ECOTOXICITY DATA FOR THE GROUPING OF NANOMATERIALS. PROJECT NanoReg2

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Necessity of ecotoxicological data for the registration of substances Example of REACH

Substance Registration: Information about substance physico-chemical properties and about hazard (toxicity and ecotoxicity) is needed

Annexes VII to IX ecotoxicological information required

Annex	Tonnage	Ecotoxicological information			
VII	**1 tonne or more**	Short term Daphnia	Algae growth inhibtion	Biodegradability	
VIII	**10 tonnes or more**	Short term Daphnia	Algae growth inhibtion	Biodegradability	
VIII	**10 tonnes or more**	Short term Fish	Sludge respiration inhibition testing	Degradation	Adsorption/desorption
IX	**100 tonnes or more**	Short term Daphnia	Algae growth inhibtion	Biodegradability	
IX	**100 tonnes or more**	Short term Fish	Sludge respiration inhibition testing	Degradation	Adsorption/desorption
IX	**100 tonnes or more**	Long term Daphnia	Fish early life stage toxicity	Fish short term toxicity on embryo	Fish juvenile growth test
IX	**100 tonnes or more**	Long term Fish	Degradation biotic	Degradation surface water	Bioacumulation in fish
IX	**100 tonnes or more**	Soil simulation testing	Sediment simulation testing	identification of degradation products	Further adsorption/desorption information
IX	**100 tonnes or more**	Short term toxicity Soil invertebrates	Effects on soil microorganisms	Short-term toxicity to plants	

NanoReg2 deals with
"Regulatory oriented activities establishing a framework of grouping approaches"

One specific objective of NanoReg2

To determine if nanomaterials (including also nanoforms of the same substance) can be grouped according to their physico-chemical properties and ecotoxicological effects

The importance given in REACH to the generation of (eco)toxicity data by means of alternative and *in vitro* methods

NanoReg2 considers ecotoxicity data generated *in vitro* using fish and mussel cells

The grouping approach using ecotoxicology data

1st step: Compilation of basic ecotoxicity data for substances (nanomaterials) according to the necessities for registration under REACH

- **Algae growth inhibition**
- **Daphnia immobilitation**
- **Fish acute toxicity**

TiO2	NM100
	NM101
	NM102
	NM103
	NM104
	NM105
ZnO	NM110
	NM111

SiO2	NM200
	NM201
	NM202
	NM203
	NM204
MWCNTs	NM400
	NM401
	NM402
	NM403

The grouping approach using ecotoxicology data in NanoReg2 WP1

Ecotoxicity data collection from available sources

- OECD dossiers for nanomaterials
  http://www.oecd.org/chemicalsafety/nanosafety/dossiers-and-endpoints-testing-programme-manufactured-nanomaterials.htm

- NanoReg2 data base
  Data from projects MARINA and Nanoreg

The grouping approach using ecotoxicology data in NanoReg2 WP1

Ecotoxicity data collection from available sources

Problem 1: lack of harmonization

- **Data obtained using different methodologies in the tests**
  - **Different duration of the same tests**
  - **Different species used in the same test**
  - **Etc…**

- **Variations in the dispersion procedures**

Problem: Available data bases and bibliography did not allow to generate a table appropriate for a grouping exercise (important data gaps)

		OECD 201	OECD 201	OECD 202	OECD 202	OECD 202	OECD 202	OECD 203	OECD 203	OECD 204	OECD 204	OECD 210	OECD 210	OECD 211	OECD 211	OECD 212	OECD 212	OECD 236	OECD 236
		EC50	EC10	EC50	EC10	EC50	EC10	EC50	EC10	EC50	EC10	EC50	EC10	EC50	EC10	EC50	EC10	EC50	EC10
				48h	48h	24h	24h	96h	96h	14d	14d	35d	35d	21d	21d	8d	8d	72h	72h
TiO2	NM100			>50	>50													>100	>100
	NM101	50,57	4,38	79,52														>100	>100
	NM102			>50	>50													>100	>100
	NM103			>1500	>1500									> 10	> 10				
	NM104			>1500	>1500									> 10	> 10				
	NM105	230	2																
	P25	28,35	2,01	>1500	>1500									12,1	0,34				
ZnO	NM110	0,07	0,02	1,87	0,89	17,6	3,05					> 0,54	> 0,54						
	NM111			1,1	1,62	> 100	5,8												
SiO2	NM200					> 10000	> 10000	> 10000	> 10000										
	NM201					> 10000	> 10000	> 10000	> 10000										
	NM202					> 1000	> 1000	> 10000	> 10000										
	NM203					> 1000	> 1000												
	NM204																		
CNTs	NM400																		
	NM401																		
	NM402							>100											

