Evaluation de la toxicité potentielle des Nanomatériaux : étude de cas

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April 2015 : 1824 consumer products containing nanomaterials

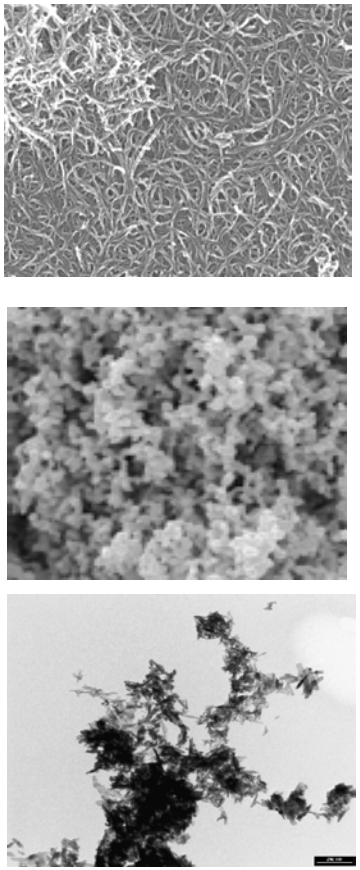
(http://www.nanotechproject.org/inventories/consumer/)

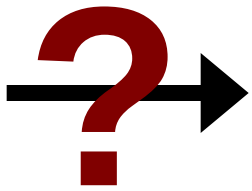
Most used NPs:

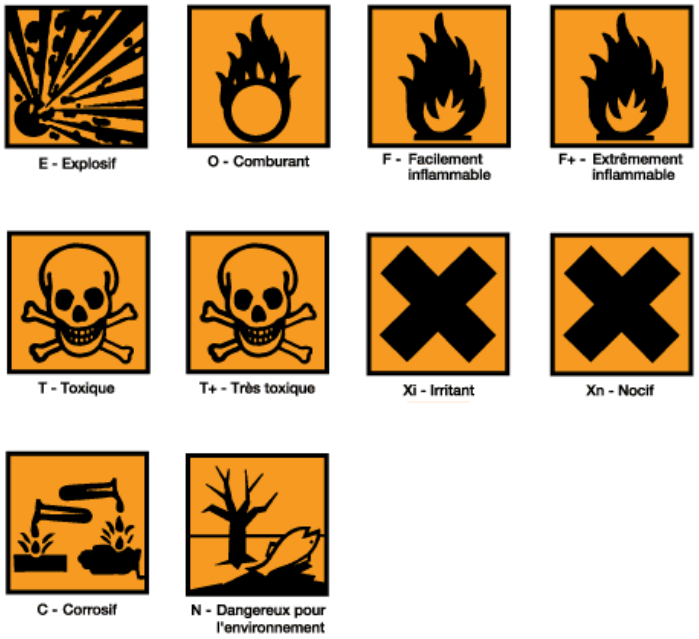
- Ag
- C
- TiO_2
- SiO_2
- ZnO

Nanoparticles

Nanoparticles and toxicity



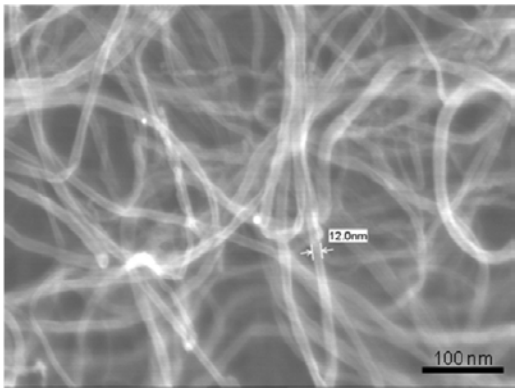




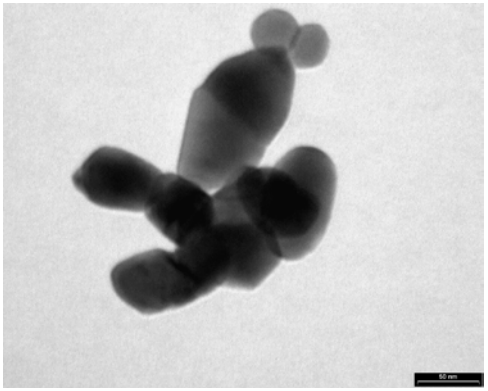
Nanotoxicology: complex discipline

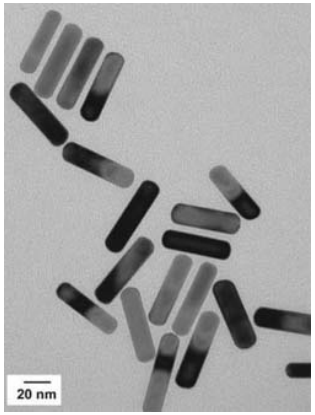
◆ **Great diversity of nanomaterials (chemical composition, size, shape, specific surface area, functionalized or not);**

Carbon nanotubes

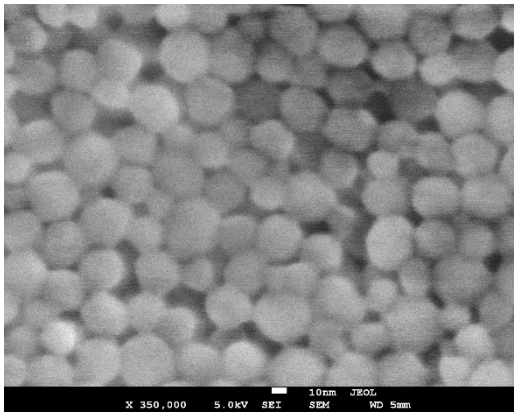


Copper oxide (spherical)

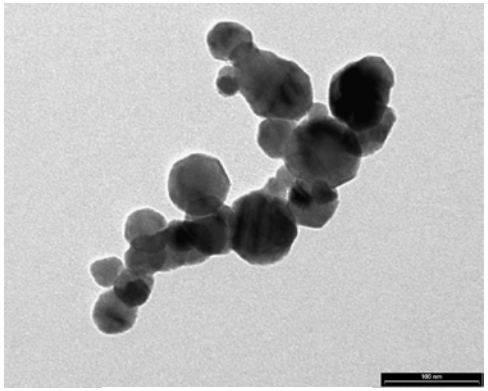




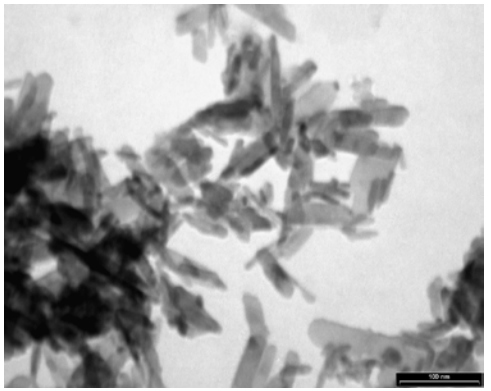
Gold sticks



Titanium carbide



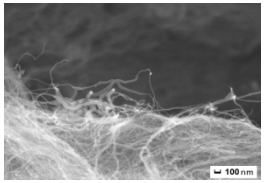
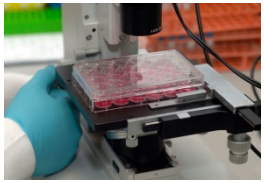
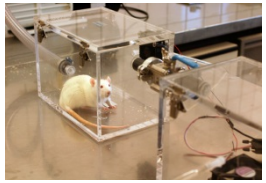
Silicon carbide



Copper oxide (rod-shaped)

Pluridisciplinary research team

Multidisciplinary platform


namur nanosafety centre
INTEGRATED PLATFORM

Coordination

Coordinator
Dr Olivier Toussaint

Characterization (PMR-LARN)

Prof. Stéphane Lucas
Dr Omar Lozano
Dr Jorge Mejia Mendoza

In Vitro Toxicology (URBC)

Dr Olivier Toussaint
Dr Jean-Pascal Piret
Dr Randa Ben Ameur
Elise Dumortier

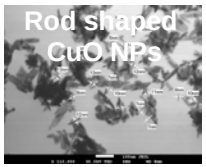
In Vivo Toxicology (Dpt. Of Pharmacy)

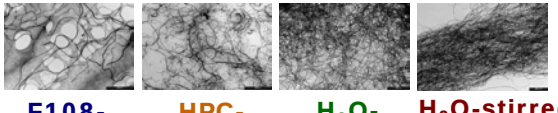
Prof. Jean-Michel Dogné
Dr Julie Laloy
Lutfiye Alpan

PMR-LARN: Nanoparticles characterization

FEG-SEM with EDX (JEOL)

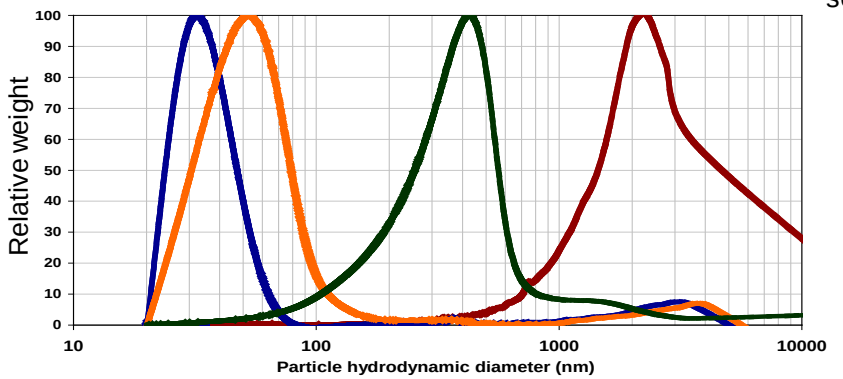
- Particle size and shape analysis.
- Selective area chemical identification (particles and/or matrix)

