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# Are standard genotoxicity tests useful for the safety evaluation of nanomaterials?

*Serão os testes de genotoxicidade convencionais úteis para a avaliação de segurança dos nanomateriais?*


**Henriqueta Louro**

R&D Unit, Departament of Human Genetics

**National Institute of Health Dr. Ricardo Jorge, Lisbon**


***1st NATIONAL MEETING- Nanotechnology: Regulate to compete***

***3 April 2014***

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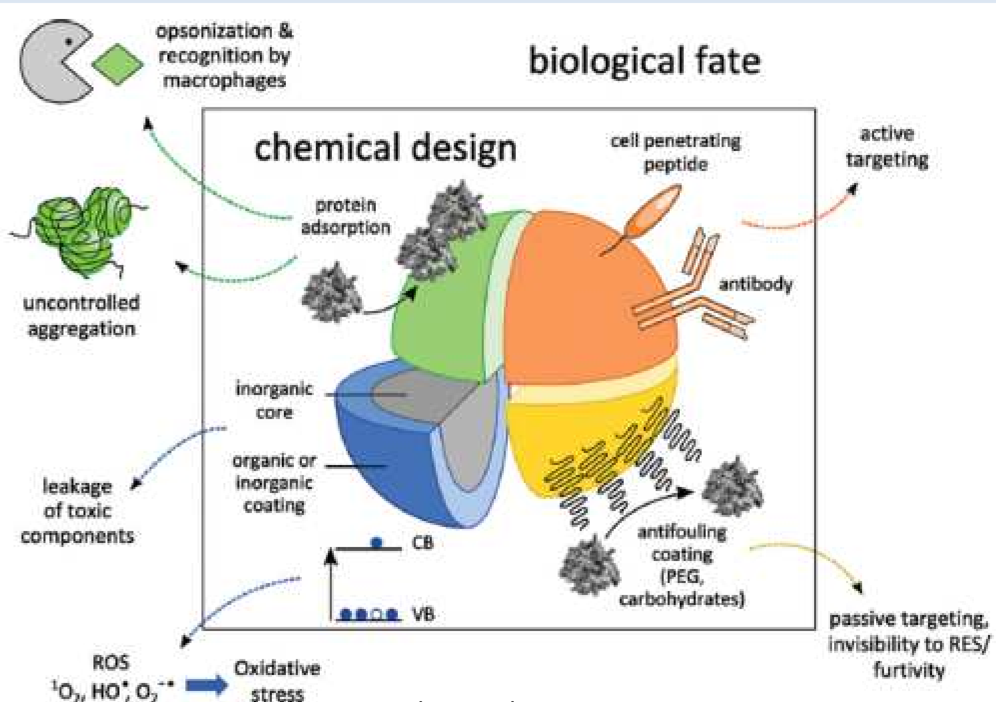
# Background

**Nanomaterials (NM)**
**-Small size**
**-High surface-to- volume ratio**

**Unique mechanical, optical, electrical and magnetic characteristics**

**Useful for innovative biomedical and industrial applications.**




Pelaz et al., 2012.

**However, such properties also influence the nano interactions with cellular components**

**Need to warrant NMs safe use**

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## Background and objectives

# Some Open Questions about NMs safety:

**-Do NMs cause genotoxic lesions that may be related to cancer development?**

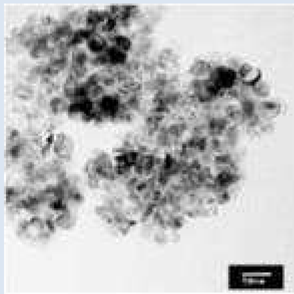
**- Are standard genotoxicity tests useful for the safety evaluation of nanomaterials?**

