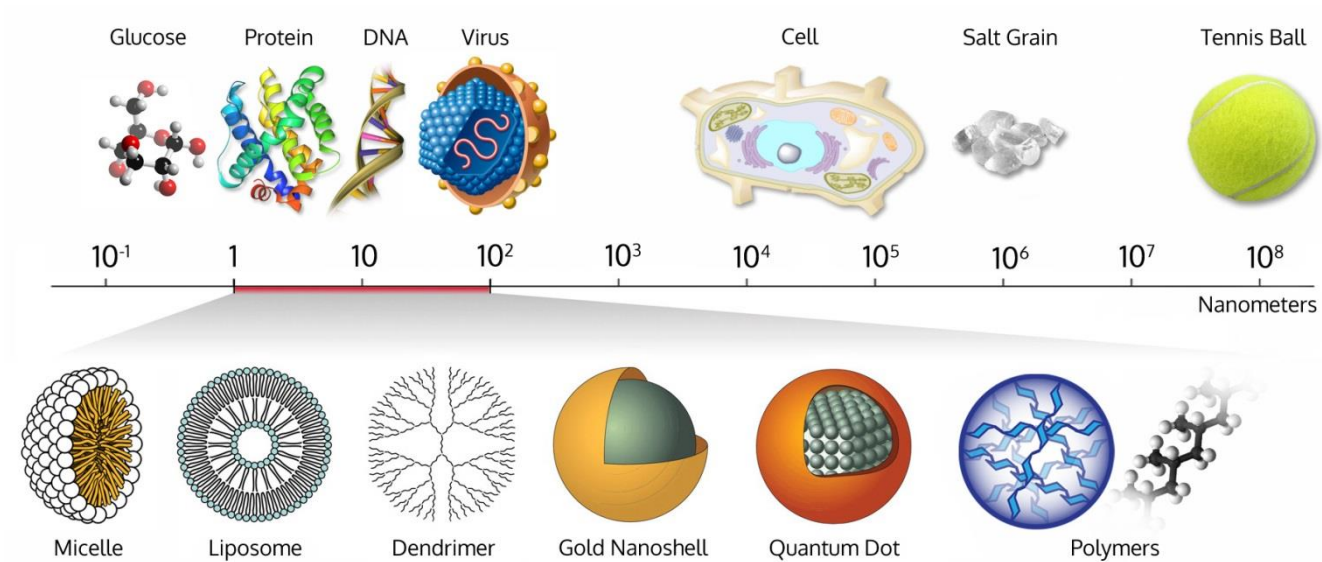


The comet assay in testing the potential genotoxicity of nanomaterials

Amaya Azqueta

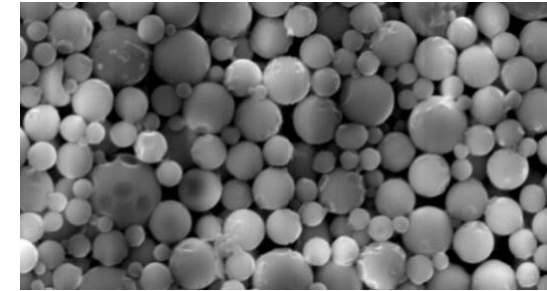
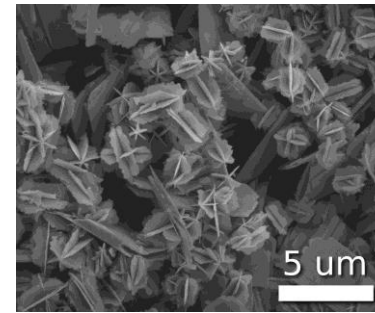
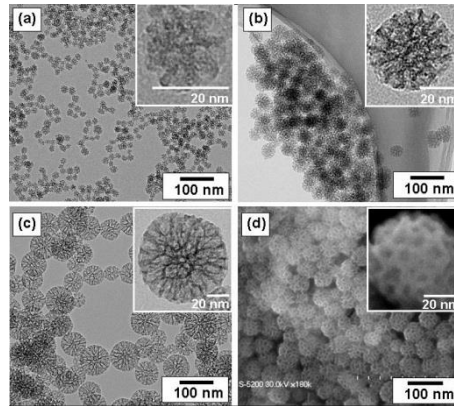
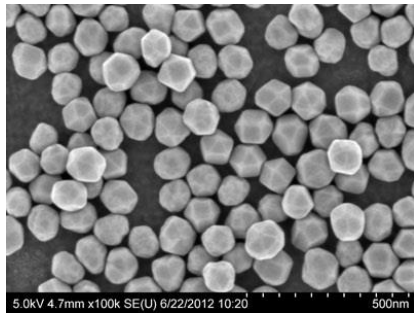
Department of Pharmacology and Toxicology, University of Navarra, Spain



Nanomaterial (NM): at least one of their dimensions is 100 nm or less.