The grouping approach using ecotoxicology data in NanoReg2 WP1

Ecotoxicity data collection from available sources

Problem 1: lack of harmonization in the ecotoxicity tests

- Data obtained using different methodologies, not only in the tests but in the dispersion procedures.
    - Different duration of the same tests
    - Different species used in the same test
    - Etc…

Problem 2: lack of data about physico-chemical properties of nanomaterials in ecotoxicity media

Solution: Generation of own COMPARABLE data

Three different laboratories: UCO, INERIS, INIA

Working with:

- **Same nanomaterials**
- **Same batch**

JRC repository of industrial representative nanomaterials (17 NMs)

TiO2	NM100
	NM101
	NM102
	NM103
	NM104
	NM105
ZnO	NM110
	NM111
SiO2	NM200
	NM201
	NM202
	NM203
	NM204
MWCNTs	NM400
	NM401
	NM402
	NM403

Solution: to generate own COMPARABLE data for a grouping exercise

DISPERSION PROTOCOL (NANOGENOTOX)

- Pre-wetting NM with EtOH
  - Dispersion MQ water (2.56 mg/ml)
  - Dispersion with 0.05 wt% BSA – MQ water (2.56 mg/ml)
- Probe sonication
- Dilution (1/10) in media (256 µg/ml)

NM CHARACTERIZATION IN TEST MEDIUM

- DLS (size frequency distribution)
- DLS (Z-potential)
- Stability through colorimetry (UCO)
- TEM in cell culture media (INIA) (Shape)

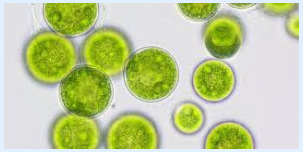
REPORTED DATA

Calculated using the REGTOX macro in excel EC_{10}; EC_{25}; EC_{50}

Solution: to generate own COMPARABLE data for a grouping exercise

Tests used

- **Algae growth inhibition test (OECD TG201)**

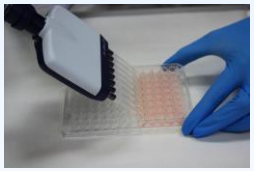


- **Daphnia acute immobilization test (OECD TG202)**

- **Fish acute toxicity test (OECD TG203)**

Ethical issues (importance of *in vitro* methods in REACH)