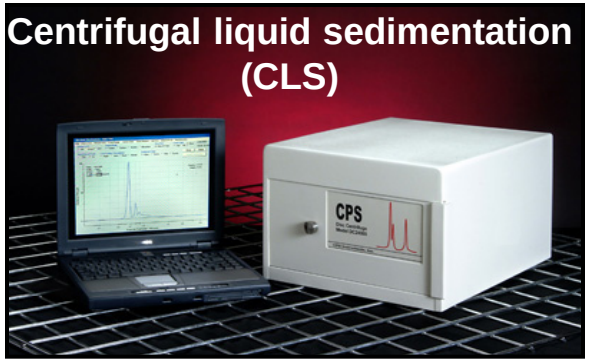




dilution in cell culture medium

centrifugal liquid sedimentation





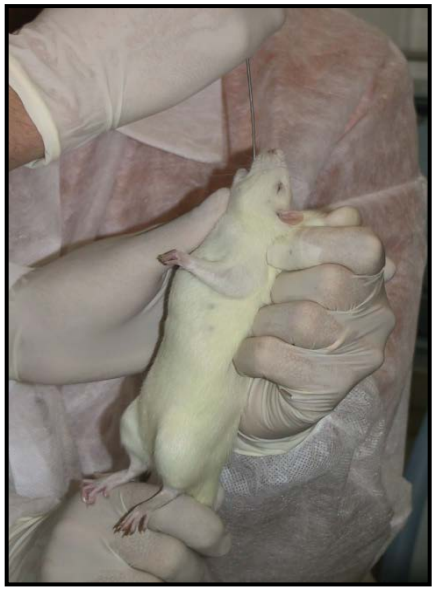
- Particle size distribution of NPs in suspension (stock solutions, dispersion in cell culture media).
- Estimation of number and mass of NPs in liquid sample.

Certified by the IRMM-JRC, in 2009, as a reference laboratory for NPs characterization with CLS, SEM and TEM

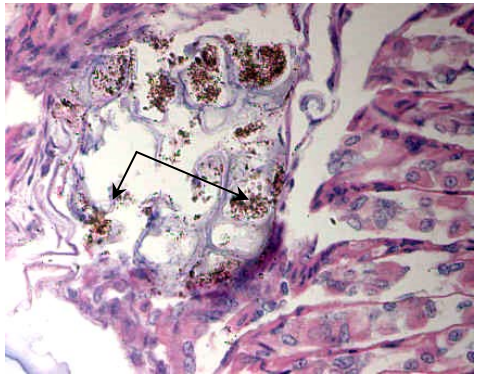
Department of Pharmacy (NAMEDIC): *In vivo* studies

Oral exposure (gavage)

Acute (1 day) and subacute (5 days/week, total 28 days) oral exposure

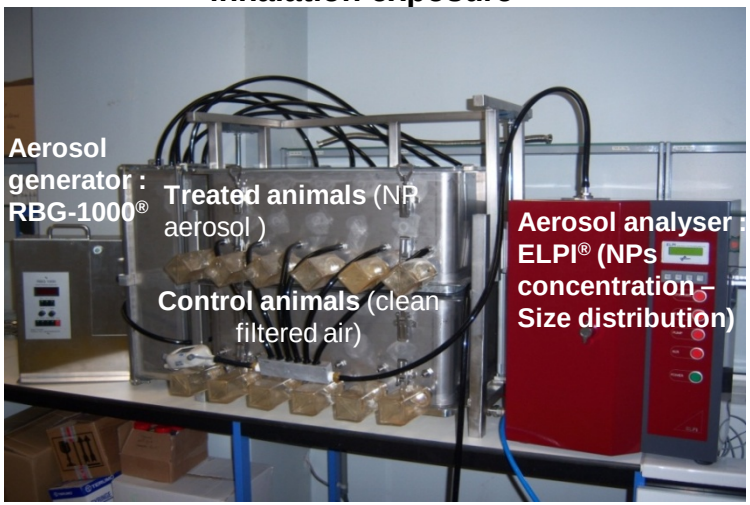


Stomach



Whole-body exposure

Acute (6h exposure + 0, 1, 3 days of recovery) and subacute (5 days exposure + 0, 3, 56 days recovery) inhalation exposure



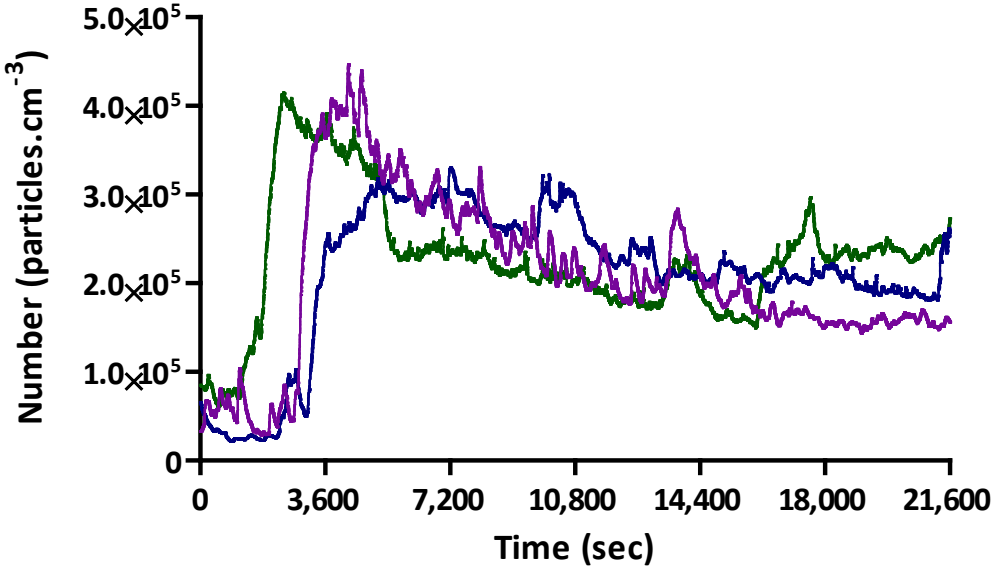
Histological analysis of the main organs

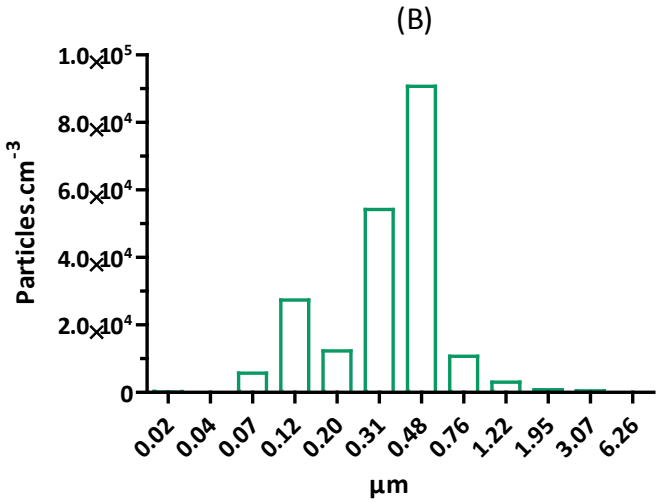
Blood analysis:

- Glucose
- Total cholesterol
- Na^+ and K^+
- Alkaline phoshatases
- Creatinine (kidney)
- Urea (kidney)
- Total proteins
- Albumin
- Bile acids (liver)
- Transaminases (liver)

→ Stable concentration of SiC generated of approximately 250,000 particles.cm^{-3}

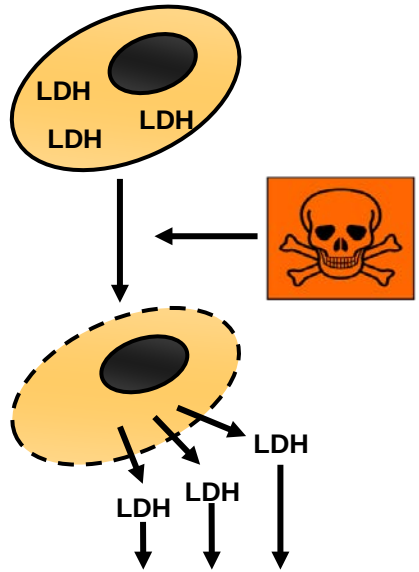
→ Bimodal distribution in number with peaks at 100 nm and 500 nm



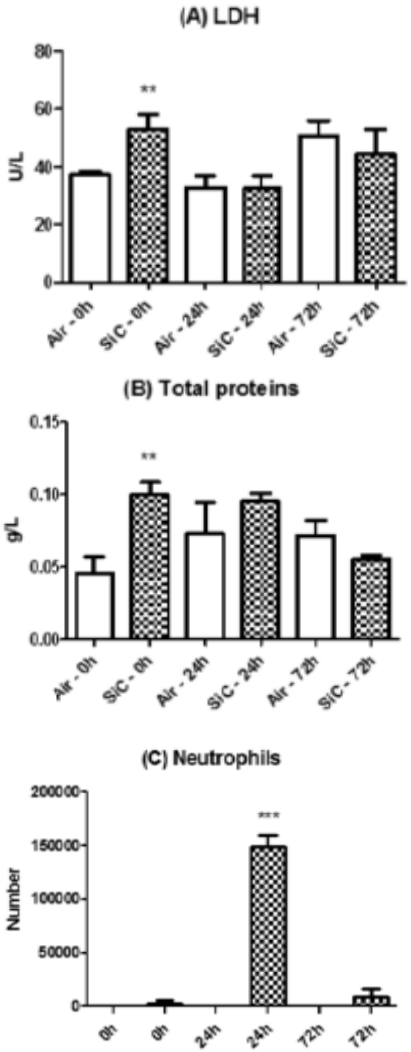


Laloy et al., 2015

Significant increase of total proteins and LDH activity in BALF immediately after exposure to SiC NPs aerosol