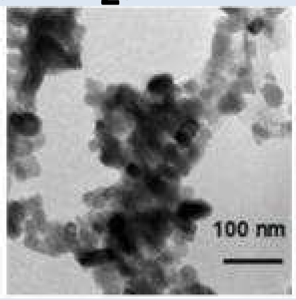
NANOGENOTOX

**European Joint Action 2010-2013**
**www.nanogenotox.eu**

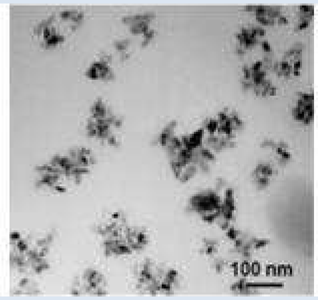
# Methods

## Titanium dioxide ($TiO_2$) nanomaterials from JRC repository

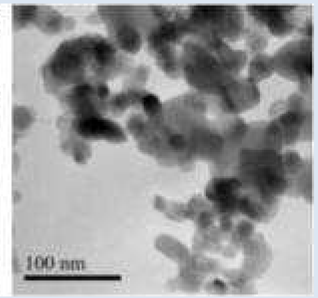

NM-102



NM-103



NM-104



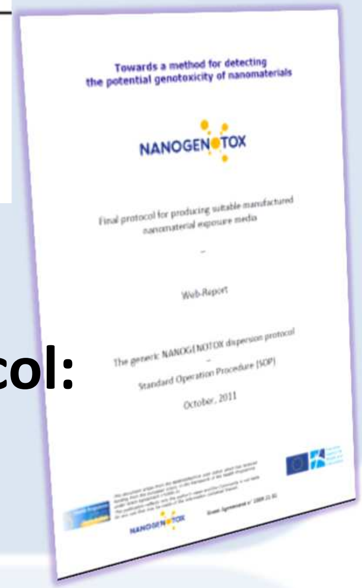
NM-105

| Nanomaterial | | Phase (and other information)[a] | Impurities/ coatings (surface modification) | Specific surface area ($m^2/g$)[a] | Primary particles | | | | Aggregates/agglomerates[d] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Feret Min ± SD (nm)[b] | Feret Max ± SD (nm)[b] | Aspect ratio ± SD[b] | N[c] | 25% (nm) | Median (nm) | 75% (nm) |
| $TiO_2$ | NM-102 | Anatase | – | 90 | 20.8 ± 1.6 | 33.0 ± 1.5 | 1.5 ± 1.3 | 59 | 43 | 54 | 72 |
| | NM-103 | Rutile (hydrophobic) | Dimethicone 2%[e] | 60 | 21.9 ± 1.4 | 37.9 ± 1.6 | 1.7 ± 1.3 | 40 | 33 | 67 | 129 |
| | NM-104 | Rutile (hydrophilic) | Glycerine[e] | 60 | 19.0 ± 1.5 | 25.8 ± 1.4 | 1.4 ± 1.3 | 47 | 33 | 60 | 112 |
| | NM-105 | Rutile-anatase (15-85%) | None[e] | 61 | 20.0 ± 1.3 | 29.6 ± 1.3 | 1.4 ± 1.2 | 42 | 55 | 90 | 144 |

*Tavares et al., Toxicology In vitro (2014)*

## $TiO_2$- Insoluble NMs

**Dispersion of NMs according a standardized protocol:**


Towards a method for detecting
the potential genotoxicity of nanomaterials

NANOGENOTOX

Final protocol for producing suitable manufactured
nanomaterial exposure media

–

Web-Report

The generic NANOGENOTOX dispersion protocol
–
Standard Operation Procedure (SOP)

