

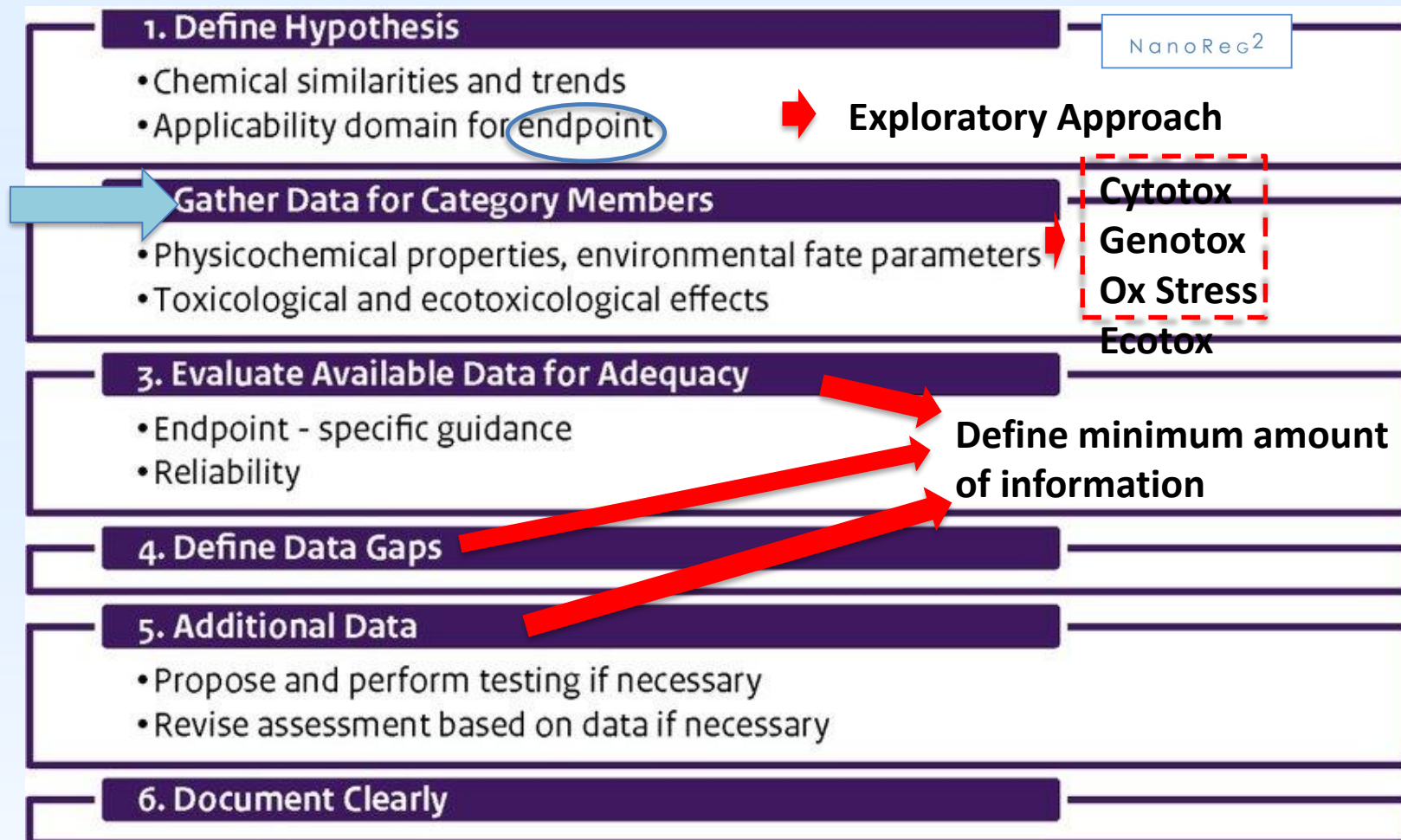
# NanoReg2 grouping case studies

## Human tox

Blanca Suarez-Merino (SCAHT/TEMAS)