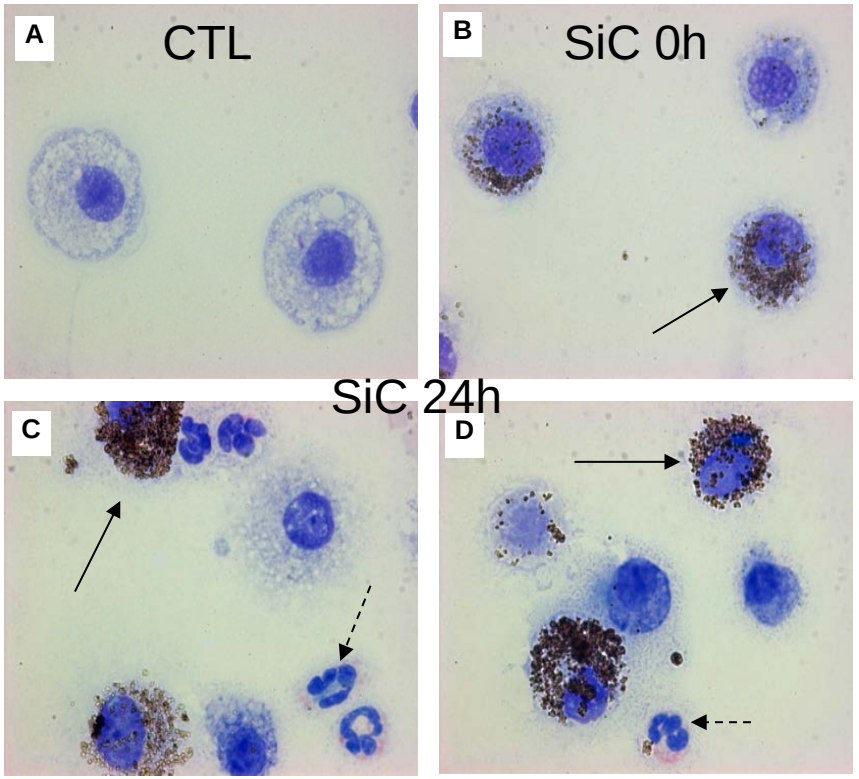


Measurement in cell culture medium of the LDH activity by spectrophotmetry



Increase number of neutrophils

Macrophages filled with SiC NPs

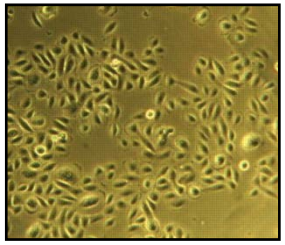


→ Limited acute inflammatory response was found up to 24 h after exposure characterized by LDH and total protein increase or presence of inflammatory cells in pulmonary lavage

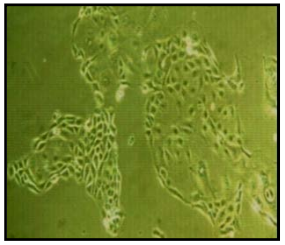
Laloy et al., 2015

In vitro cellular models for NPs toxicity evaluation

cutaneous contact

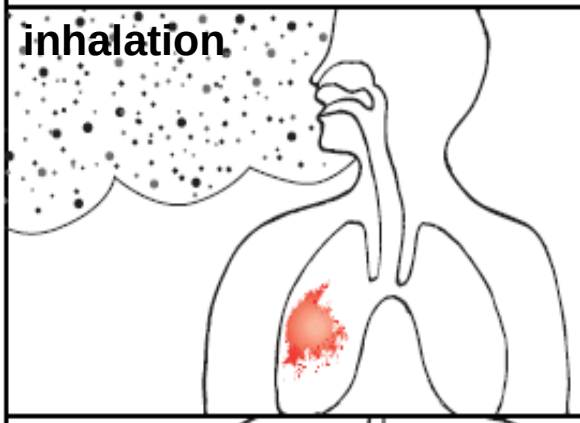


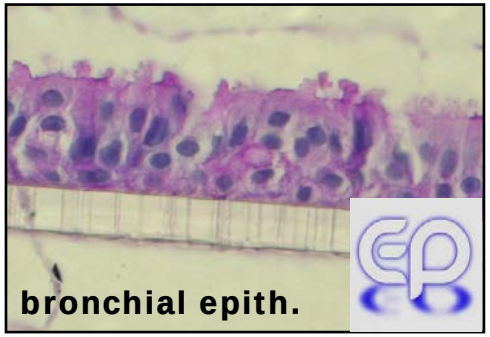
N-hTERT keratinocytes

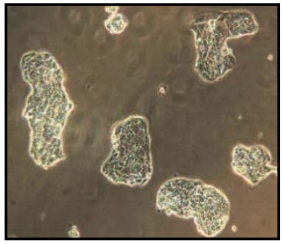


SZ95 sebocytes

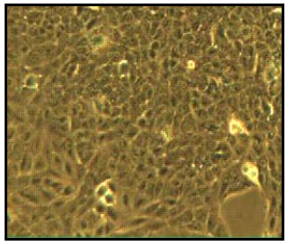
inhalation





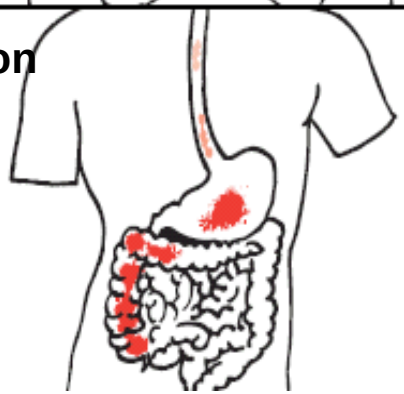


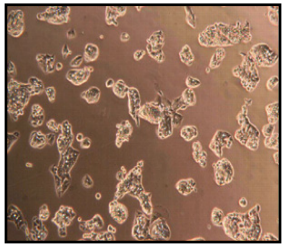
Calu-3 bronchial epithelial cells



A549 alveolar cells

ingestion





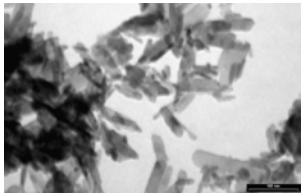
HepG2 hepatoma cells

In vitro study of NPs toxicity

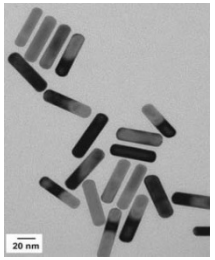
NPs dispersion protocols

NPs characterization in *in vitro* toxicity evaluation

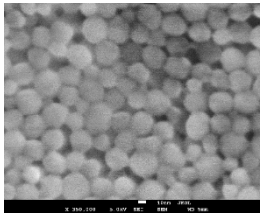
Copper oxide (rod-shaped)



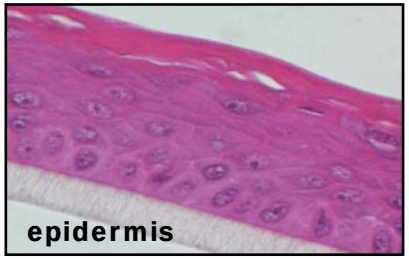
Gold sticks



Titanium carbide



In vitro cell models



In vitro assays interference

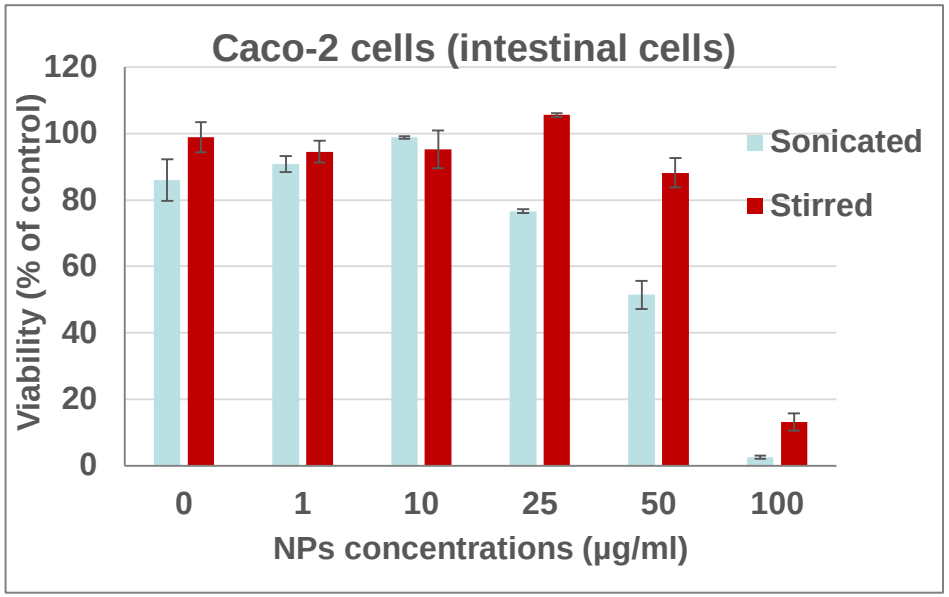
Arch Toxicol (2012) 86:1123–1136
DOI 10.1007/s00204-012-0837-z