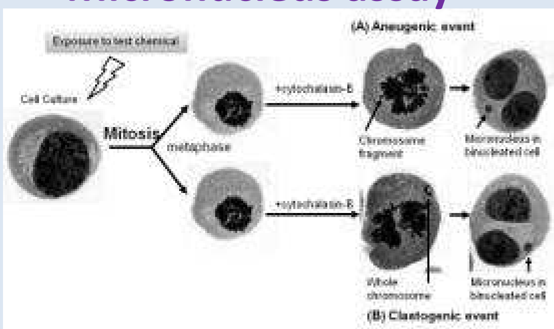
October, 2011

**www.nanogenotox.eu**

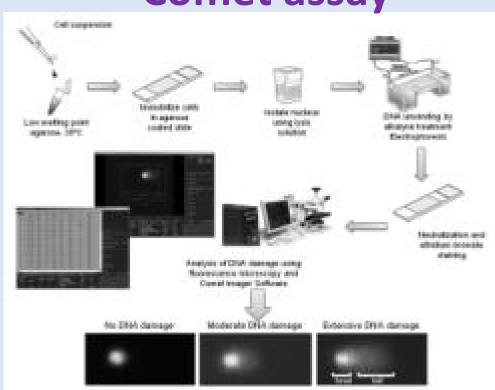
# Methods

## In vitro testing of $TiO_2$

### Micronucleus assay




### Comet assay

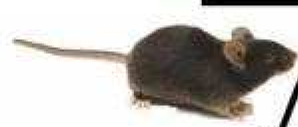



**Human lung cell lines (BEAS-2B, A549)**
**Human lymphocytes**

## In vivo testing of $TiO_2$

### Integrated Approach Using LacZ Plasmid-Based Transgenic Mice

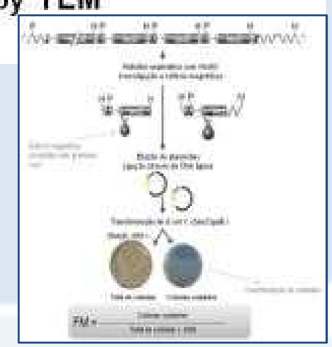
Exposure of groups of mice to 0, 10 or 15 mg/kg of NM-102, i.v. on 2 consecutive days



Sacrifice 28 days after last i.v.

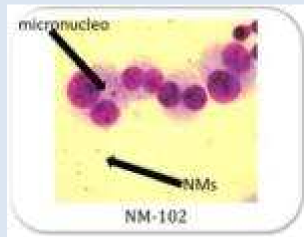
Micronucleus assay in tail blood, 42h after last i.v.

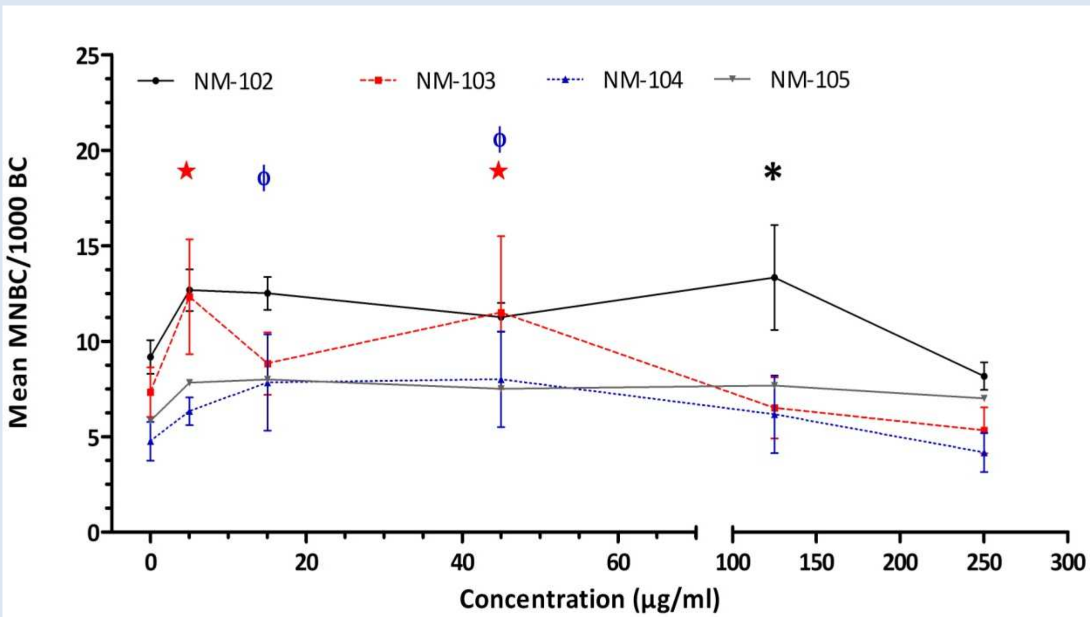
Comet assay in liver
*LacZ* mutation assay in liver and spleen
Detection of NM-102 in liver by TEM
Histological analysis of liver