Cytotoxicity assays on fish and mussel cells

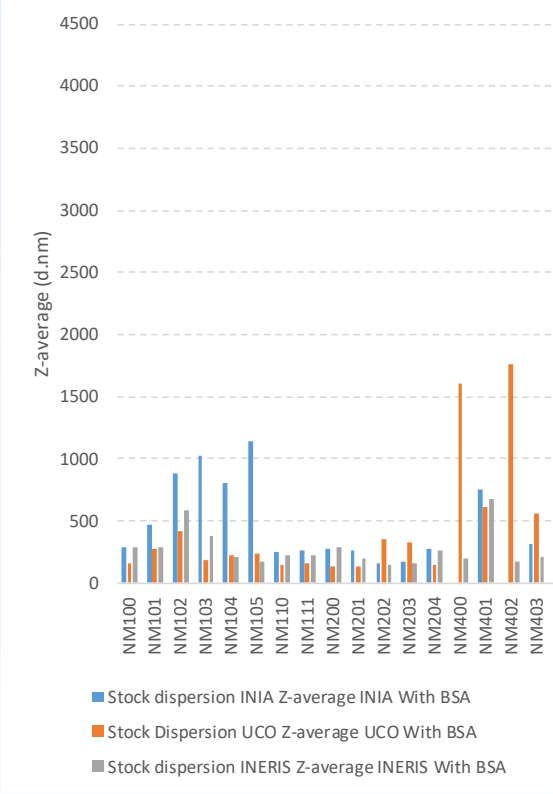


Fish cell lines:
- **AlamarBlue**
- **Neutral Red**
- **CFDA-AM**

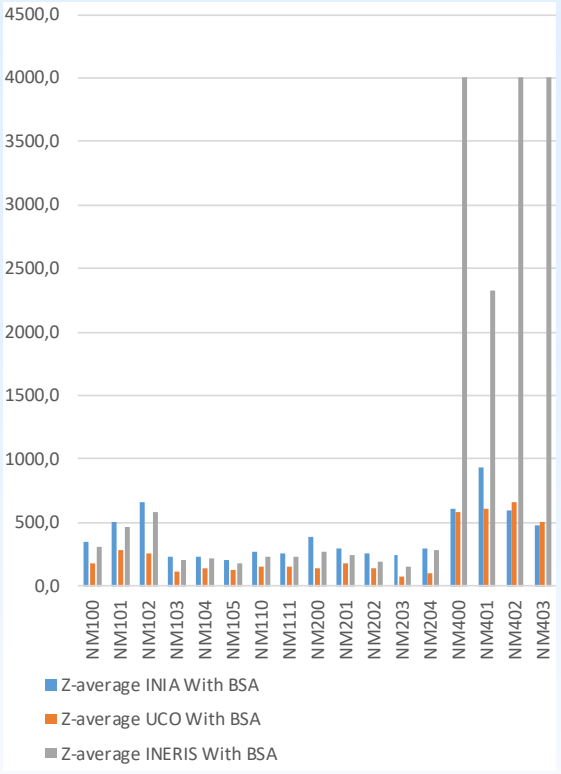
Mussel cells:
- **Alamar Blue**
- **CFDA-AM**