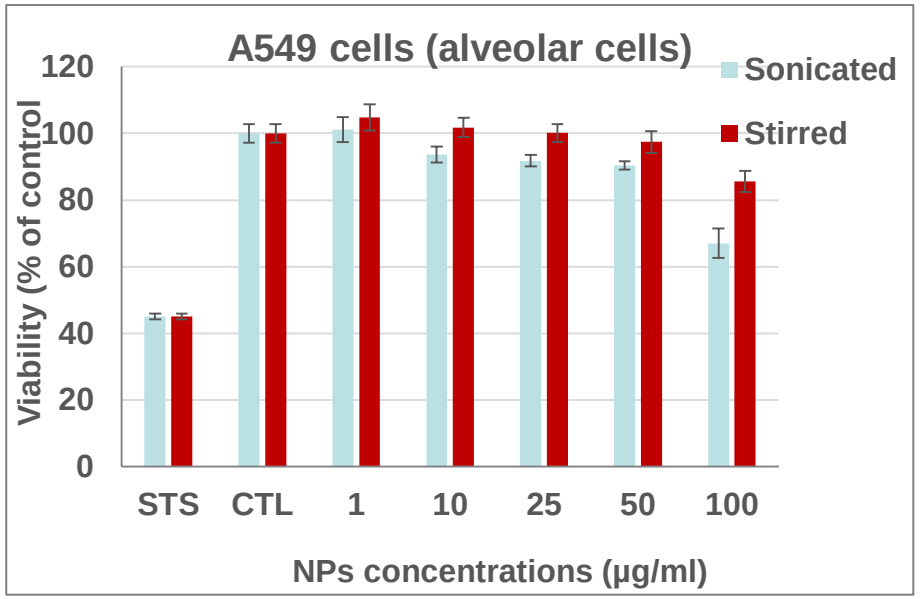
INORGANIC COMPOUNDS

Interference of engineered nanoparticles with in vitro toxicity assays

Alexandra Kroll · Mike Hendrik Pillukat · Daniela Hahn · Jürgen Schnekenburger

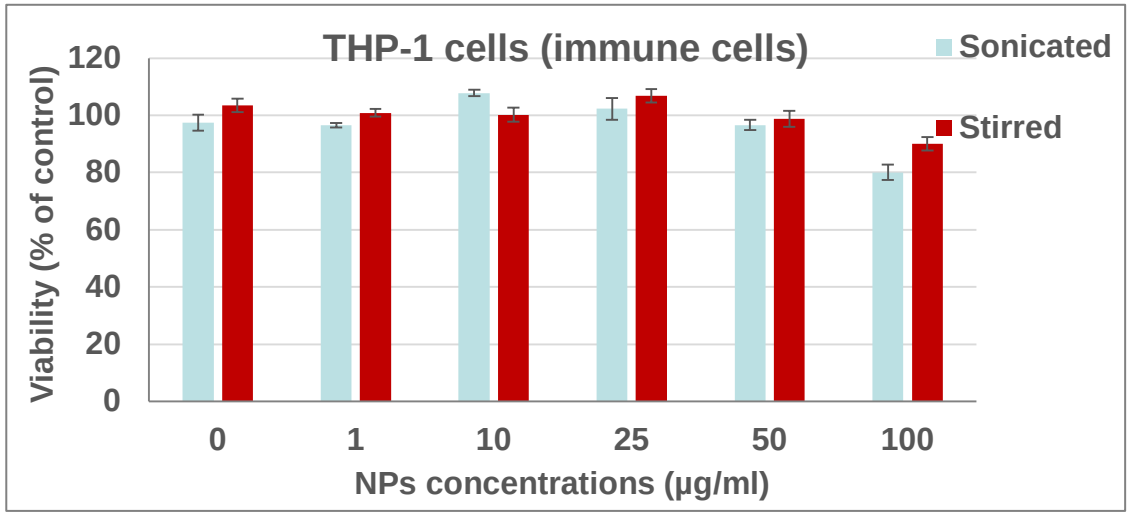
Evaluation of potential toxicity of sonicated or stirred ZnO NPs on different cell lines





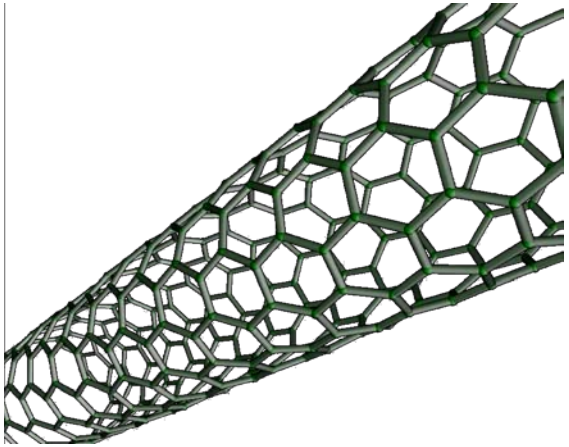
Stirred

Sonicated



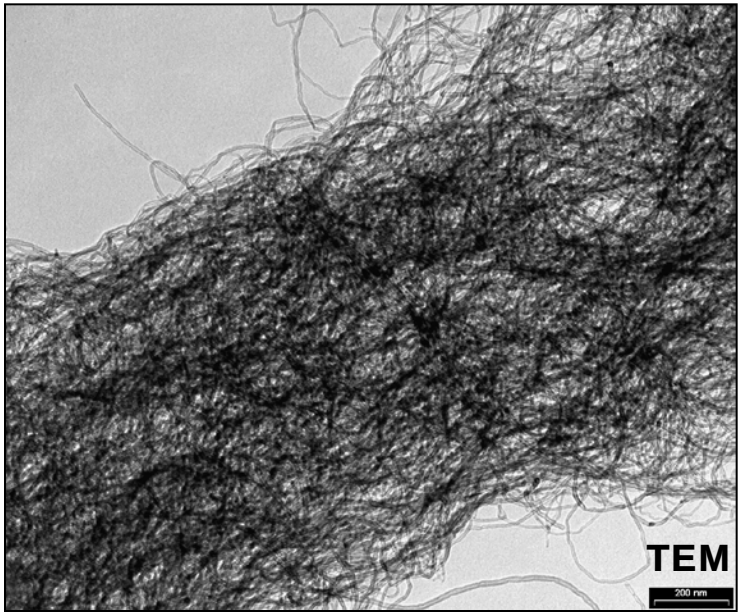
Different toxic effect depending on dispersion method
Cell type specific effect

MWCNT behaviour in water

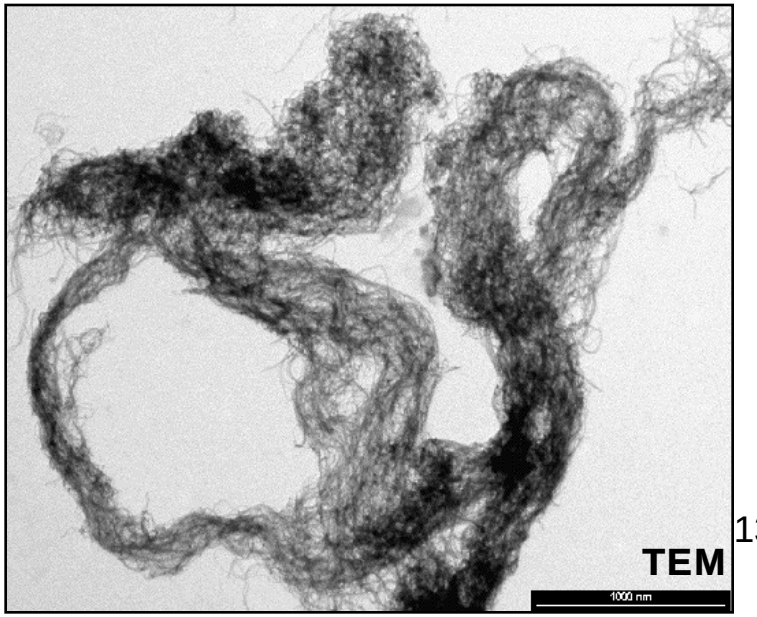


- **highly hydrophobic**
- **Very difficult to disper in aqueous liquid**
- **highly agglomerated**