Rodríguez-Llopis I and Gómez-Fernández P (GAIKER), Haase A and Giusti, A (BfR), Jacobsen NR and Jensen K (NRCWE), Dusinska M, Rundén-Pran E and Mariussen E (NILU), Sandström J and Aicher L (UniBas), Gromelski M and Puzyn T (UG), Carnovale C and Balusamy B (IIT), Apostolova M (IMB-BAS) De Angelis I, Barone F, Battistelli C, Bossa C, Zijno A and Giuliani, A (ISS) Grall R (CEA), Tanasescu S (IPC)

# ECHA Guidelines for Read-across and Grouping (2017)



Source: Sellers, K & Deleebeeck, Nele & Messiaen, Marlies & Jackson, M & Bleeker, Eric A. J. & Sijm, Dick & A. van Broekhuizen, F. (2015). Grouping Nanomaterials - A strategy towards grouping and read-across.

**Data gathering for category member**

**(Cytotox, Genotox, Ox Stress)**

- Data download templates (harmonised)
- NanoReg2 database
- Data scoring (based on NanoSolutions)

**Evaluate Data for adequacy**

**(manual curation)**

- Phys chem characterization
- SOPs
- Exposure times
- Concentrations (metrics)
- Cell lines

**Identification of Data Gaps**

- Data matrix (per assay)

**Additional data**

- Identification of most relevant assay per end point
- Perform testing for gap filling



