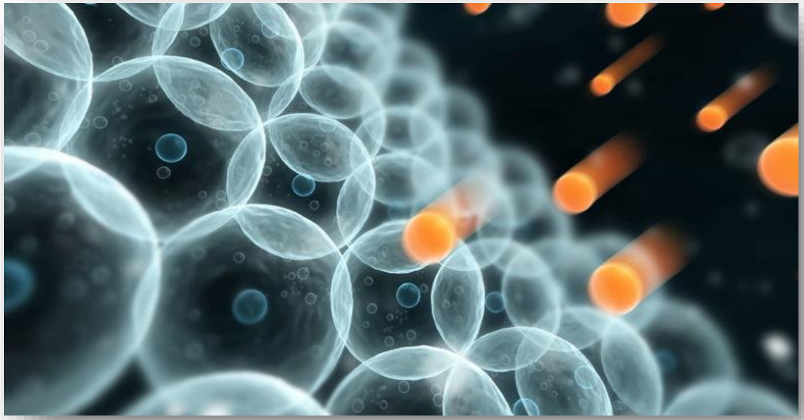
Toxicological profile assessment of ENMs for polymer industry in the context of NanoDesk SUDOE project



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Outline

1. Key aspects on nanomaterial and toxicity
2. NanoDesk SUDOE Project contextualization
3. Methodology for the toxicology profile assessment
4. Results and conclusions

❑ Importance of nanomaterials:

New developments have arisen based on the use of the nanotechnology, which brings innovative opportunities to the industry in general, and in the plastic one in particular

NEW FUNCTIONAL MATERIALS

The incorporation of **nanofillers** allows the development of **high performance polymer based materials**

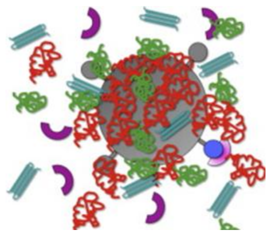
- Mechanical
- Gas Barrier
- Antimicrobial
- Temperature Stability
- UV protection
- Flexibility

- Flame-retardant
- Viscosity
- Shelf life
- Permeability
- Electric and electromagnetic
- Conductivity

❑ Why ENMs can be a risk?

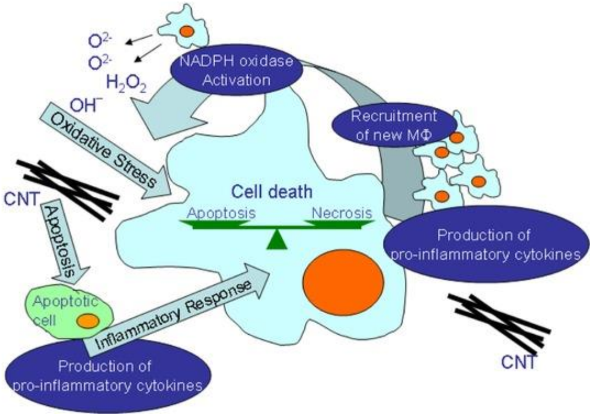
Nanomaterials have very different properties from those of the same bulk substance, and they also have **complex interactions with biological processes**.
So, it could affect their physicochemical and biological behavior, which results in more toxic properties of that nanomaterials.

- ✓ Due to their **small size**, nanomaterials can **translocate** from the skin, lungs or the gastro-intestinal tract into the circulatory and lymphatic systems, and ultimately to body tissues and organs.

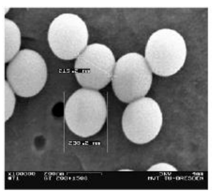


- ✓ Some nanoparticles, depending on their composition and size, can produce **irreversible damage** to **cells** by oxidative stress or/and organelle injury

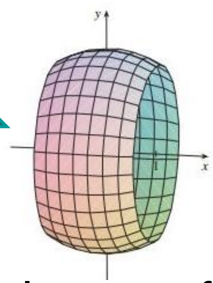
- ✓ Nanomaterials have the greatest potential to **enter the body through the respiratory system** if they are airborne and in the form of respirable-sized particles



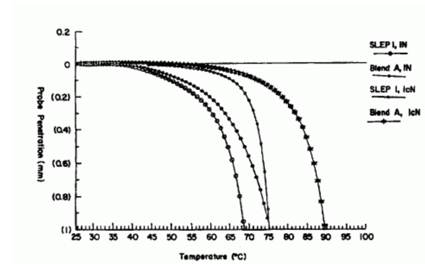
❑ Why ENMs can be a risk?