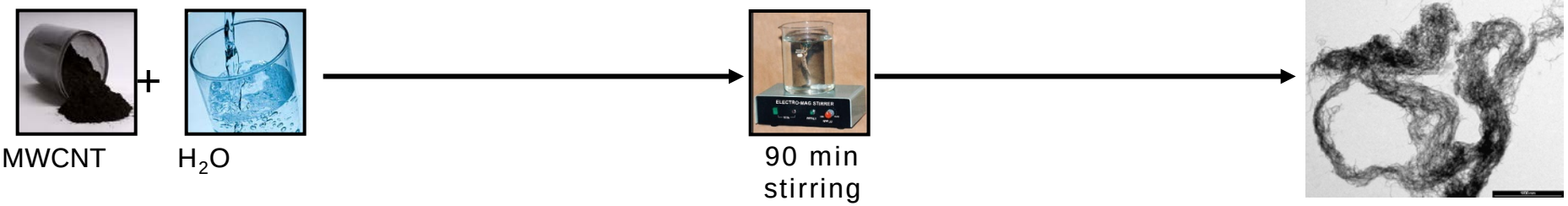
Raw MWCNT powder

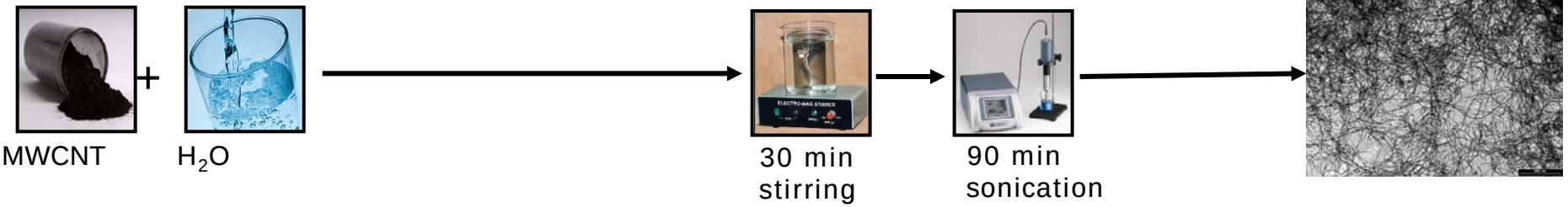


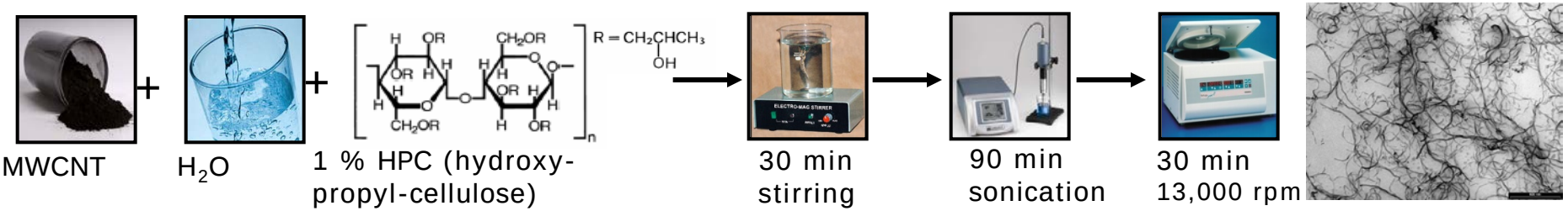
MWCNT stirred in water for 90 min

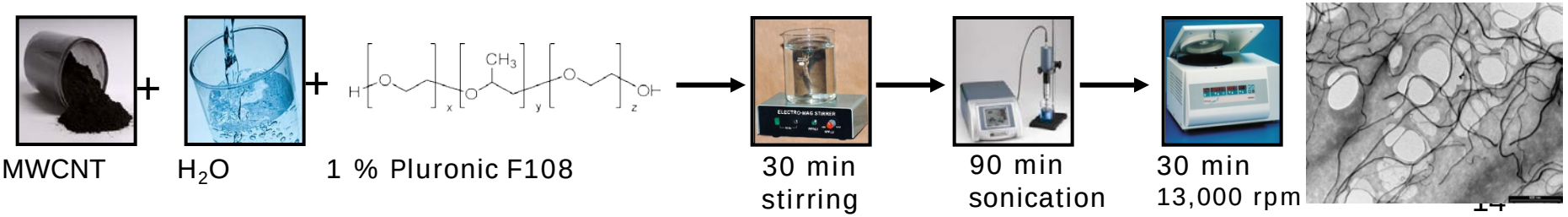


Four dispersion methods of MWCNT



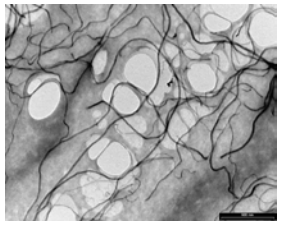




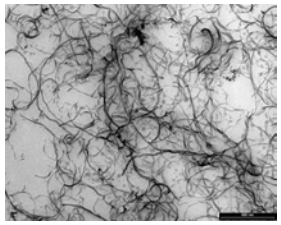


Piret, Detriche et al., 2010; J. Part. Res.

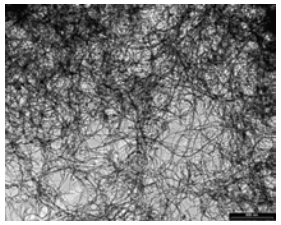
Size distribution and agglomeration state of MWCNT in cell culture medium