Nanoparticles: are nanomaterials with the three dimensions of 100 nm or less.

(In nanomedicine: particles within the range of nm are considered nanomaterials)



Increase in the surface area: **high reactivity** with the environment

Different physical, chemical and biological properties compared to bulk materials of the same composition

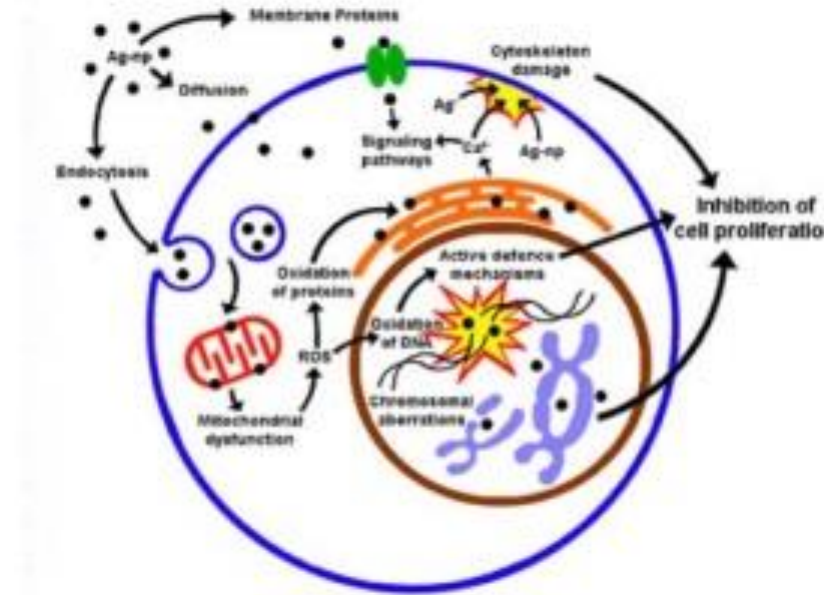
Applications: technology, energy, construction, electronics, agriculture, optics, paints, textiles, food, cosmetics, medicine (diagnosing, monitoring, treating -e.g., drug carriers-, relieving pain...)

Potential toxicity:

- Small size: High cellular uptake

Cellular accumulation → Chronic Toxicity

- High reactivity with the environment (cellular components including DNA)





Organisation for Economic Co-operation and Development

OECD publishes internationally agreed guidelines for several validated and standardized in vitro and in vivo toxicity methods to ensure the safety of chemicals



Organisation for Economic Co-operation and Development

Assay	Guideline
Ames test	No. 471
<i>In vitro</i> chromosome aberration assay	No. 473
<i>In vivo</i> micronucleus test	No. 474
<i>In vivo</i> chromosome aberration assay	No. 475
Cell gene mutation test	No. 476
Unscheduled DNA synthesis (UDS) test	No. 486
<i>In vitro</i> micronucleus test	No. 487
Transgenic rodent somatic and germ cell gene mutation assays	No 488
<i>In vivo</i> comet assay	No. 489 (*)

(*) Adopted on September 2014



Organisation for Economic Co-operation and Development

Assay	Guideline
Ames test	No. 471
<i>In vitro</i> chromosome aberration assay	No. 473
<i>In vivo</i> micronucleus test	No. 474
<i>In vivo</i> chromosome aberration assay	No. 475
Cell gene mutation test	No. 476
Unscheduled DNA synthesis test	No. 486
<i>In vitro</i> micronucleus test	No. 487
Transgenic rodent and germ cell gene mutation assays	No. 488
<i>In vivo</i> comet assay	No. 489 (*)

Are these assays suitable for testing NMS?