NanoReg2 Ecotoxicological phys-chem data set – system dependent characterization

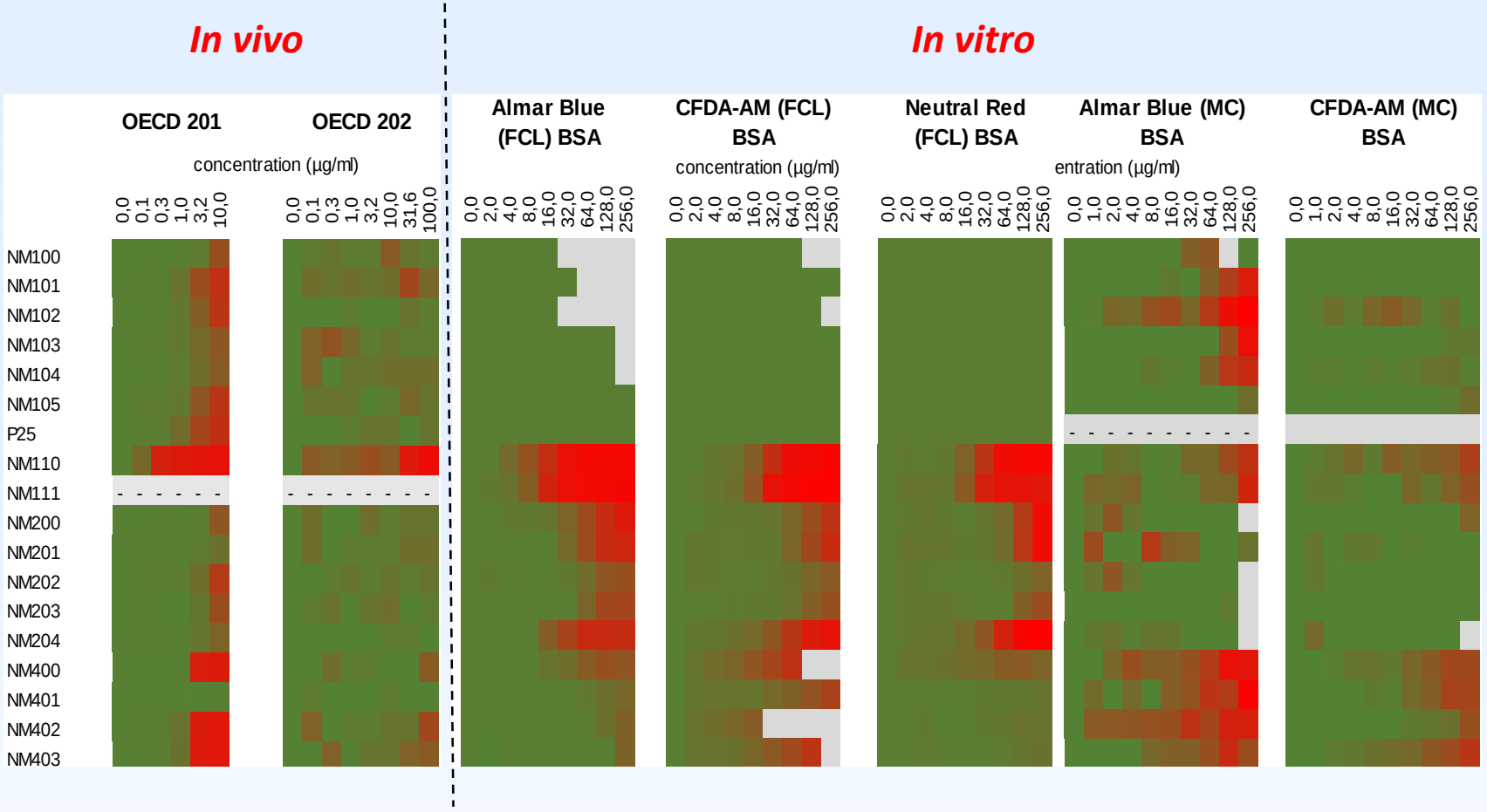
Initial stock dispersion



Ecotoxicological media



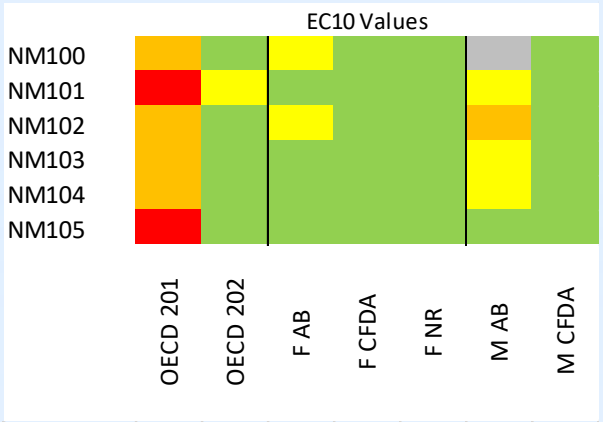
NanoReg2 ecotoxicity data set

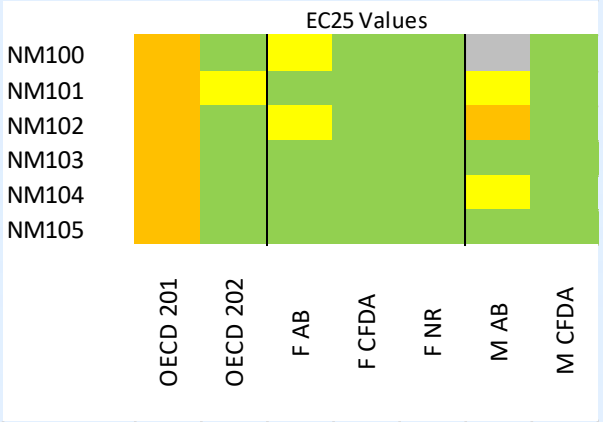


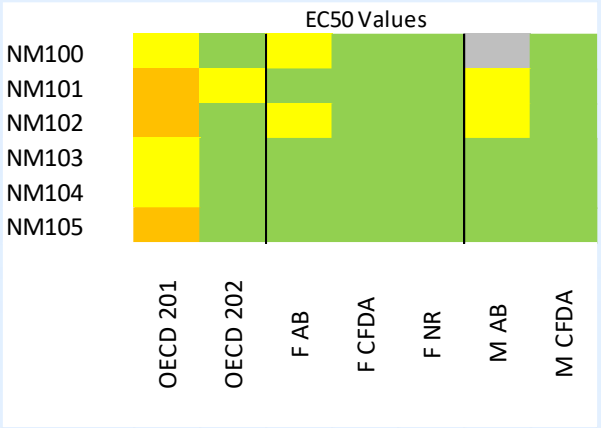
→ **Dose-response used for EC_{10}, EC_{25} and EC_{50} estimation**

Example with TiO_2 NPs

Non toxic	> 100	Mod. toxic	1-10
Slightly toxic	10-100	Highly toxic	<1







- Alage growth inhibition test (TG201) EC_{10}, most sensitive endpoint. 2 groups: [101, 105] / [100, 102, 103, 104]
- Daphnia acute immobilization test (TG202). Low sensitivity. 2 groups: [101] / [100, 102, 103, 104, 105]
- *In vitro* FCL Alamar Blue test. Low senstiivty. 2 groups: [100, 102] / [101, 103, 104, 105]
- *In vitro* mussel cells Mussel Alamar Blue test, EC10, 3 groups: [102] / [101, 103, 104] / [105]
- *In vitro* CDFA-AM and NR in fish or mussel: no toxicity detected. No groups could be established.

Different grouping results depending on:
- Assay
- EC_x considered

Differences could be related to:
- Differences in test media
- Differences in mechanism of toxic action

Question:
- How to integrate in a grouping strategy, results obtained with different tests and EC_x?

Need to go further and analyse data considering phys-chem properties

Similarity analysis of nanomaterials

- The results of ecotoxicity measurements were sent to Gdansk University team in order to perform similarity analyses
- At start, metal and metal oxide manufactured nanomaterials from the JRC repository are being analyzed.
- Analyses are still in progress, however there are already some preliminary results.

Similarity analysis of nanomaterials

- Analyses are done per independent assay and variable, e.g.
  - Algae growth inhibition test EC_{10},
  - Algae growth inhibition test EC_{25},
  - …

- The dataset for pristine and system-dependent characterization of nanoparticles comprises measured and computed descriptors.

MARINA, NanoReg2 Approach

What they are?
(Intrinsic physico-chemical properties descriptors)

Where they go?
(System dependent physico-chemical descriptors)

What they do?
(Ecotoxicity descriptors)