# Results – *in vitro*

**Human Lymphocytes exposed to a panel of $TiO_2$**




*Tavares et al., Toxicology In vitro (2014)*

**Significant increase in the micronucleus frequency in:**

***NM-102: 125 µg/ml** (*p*=0.038),

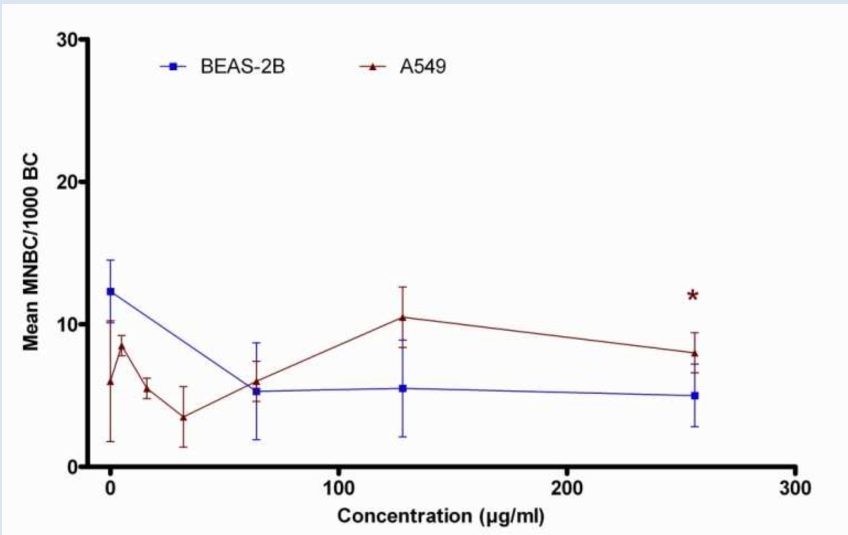
**★NM-103: 5 e 45 µg/ml** (*p*=0.007 and 0.039)

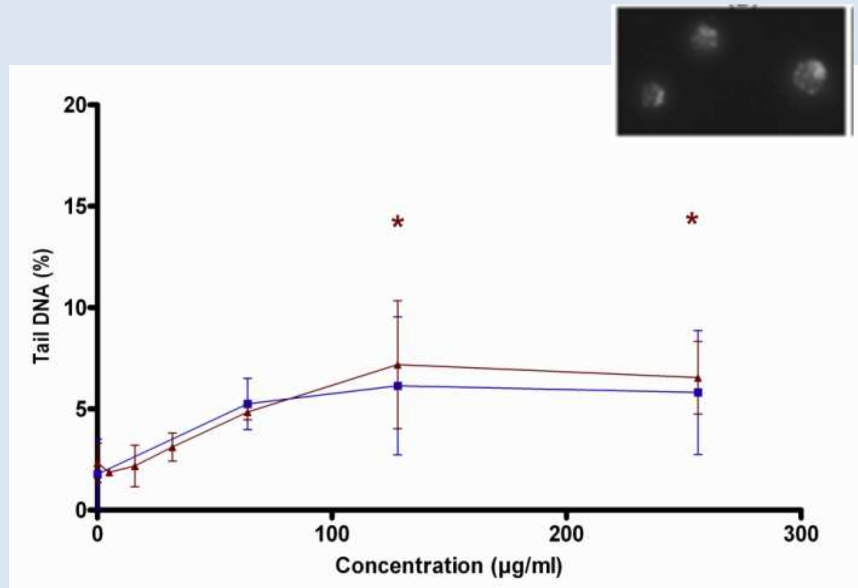
**ϕNM-104: 15 e 45 µg/ml** (*p*= 0.037 and 0.048)