(*) Adopted on September 2014



Organisation for Economic Co-operation and Development

2006: Working Party on Manufactured Nanomaterials (WPMN)



Organisation for Economic Co-operation and Development Working Party on Manufactured Nanomaterials

2009

Assay	Guideline
Ames test	No. 471
<i>In vitro</i> Chromosome aberration assay	No. 473
<i>In vivo</i> micronucleus test	No. 474
<i>In vivo</i> Chromosome aberration assay	No. 475
Cell gene mutation test	No. 476
Unscheduled DNA synthesis (UDS) test	No. 486
<i>In vitro</i> micronucleus test	No. 487
Transgenic rodent somatic and germ cell gene mutation assays	No 488
<i>In vivo</i> comet assay	No. 489 (*)

(*) Adopted on September 2014



Organisation for Economic Co-operation and Development Working Party on Manufactured Nanomaterials

2014

Assay	Guideline
Ames test	No. 471
<i>In vitro</i> Chromosome aberration assay	No. 473
<i>In vivo</i> micronucleus test	No. 474
<i>In vivo</i> Chromosome aberration assay	No. 475
Cell gene mutation test	No. 476
Unscheduled DNA synthesis (UDS) test	No. 486
<i>In vitro</i> micronucleus test	No. 487
Transgenic rodent somatic and germ cell gene mutation assays	No 488
<i>In vivo</i> comet assay	No. 489 (*)

(*) Adopted on September 2014



Organisation for Economic Co-operation and Development Working Party on Manufactured Nanomaterials

What about the comet assay?

- The in vitro comet assay: under validation (does not have a OECD guideline yet).
- The in vivo comet assay: OECD guideline adopted on September 2014 (2 months before the last WPMN report)

The comet assay in nanogenotoxicity testing

	Azqueta et al, 2014 102 papers - nanomedicine
<i>In vitro studies</i>	81
Comet assay	52
Micronucleus assay	30
Chromosome aberration test	9
Ames test	9
γ -H2AX by immunostaining	9
<i>In vivo studies</i>	16
Micronucleus assay	11
Comet assay	6
Sporadic Techniques	chromosome aberration assay, gene mutation assay, sister chromatid exchange, γ -H2AX assay by immunostaining in vivo and others.

Azqueta, A. et al. (2014). Genotoxicity of nanoparticles. A,” in *Nanomedicine: Current View, Present and Future Main Regulatory*, eds V. B. Sutariya and Y. Pathak (Boca Raton, FL: Taylor and Francis), 353-363.

The comet assay in nanogenotoxicity testing

	Azqueta et al, 2014 102 papers - nanomedicine	Magdolenova et al, 2014 112 papers
<i>In vitro studies</i>	81	94
Comet assay	52	58
Micronucleus assay	30	31
Chromosome aberration test	9	10
Ames test	9	13
γ -H2AX by immunostaining	9	--
<i>In vivo studies</i>	16	22
Micronucleus assay	11	14
Comet assay	6	9
Sporadic Techniques	chromosome aberration assay, gene mutation assay, sister chromatid exchange, γ-H2AX assay by immunostaining in vivo and others.	chromosome aberration assay in vivo, gene mutation assay, sister chromatid exchange, γ- H2AX assay and others.

Azqueta, A. et al. (2014). Genotoxicity of nanoparticles. A, in *Nanomedicine: Current View, Present and Future Main Regulatory*, eds V. B. Sutariya and Y. Pathak (Boca Raton, FL: Taylor and Francis), 353-363.

Magdolenova, Z. et al. (2014). Mechanisms of genotoxicity. A review of *in vitro* and *in vivo* studies with engineered nanoparticles. *Nanotoxicology* 8, 233–278.

Potential interferences of NMs with the comet assay

- Interaction of NMs with naked DNA: unlikely to be significant (*Karlsson et al, 2015*).
- Interaction of NMs with the staining of the comets; no indication of this phenomenon (visual scoring) (*Karlsson et al, 2015*).
- NMs can interfere with FPG: unlikely when applied correctly (*Magdolenova et al, 2012*).
- Photocatalytic nanomaterials + light → extra breaks (*Karlsson et al, 2014*).

Karlsson H. L. et al. (2015) Can the comet assay be used reliably to detect nanoparticle-induced genotoxicity? Environ. Mol. Mutagen. 56:82-96.

Magdolenova, Z. et al. (2012). Can standard genotoxicity tests be applied to nanoparticles? J. Toxicol. Environ. Health A 75, 800–806.

The comet assay in nanogenotoxicity testing

- Complete the battery of assays to assess the genotoxicity of NMs: MoA.
- Comet assay + lesion-specific enzymes: different DNA lesions such as oxidized bases can be detected, additionally to DNA breaks.



In vivo comet assay: DNA damage induced by secondary mechanisms (inflammation in several organs).

**Thank you very much for your
attention!**



Halley's comet