assess if there is an impact of ENMs on the effects of endotoxins on a specific endpoint
Filtrate only control	Assess potential toxicity of contaminants on and dissolution from ENMs from the synthesis, storage, and dispersion processes
Larger/Bulk particle control	Allows for comparison of endpoints with ENMs and if nano-specific effects are observed

Potential Control Experiments

Potential control experiments	Purpose(s)
Mixing control	Assess extent of mixing using inert markers
Nutrient depletion control	Assess extent to which adsorption of media constituents by ENMs could have an indirect toxicity effect on endpoints
Shading control	Assess light intensity reduction caused by ENMs and if that could impact the endpoints being studied
Sonication control with media and organic chemicals/coatings	Investigate possible changes to media constituents or toxicological properties of organic chemicals from sonication
Mixing control	Assess extent of mixing using inert markers