Similarity analysis of nanomaterials

Examples of descriptors used in analyses

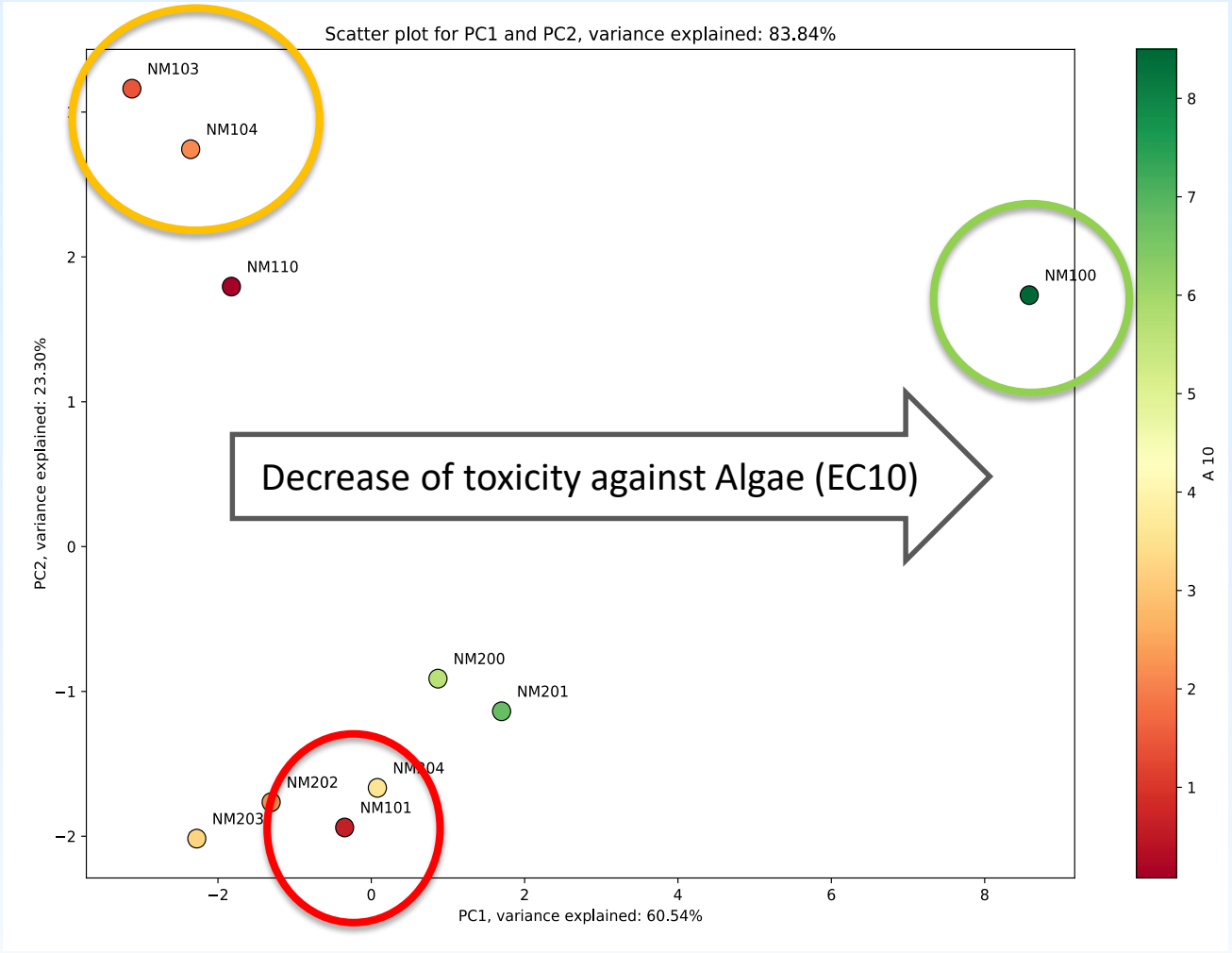
TEM	Diameter Mean (nm)	DM Mean	mean value in measurements
		DM Max	maximum value in measurements
		DM Min	minimum value in measurements
		DM Median	median value in measurements
	Diameter Max (nm)	DMAX Mean	mean value in measurements
		DMAX Max	maximum value in measurements
		DMAX Min	minimum value in measurements
		DMAX Median	median value in measurements
QM Descriptors MeOx		Hof	heat of formation
		total	total energy
		area	surface area
		volume	total volume
		HF	Hartree Fock energy
System-dependent characteristics		Zeta Pot medium	measured zeta potential in medium
		Diameter medium (d-nm)	size in medium
		PdI medium	Polydispersity index in medium

Similarity analysis of nanomaterials

Example with algae test EC_{10} values

OECD 201	
	EC10
NM100	8,50
NM101	0,61
NM103	1,44
NM104	2,14

PC2: System dependent characteristics (*Z-average medium, DLS peak 1 in medium, Zeta pot medium*)



PC1: Pristine material characterization (*Elongation, Aspect ratio, Central Distance, Convexity, Shape*)

Similarity analysis of nanomaterials

- There are diversified trends in distribution of toxic and nontoxic nanoparticles for most endpoints and even for different measures (EC_{10}, EC_{25} and EC_{50}).

- The approach is mainly explorative, nonetheless, the main aim is to systematize the knowledge about the relationships between physicochemical structure and toxic properties of metal oxides nanoparticles.

- By applying a homogenous scale – a scoring system, the toxic characterization of each nanoparticle will be more informative and simultaneously, comparisons among nanoparticles' toxicity (including different assays) will be more efficient.

Thank you very much!!!

Happy to take your questions.