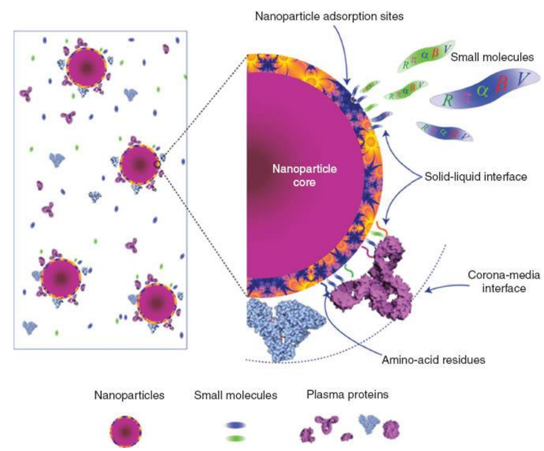
Size particle



Relative surface area

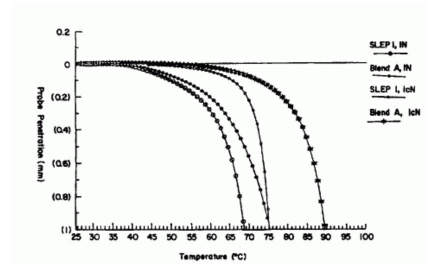


novel and enhanced material properties

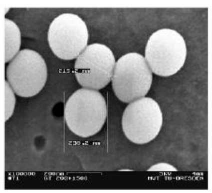


biological reactivity

❑ Why ENMs can be a risk?