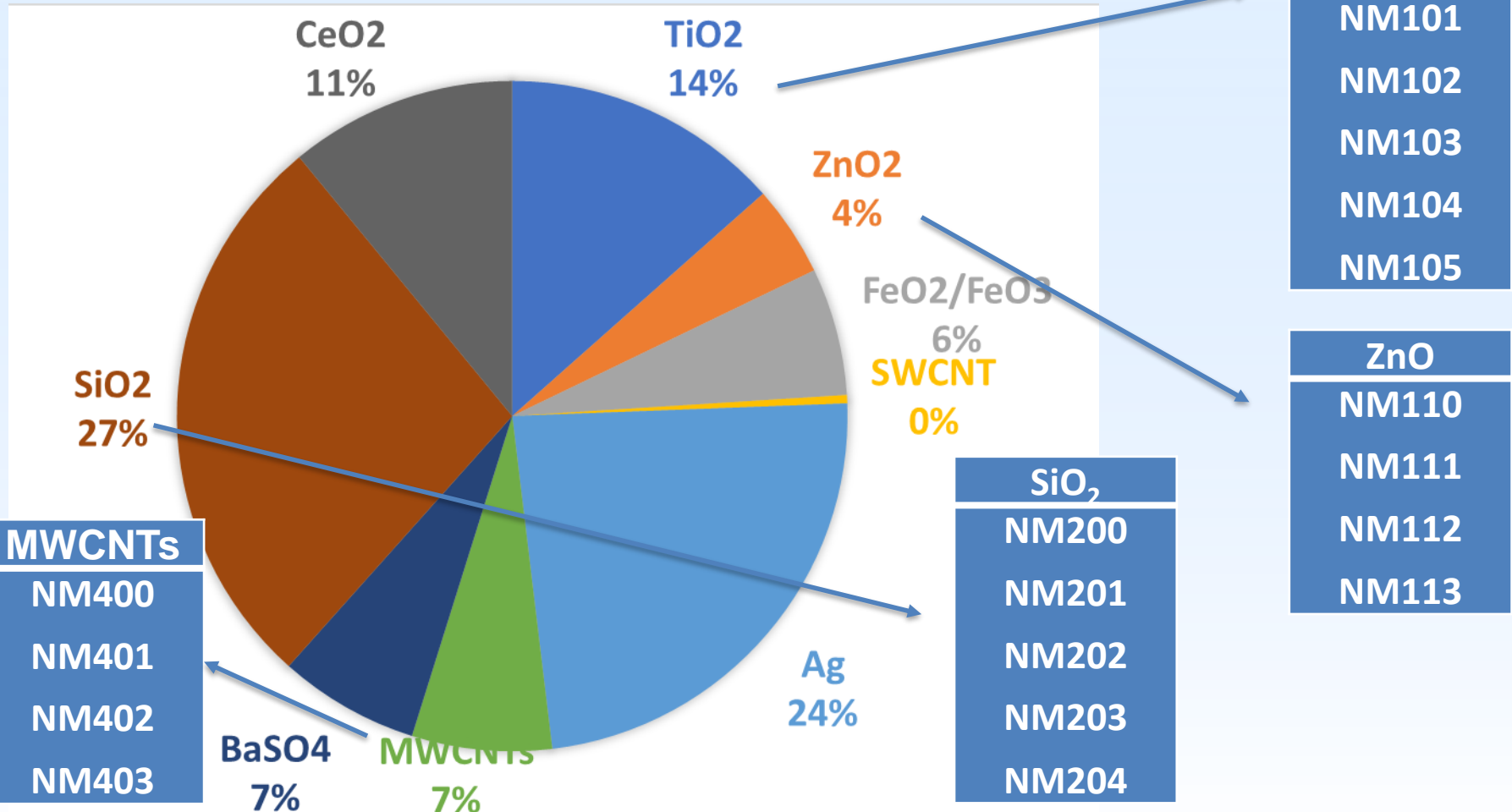
**Reporting**

# Data gathering for category members

Selection of nanomaterials based on availability of data (availability of several nanoforms)

## *Industrially relevant materials*

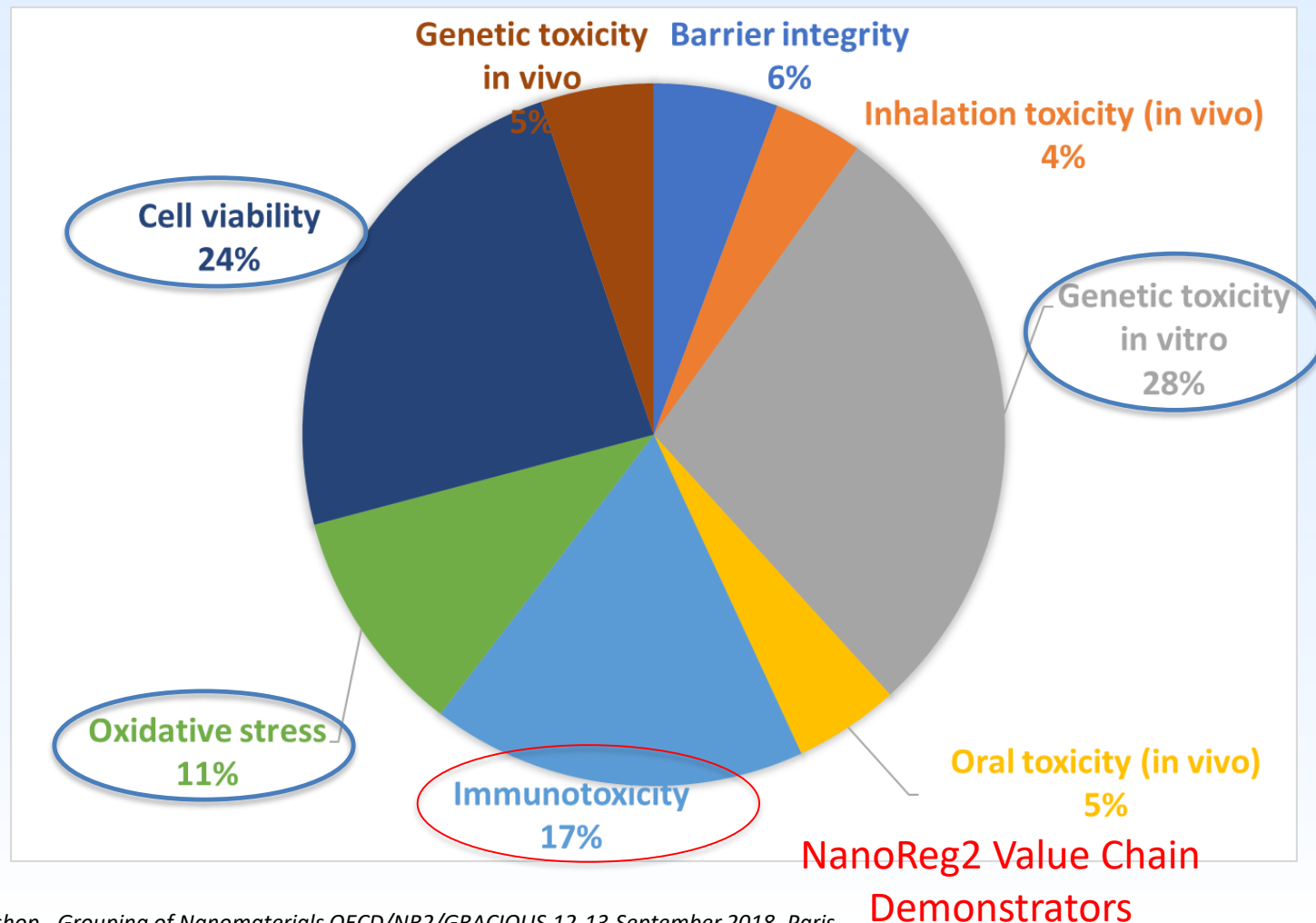
Data retrieved from the NanoReg2 database