**Closely related NMs have distinct genotoxic effects**

# Results – *in vitro*

## Human lung cells exposed to one $TiO_2$ (NM-102)



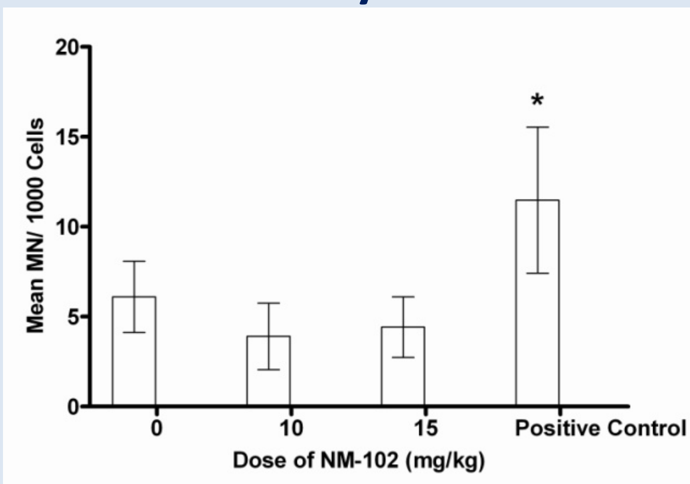




- **Significant increase in the micronucleus frequency in A549 cells exposed to 256 µg/ml.**
- **Significant increase in DNA breaks in A549 cells exposed to 125 and 256 µg/ml.**

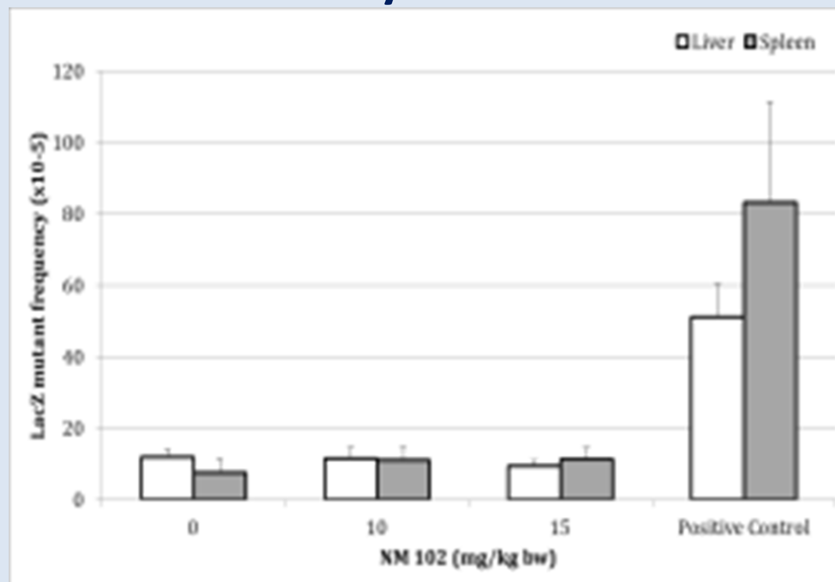
**The same NM has distinct genotoxic effects on different cell types**