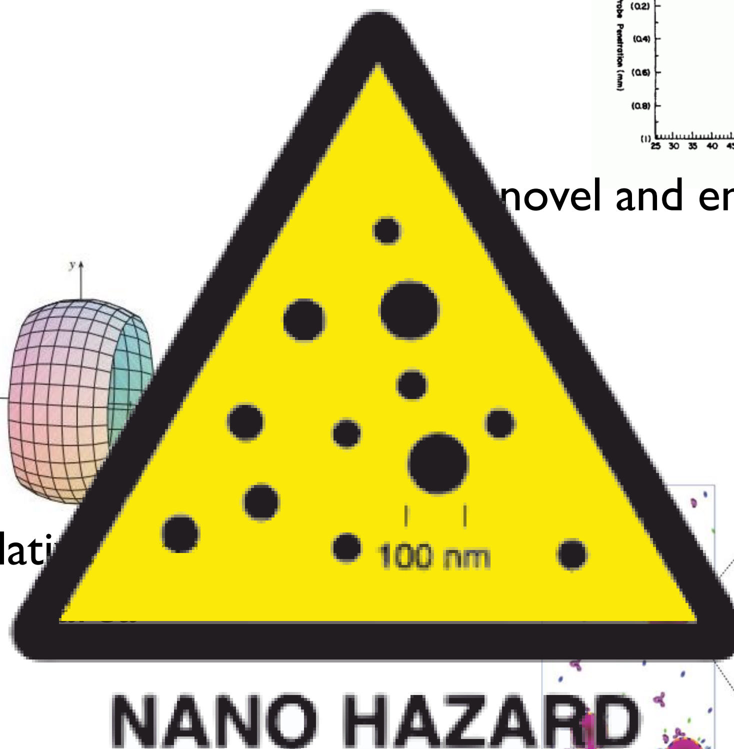


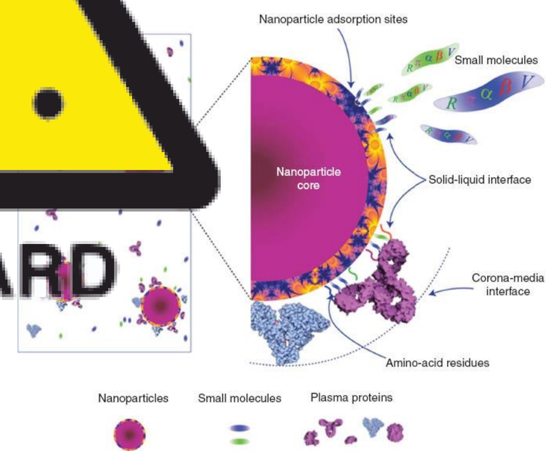
novel and enhanced material properties



Size particle

Relati



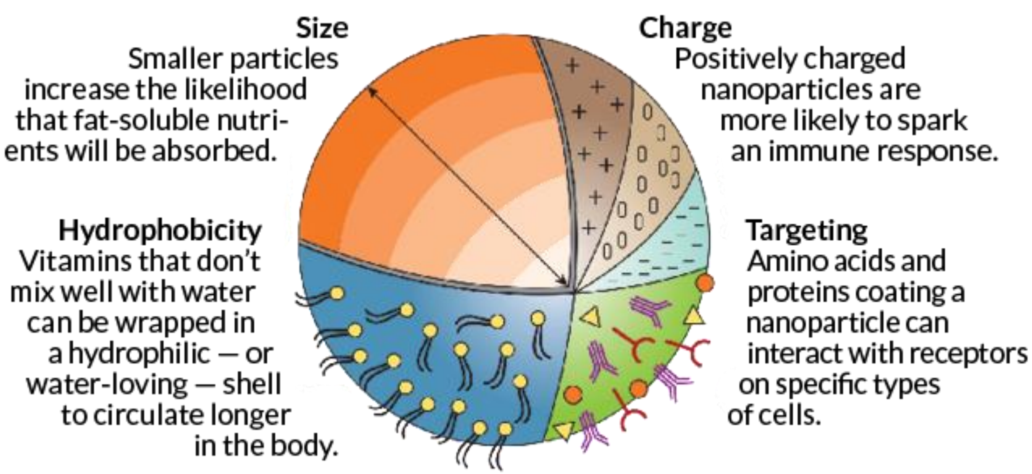


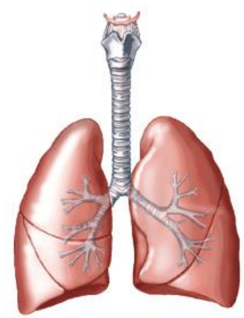
biological reactivity

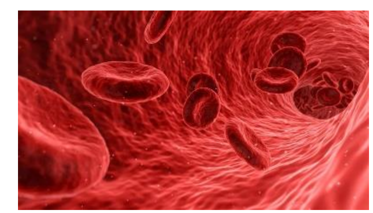
❑ Exposure routes and effects

Nanomaterials can **penetrate the body** through three main routes:

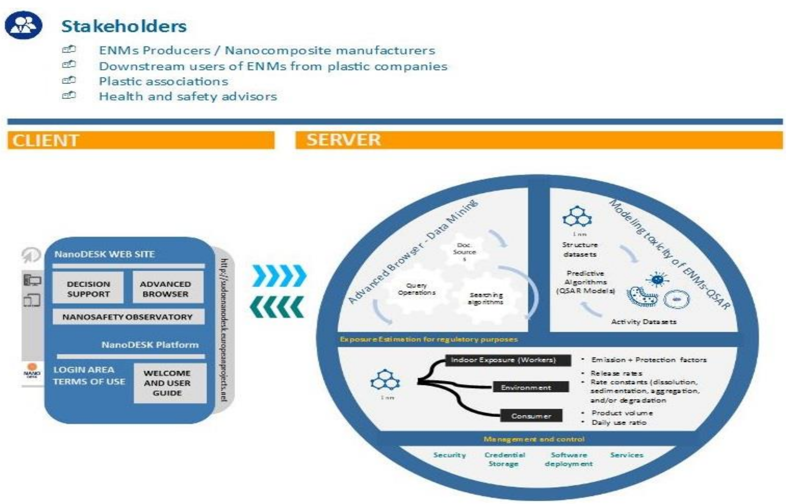
- *Inhalation*
- *Oral*
- *Dermal*





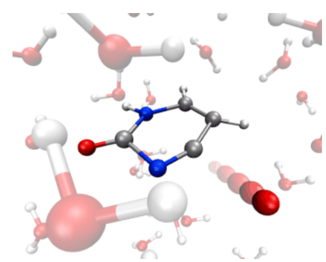


The main objective of the **Interreg SUDOE NanoDesk** project is the development of a series of **web tools** that are easy to access and use. The idea behind these tools is to equip the **plastics industry** with the knowledge necessary for the **safe use of nanomaterials** to improve their products.



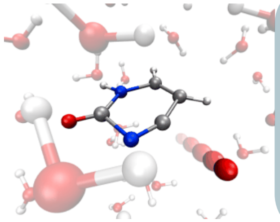
Toxicology as key factor for the safety use of ENMs

TOXICOLOGICAL PROFILE ASSESSMENT



QSAR Model computational predictive toxicology

2. NanoDesk SUDOE Project contextualization



QSAR Model
computational predictive toxicology

Physicochemical ENMs properties ⟷ **Adverse biological effects**

TOXICOLOGICAL PROFILE ASSESSMENT

- ✓ Predictive approach
- ✓ Reduce number of *in vivo* test

Criteria selected:

- Annual production
- Form
- Shape and size
- Forms in the market
- Uses and application
- Toxicological and ecotoxicological profile

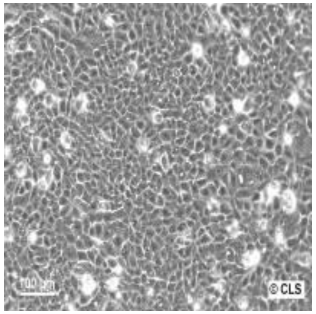
Targeted nanomaterials:

- SWCNT
- MWCNT
- Ag NPs
- Au NPs
- TiO_2
- ZnO
- SiO_2
- Al_2O_3
- $CaCO_3$
- CuO
- Graphene
- Carbon black
- Fullerenes
- Nanocellulose
- Nanoclays
- $Sb_2O_5SnO_2$

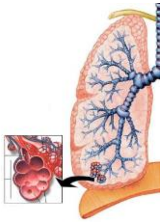
3. Methodology for the toxicological profile assessment

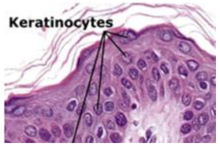
❑ Toxicological profile

With **inhalation** and **skin contact** considered to be the main routes of exposure in an occupational setting in the plastic industry, toxicological testing has focused on the most sensitive cells in the lungs and the skin.



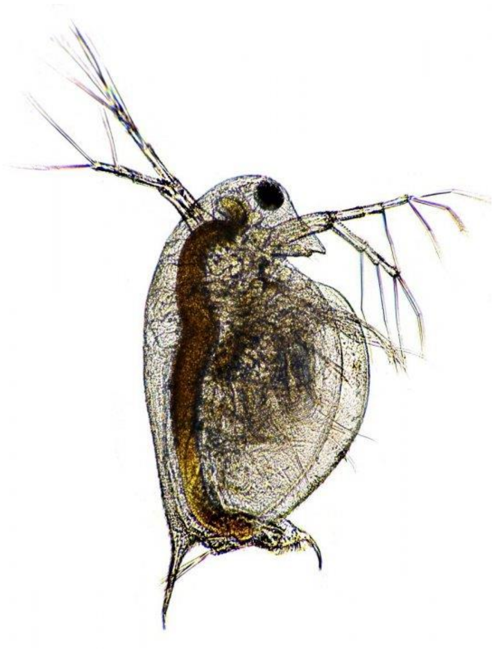
LINE CELLS	**ENDPOINT**	**METHOD**
Adenocarcinomic human alveolar basal epithelial cells **A549**	Cellular damage (**cytotoxicity**)	MTT Proliferation Assay
Spontaneously immortalized aneuploid human keratinocyte cell line **HaCaT**	DNA damage (**genotoxicity**)	Comet Assay