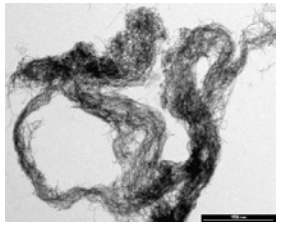
NT soniqués F108



NT soniqués HPC



NT soniqués H_2O

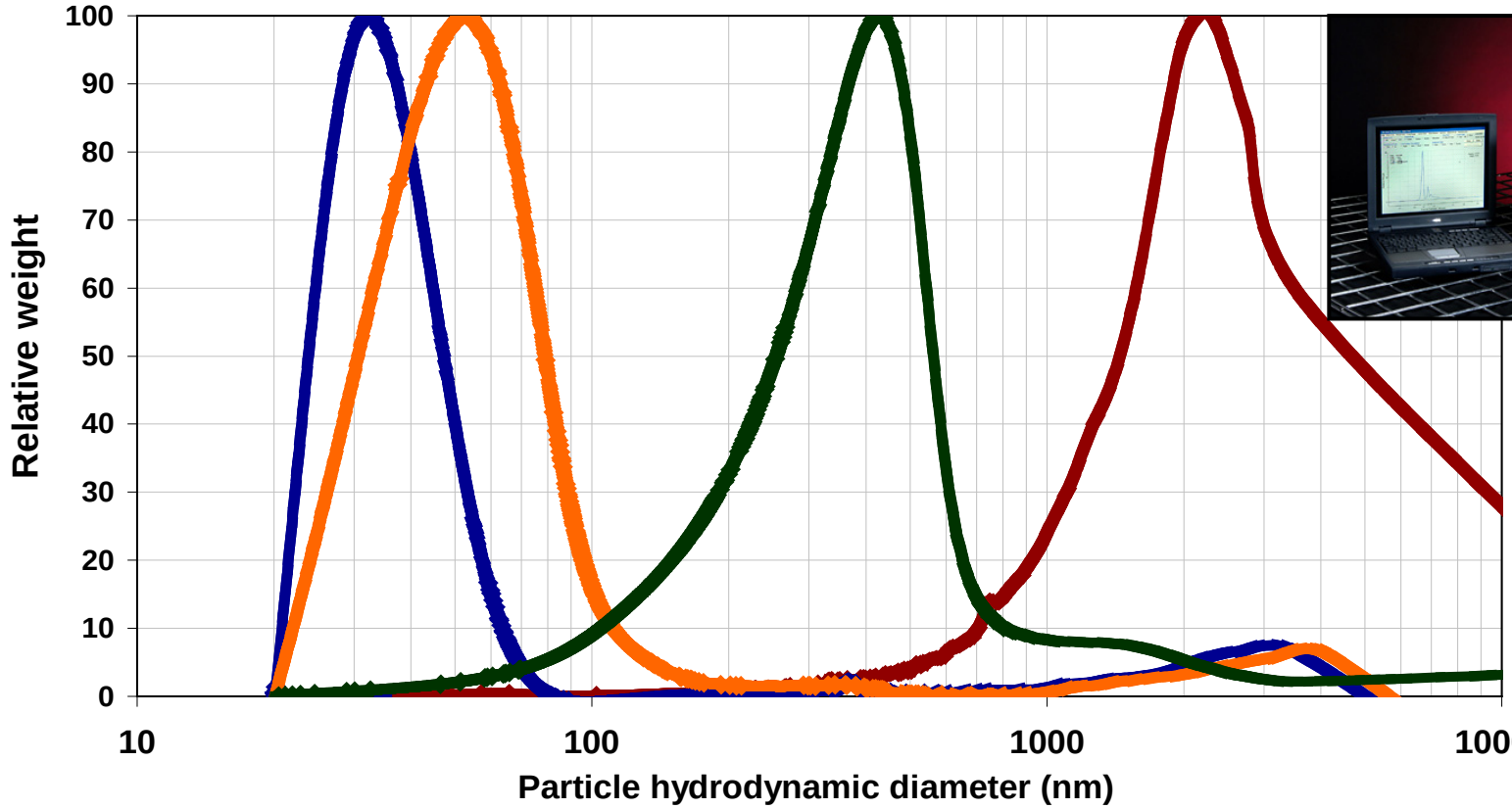


NT agités H_2O

dilution à 100 µg/ml

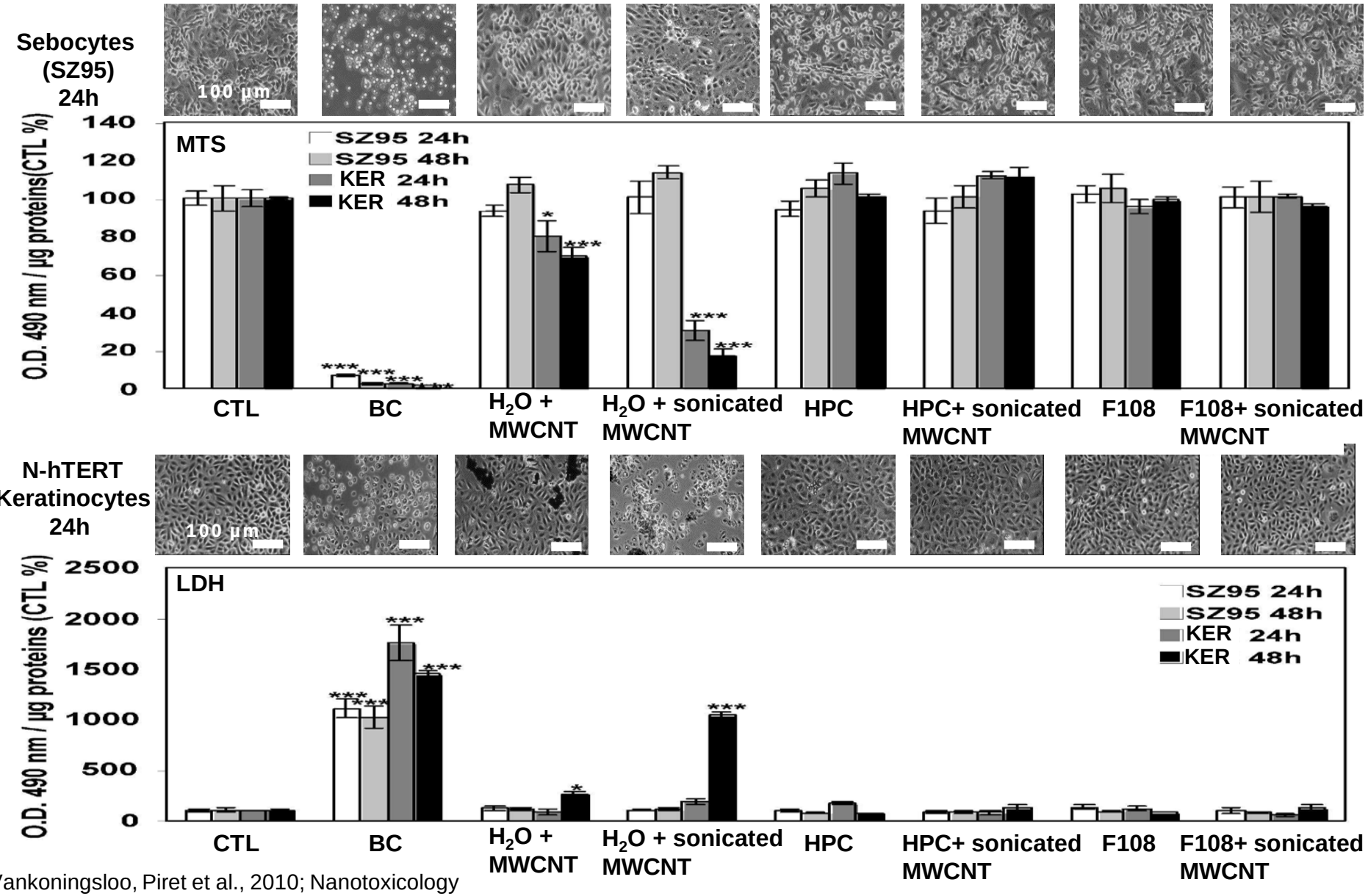
(Epilife)

sédimentation différentielle



Vankoningsloo, Piret et al., 2010 Nanotoxicology

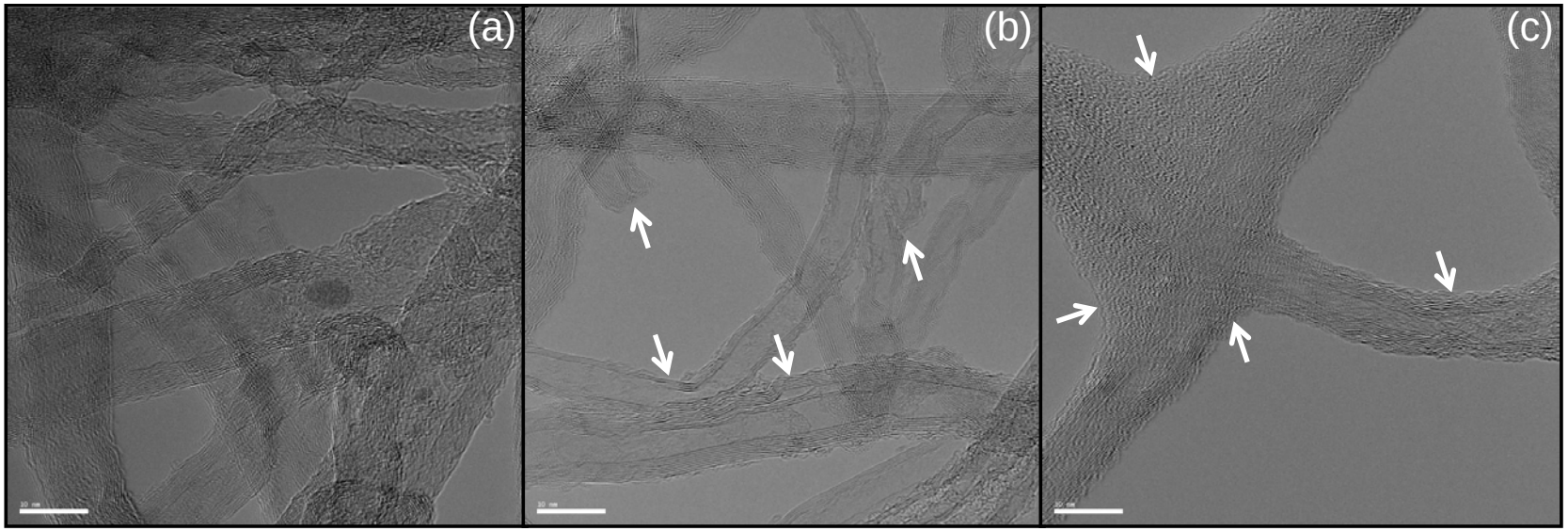
Effects of MWCNT on the viability of N-hTERT keratinocytes or sebocytes (24h or 48h)



Vankoningsloo, Piret et al., 2010; Nanotoxicology

TEM analysis of sonicated MWCNT in water or in F108 pluronic

Pristine MWCNT **H_2O + sonicated MWCNT** **F108 + sonicated MWCNT**



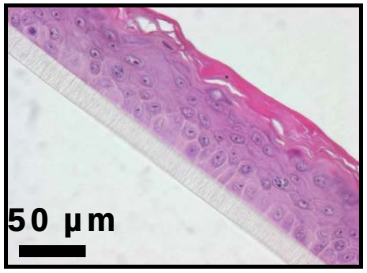
Sonication induces degradation (even breaking) in MWCNT outermost wall

Pluronic F108 thighly wraps the MWCNT surface

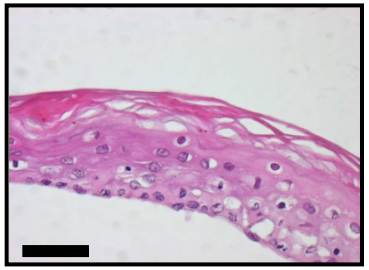
Classical dispersion methods (sonication; presence of dispersants) could alter the biological effects of MWCNT making them more or less toxic, in a way not relevant to the actual exposure situation

Meija, Tichelaar et al., 2011, J. Nanopart. Research

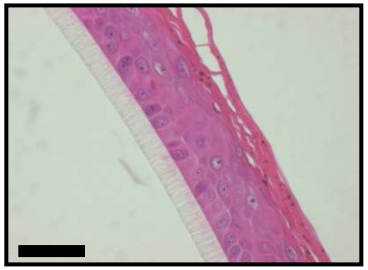
Effects of MWCNT on the morphology of *in vitro* reconstituted epidermises after 24h of incubation



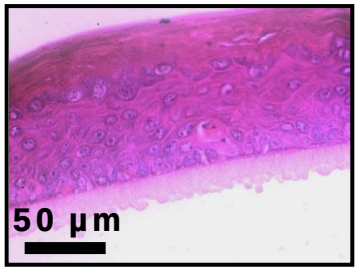
CTL



BC 1 mg/ml



MWCNT 2.5 mg/cm²



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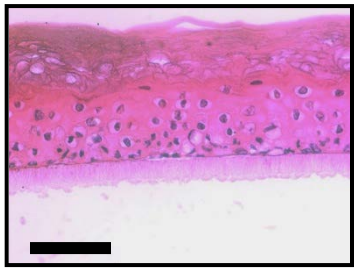
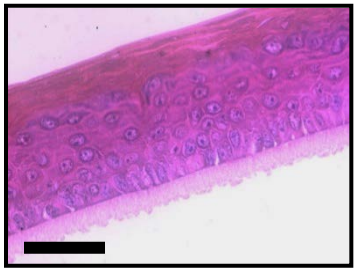
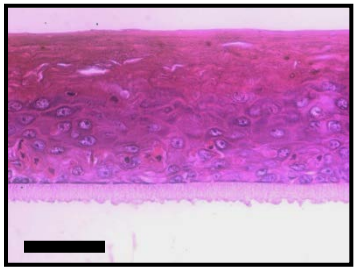