# Results – *in vivo*

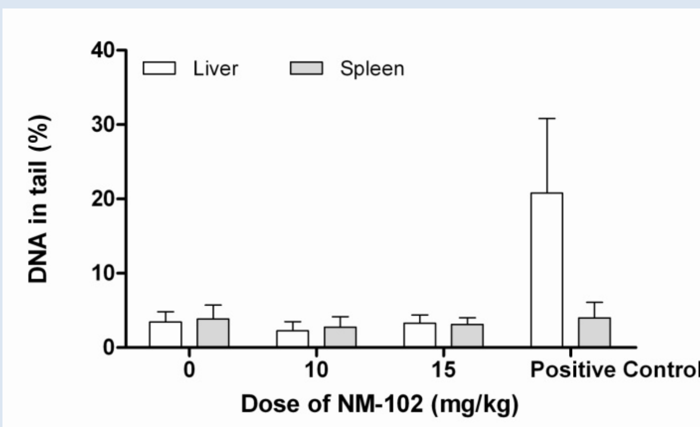
## Micronucleus assay in mouse blood




## Mutation assay in mouse liver and




## Comet assay in mouse liver and spleen

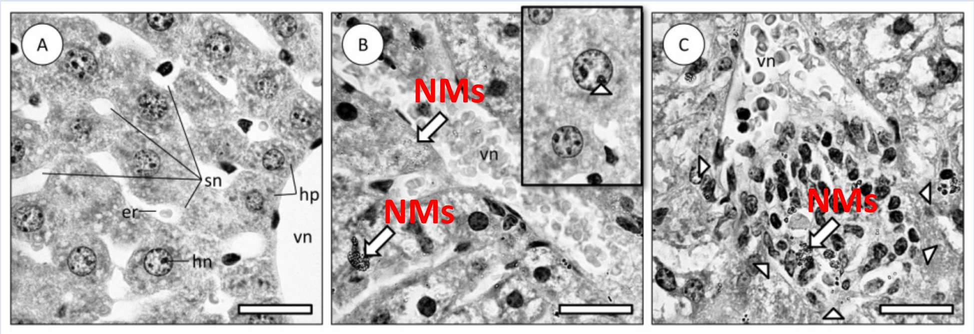


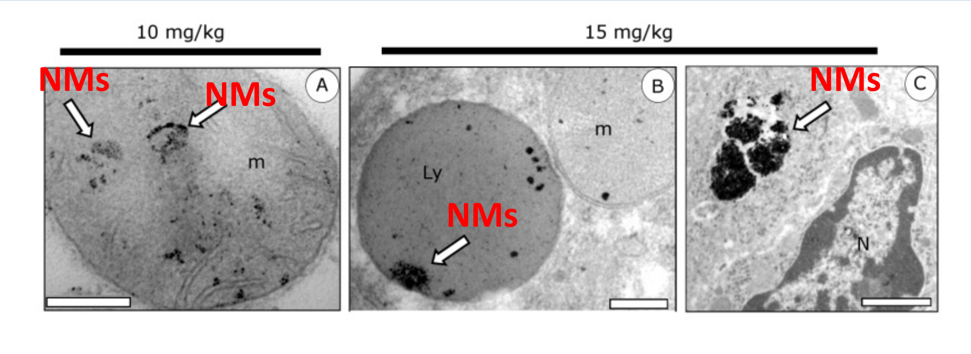

**No genotoxic effects of NM-102 *in vivo***

*Louro et al., EnvironMol Mut (2014)*

# Results – *in vivo*

**Microscopic analysis of mouse liver**




*Louro et al., EnvironMol Mut (2014)*

**Bioaccumulation of NM in mouse liver and mild inflammatory effects**

**Possibility of a secondary genotoxic effect?**

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# Conclusions

- **Differential genotoxicity observed for closely related NMs** - importance of investigating the toxic potential of each NM individually, instead of assuming a common mechanism and equal genotoxic effects for a set of similar NMs.

- **Standard genotoxicity tests are useful, an can be applied, for the safety evaluation of nanomaterials –** provided that standardized protocols for NM preparation are used and the physicochemical characteristics of NMs are considered.

- **Predictivity of the *in vitro* genotoxicity assays for *in vivo* situation with NMs?** - to be clarified.

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Safety evaluation of manufactured nanomaterials by characterisation of their potential genotoxic hazard