❑ Ecotoxicological profile

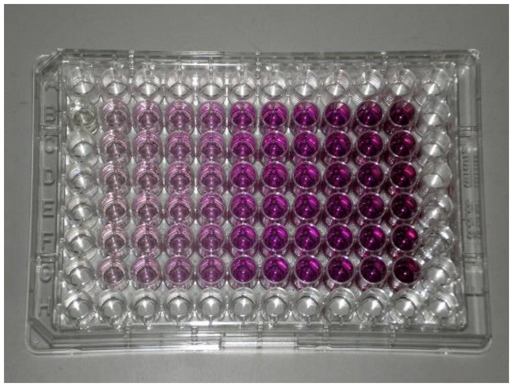
To assess ecotoxicological impact, we performed **acute toxicity assays:**



COMPARTMENT	Freshwater
ORGANISM	Daphnid (Daphnia magna)
ENDPOINT	EC_{50}
DESCRIPTION	Acute immobilisation test
STANDARD	OECD 202

4. Results and conclusions

❑ Toxicological profile assessment



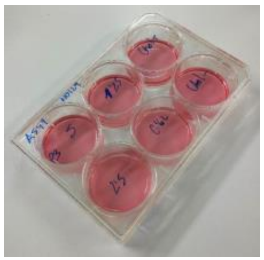
MTT Proliferation Assay

		A549				HaCaT		
COMPOUND	CONCENTRATION (ppm)	IC_{50}		IC_{10}		IC_{50}		IC_{10}
Nanobyk SiO2	100-6,25	27,619	**Low**	7,881	Moderate	Not peformed yet		
Antimony tin oxide	100-6,25	72,628	**Low**	7,003	Moderate	119,95	**Null**	-
Graphene (I)	100-6,25	86,988	**Low**	0,968	High	1,744	**Moderate**	-

✓ Graphene is presented as the compound tested with a higher level of cytotoxicity if it is considered IC_{10}