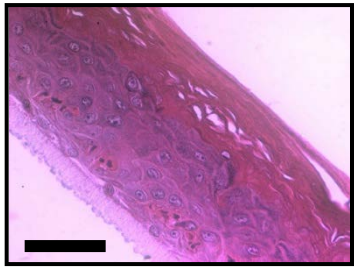
BC 1 mg/ml



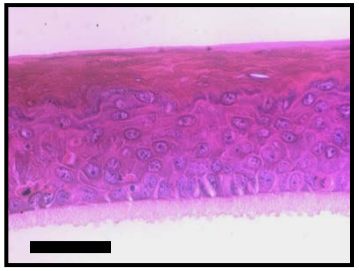
HPC



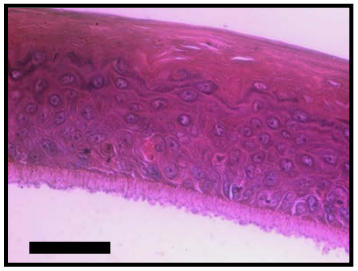
F108



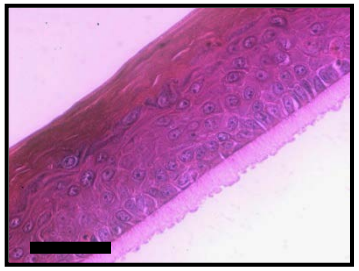
H_2O + MWCNT 100 µg/ml



H_2O + sonicated MWCNT 100 µg/ml



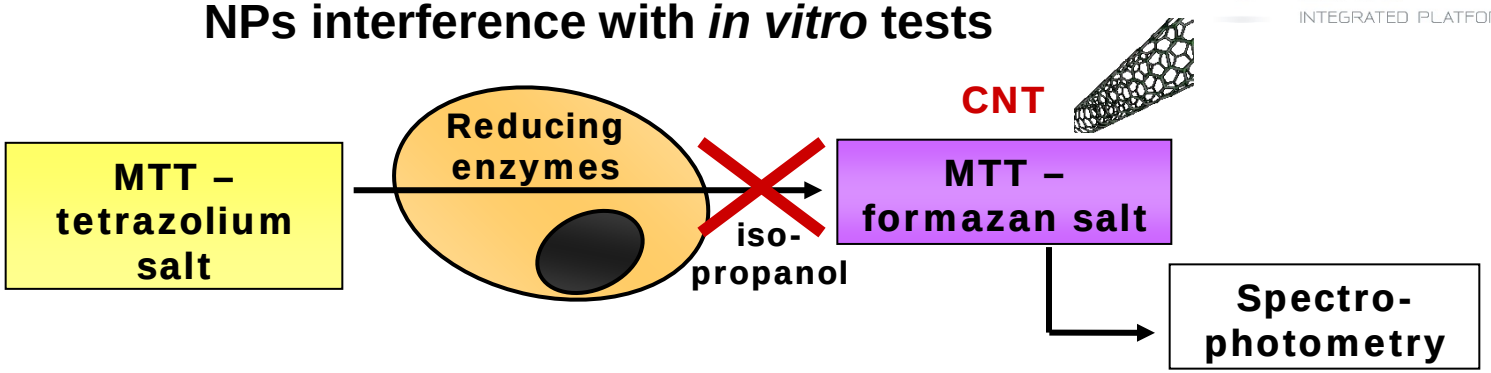
HPC + sonicated MWCNT 100 µg/ml



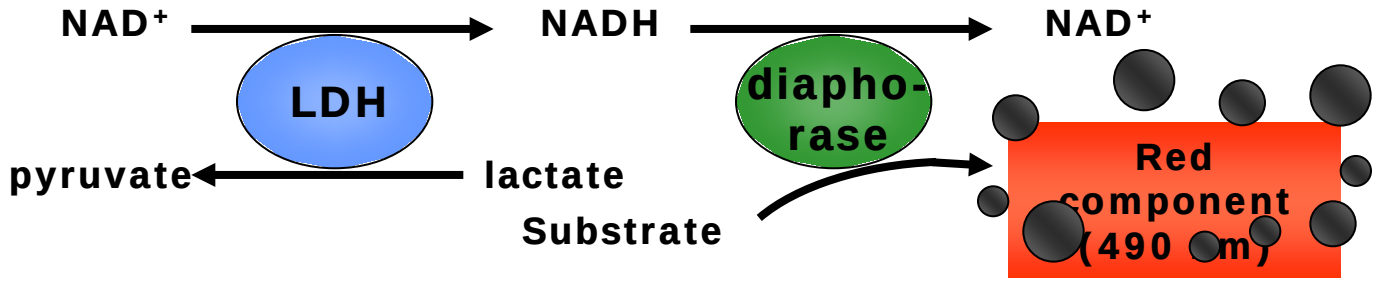
F108 + sonicated MWCNT 100 µg/ml

NPs interference with *in vitro* tests

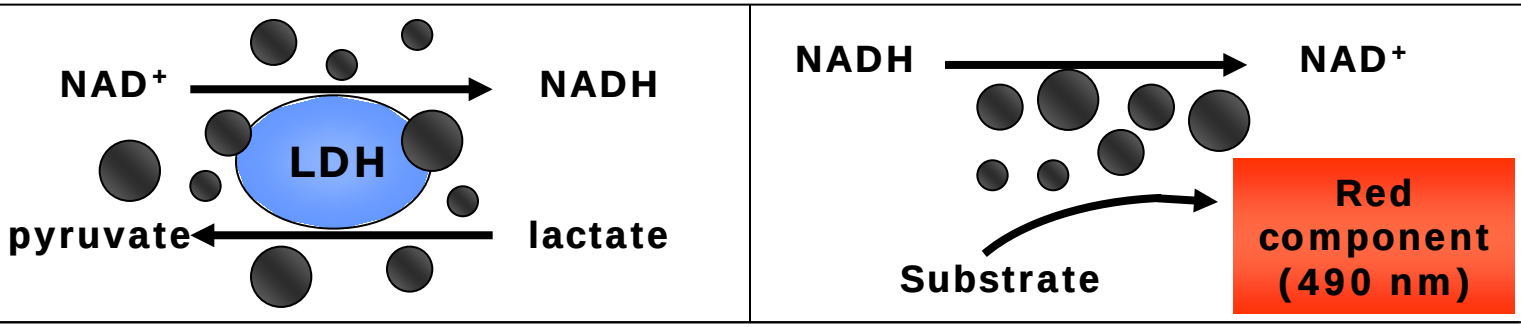
1 Physical interaction



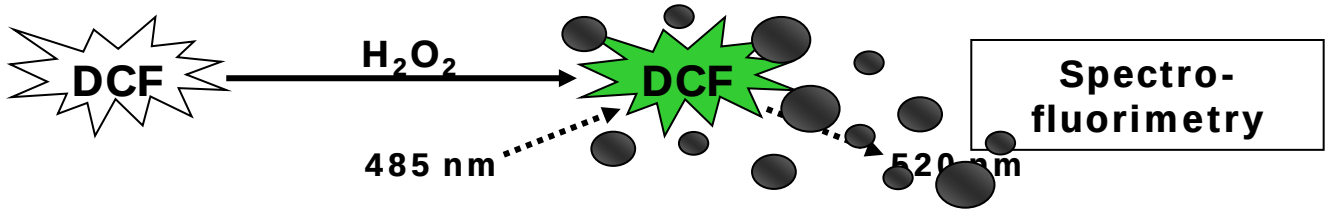
2 colorimetric interference



3 Enzymatic interference

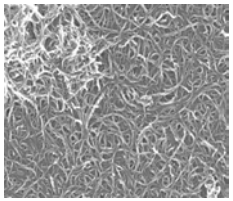


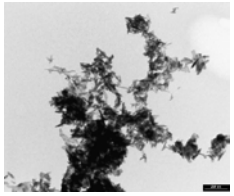
4 Fluorimetric interference



Development of standardized operating procedure (SOPs) for *in vitro* assays

In summary

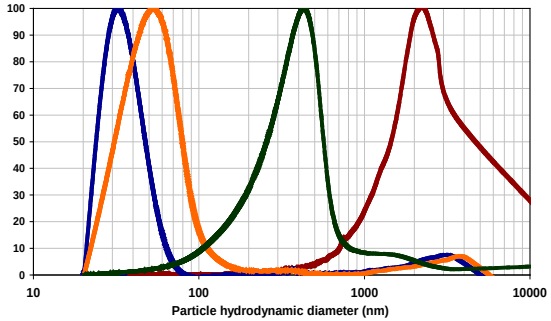




NPs brutes

caractérisation physico-chimique

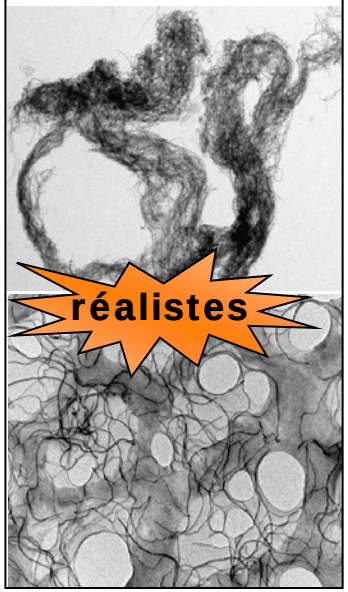
Nanotubes multi-paroi série 7000 (Nanocyl)						
Diamètre moyen	*SEM*	12 nm				
Longueur	*SEM*	> 1 µm				
Surface spécifique	*BET*	323.7 m2/g				
Composition chimique		**C**	**O**	**Al**	**Fe**	**Co**
Global (atom %)	*EDX*	90.9	6.9	2.1	0.1	0.1
	PIXE			2.6	0.4	0.1
Surface (atom %)	*XPS*	97.5	2.5			
Valeurs attendues	*TGA*	90.0	10.0			



?

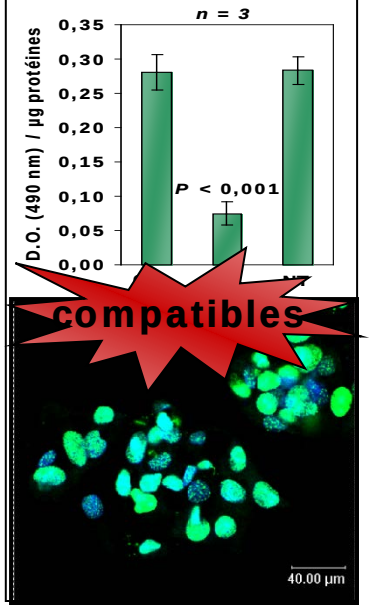
T - Toxique

suspensions



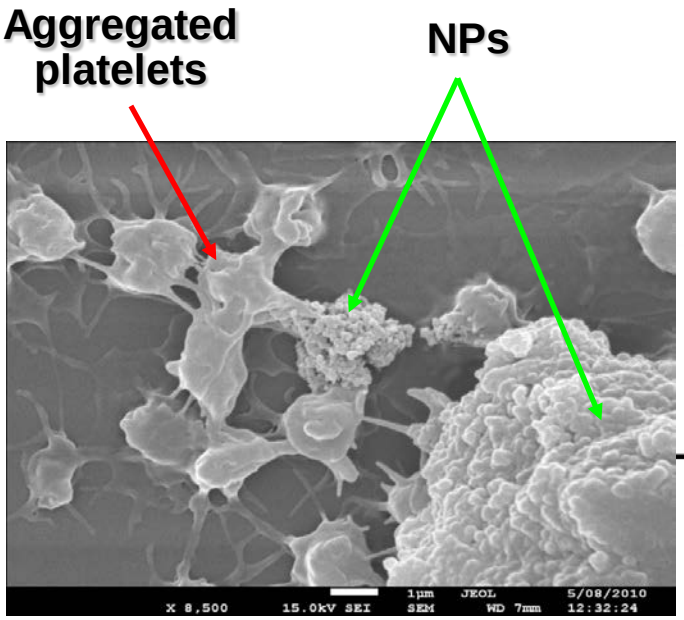
modèles

tests



SOPs

Guidelines

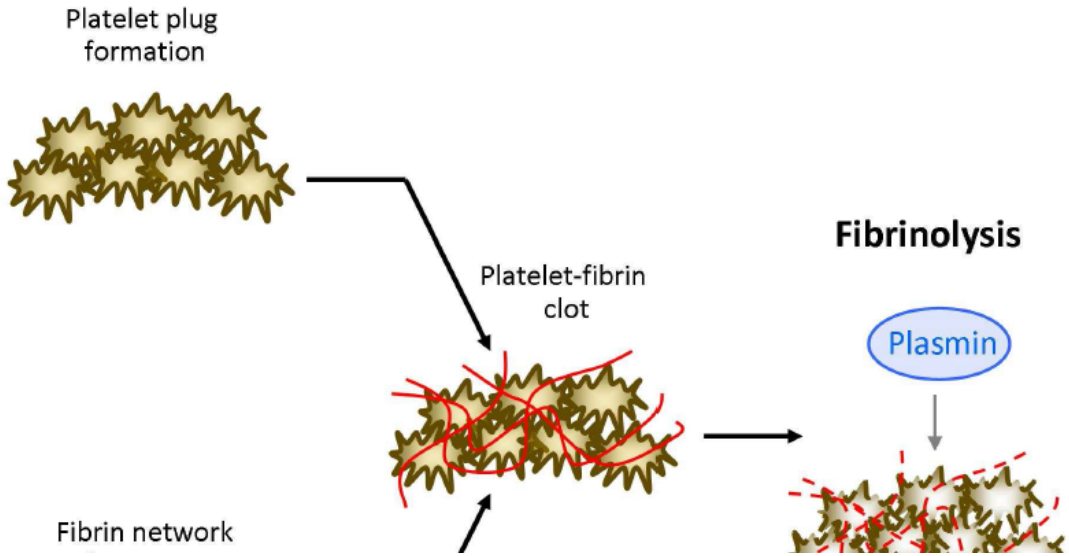


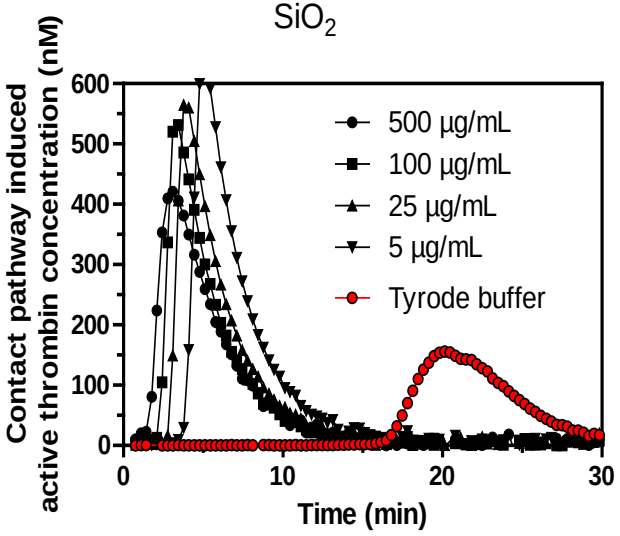
Nanotoxicology. 2013 Apr 15. [Epub ahead of print]

A comparison of six major platelet functional tests to assess the impact of carbon nanomaterials on platelet function: A practical guide.

Laloy J, Mullier F, Alpan L, Mejia J, Lucas S, Chatelain B, Toussaint O, Masereel B, Rolin S, Dogné JM.

Department of Pharmacy, Namur Nanosafety Center (NNC), NAmur MEdicine & Drug Innovation Center (NAMEDIC), Namur Thrombosis and Hemostasis Center (NTHC), University of Namur (FUNDP) , Namur , Belgium.





Nanotoxicology. 2012 Mar;6(2):213-32. doi: 10.3109/17435390.2011.569096. Epub 2011 Apr 13.

Validation of the calibrated thrombin generation test (cTGT) as the reference assay to evaluate the procoagulant activity of nanomaterials.

Laloy J, Robert S, Marbehant C, Mullier F, Mejia J, Piret JP, Lucas S, Chatelain B, Dogné JM, Toussaint O, Masereel B, Rolin S.

Department of Pharmacy, Drug Design & Discovery Center (D3C), Namur Research Institute for Life Sciences (NARILIS), Namur Thrombosis and Hemostasis Center (NTHC), FUNDP-University of Namur, Namur, Belgium. julie.laloy@fundp.ac.be

→ Guidelines proposal

Le NNC, acteur majeur dans le domaine de la « nanosafety »

Toxicologie

Nanotechnologies

nanotoxico

Programme d'excellence (Jan. 2006 – Dec. 2011)
co-financed by DGO6-SPW and University of Namur

namur nanosafety centre
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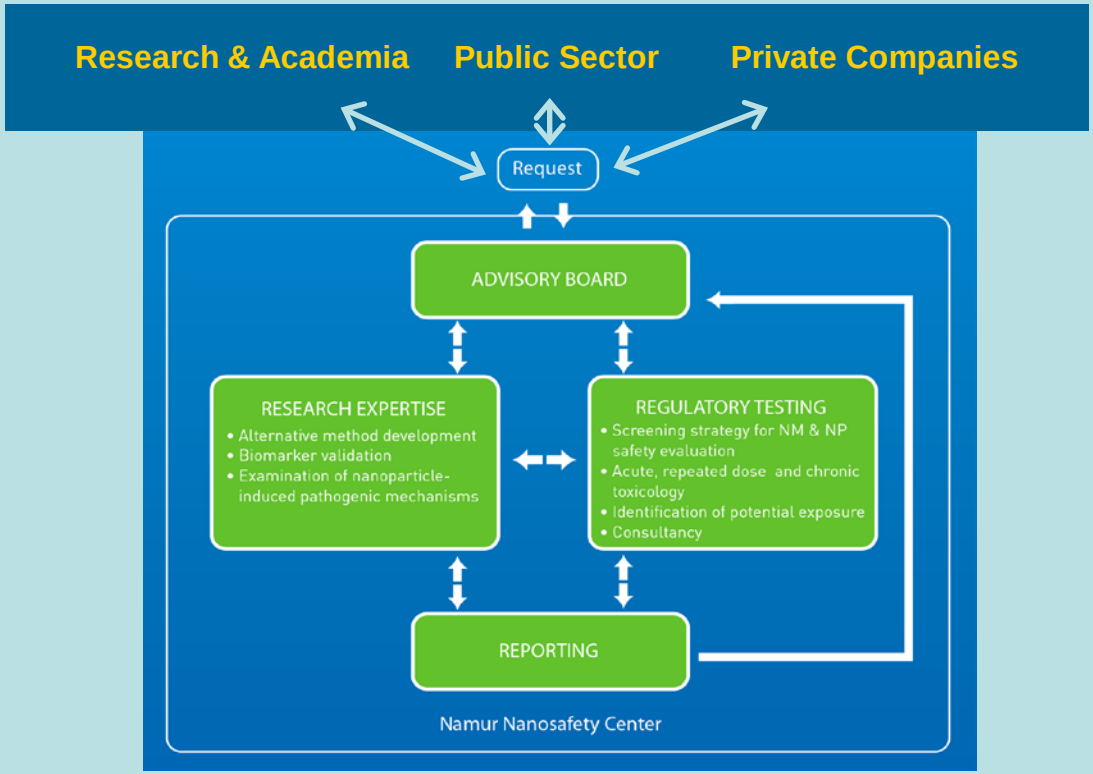
Jan. 2012
Centre multidisciplinaire

Le NNC: acteur majeur dans le domaine de la « nanosafety »



Notre futur (vision)

- Occupational safety:
  - projet NANOGECO (Integration of nanomaterials in paints)
  - Partenariat (non exclusif) avec SPF santé (mesures sur site, exposition travailleurs)
- Ecotoxicology (DG03-SPW): Etat des connaissances sur les impacts des NPs sur l'environnement en Wallonie
- Food safety (Master thesis)
- Laboratoire aux normes GLP

Vous êtes les bienvenus

namur nanosafety centre
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Dessau medical center (Germany)

Prof Christos C. Zouboulis

Jean-pascal.piret@unamur.be
nnc@unamur.be

Video: Lab'InSight Toxicological Risk Assessment (Unamur)