4. Results and conclusions

❑ Toxicological profile assessment

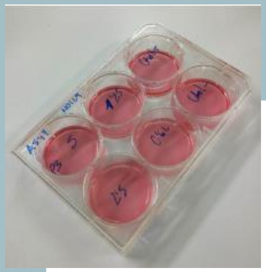


COMET ASSAY

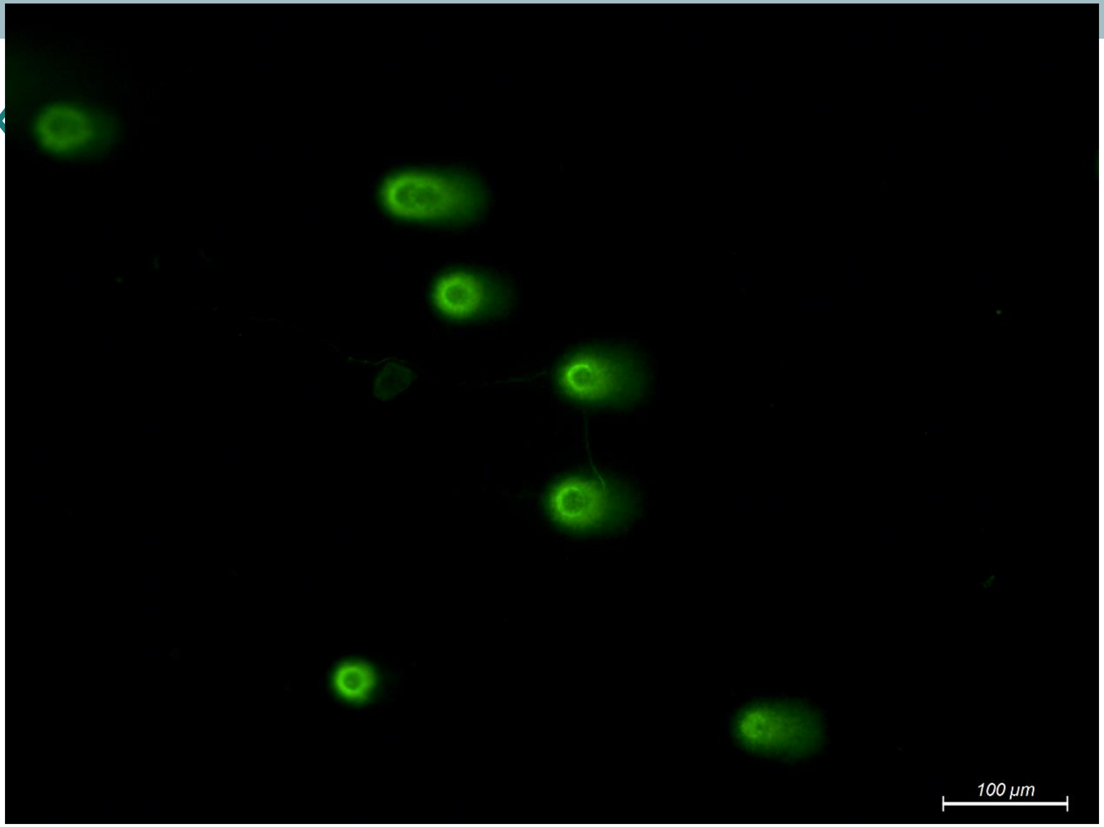
		A549		HaCaT	
COMPOUND	CONCENTRATION (ppm)	LEVEL OF DAMAGE	% AFFECTED CELLS	LEVEL OF DAMAGE	% AFFECTED CELLS
Graphene (I)	5	Low	20%	Moderate	65%
	1	Null	10%	Low	60%
Graphene (II)	5	Low	50%	High	70%
	1	Low	50%	High	95%
Antimony tin oxide	5	Moderate	35%	High	99%
	1	Low	60%	High	99%
Titanium oxide	5	Moderate	25%	Not performed yet	
	1	Low	40%		

- ✓ For all tested compounds, toxicity is observed at a concentration of 5ppm.
- ✓ For all the damage level is higher in HaCaT cell line than in A549 cells, which leads to think that these are more sensitive

4. Results and conclusions



❑ Tox



Fluorescence Microscopy (LAS CORETM, LEICA Microsistems) image example: TiO2 studied nanoparticle by Comet Assay (5ppm concentration). Long comet tails extending toward the anode were observed as an indicator of DNA damage.

❑ Ecotoxicological profile assessment

COMPOUND	DAFNIA (EC50 24h) mg/L	LEVEL OF DAMAGE	DAFNIA (EC50 48h) mg/L	LEVEL OF DAMAGE
Graphene (I)	16,410	Low	0.054	Very high
Graphene (II)	100.96	Null	33,060	Low
Antimony tin oxide	-	Null	0.007	Very high
Titanium oxide	9,278	Moderate	0.296	High
Calcium carbonate	>100	Null	0,181	High
Titanium oxide	>100	Null	>100	Null
Silicon dioxide	-		16,432	Null
Aluminum oxide	>100	Null	>100	Low
Nanoclay	>100	Null	>100	Null

✓ At 48 hours of testing, the compounds that have a higher EC50 are:

graphene

antimony tin oxide

4. Results and conclusions

❑ Overview

- ✓ The nanometric properties of ENMs are very useful for improving the properties of plastic, but they can also pose a greater risk to health and the environment
- ✓ A specific toxicological evaluation is necessary for the ENMs (different from what is done in its bulk form)
- ✓ Knowing the toxicological and ecotoxicological properties of ENMs encourages safer use of them, and therefore, involves greater application of nanotechnology in plastic
- ✓ In order to develop QSAR models framed in the project, apart from the bibliographic data, batteries of toxicological and ecotoxicological tests are being developed to study damage in marine ecosystems, cytotoxicity and genotoxicity
- ✓ Although the status of toxicological studies in the project is still early, there are two compounds that stand out above the rest for their level of toxicity: graphene and antimony tin oxide
- ✓ More studies are needed focusing on the toxicological risk assessment of nanomaterials, as well as standardized test guides.

Thanks for your attention!

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