# Data gathering for category members

Data availability per end point

Data retrieved from the NanoReg2 database  
(MARINA, NANoREG, NanoGenotox, NanoTest)



# Initial Approach – Data gathering and identification of gaps

(A549, Caco-2, THP-1, BEAS-2B)

**Cytotox**

- MTT
- AB
- CFE
- NRU
- LDH
- Impedance

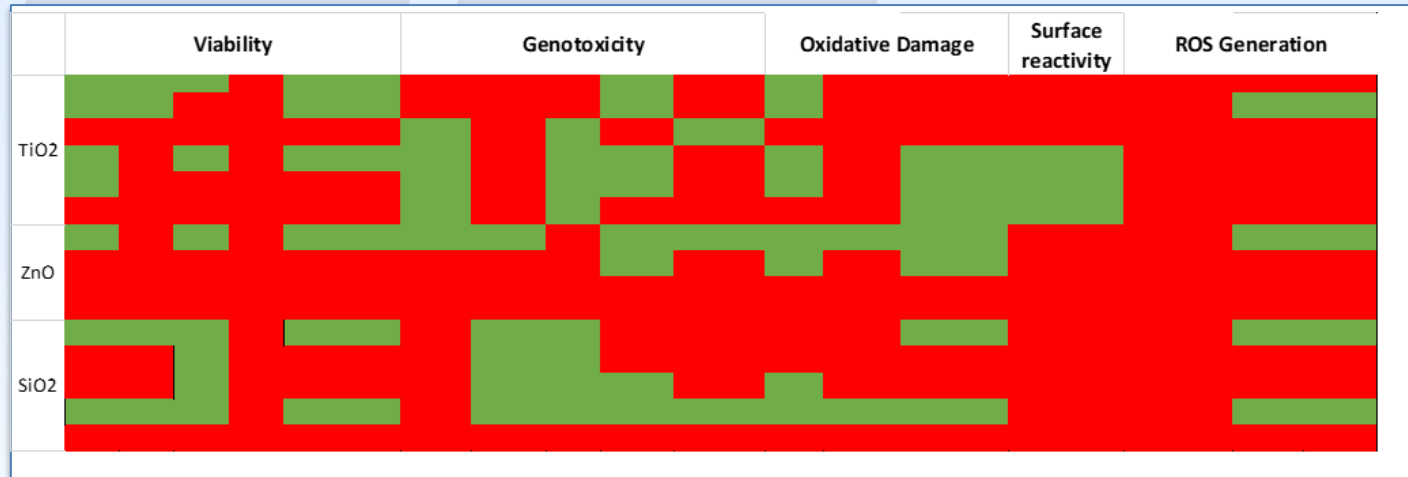
**Genotox**

- MN
- Comet
- Comet Fpg

**Ox Stress**

- DCFH2-DA

**19 materials!**



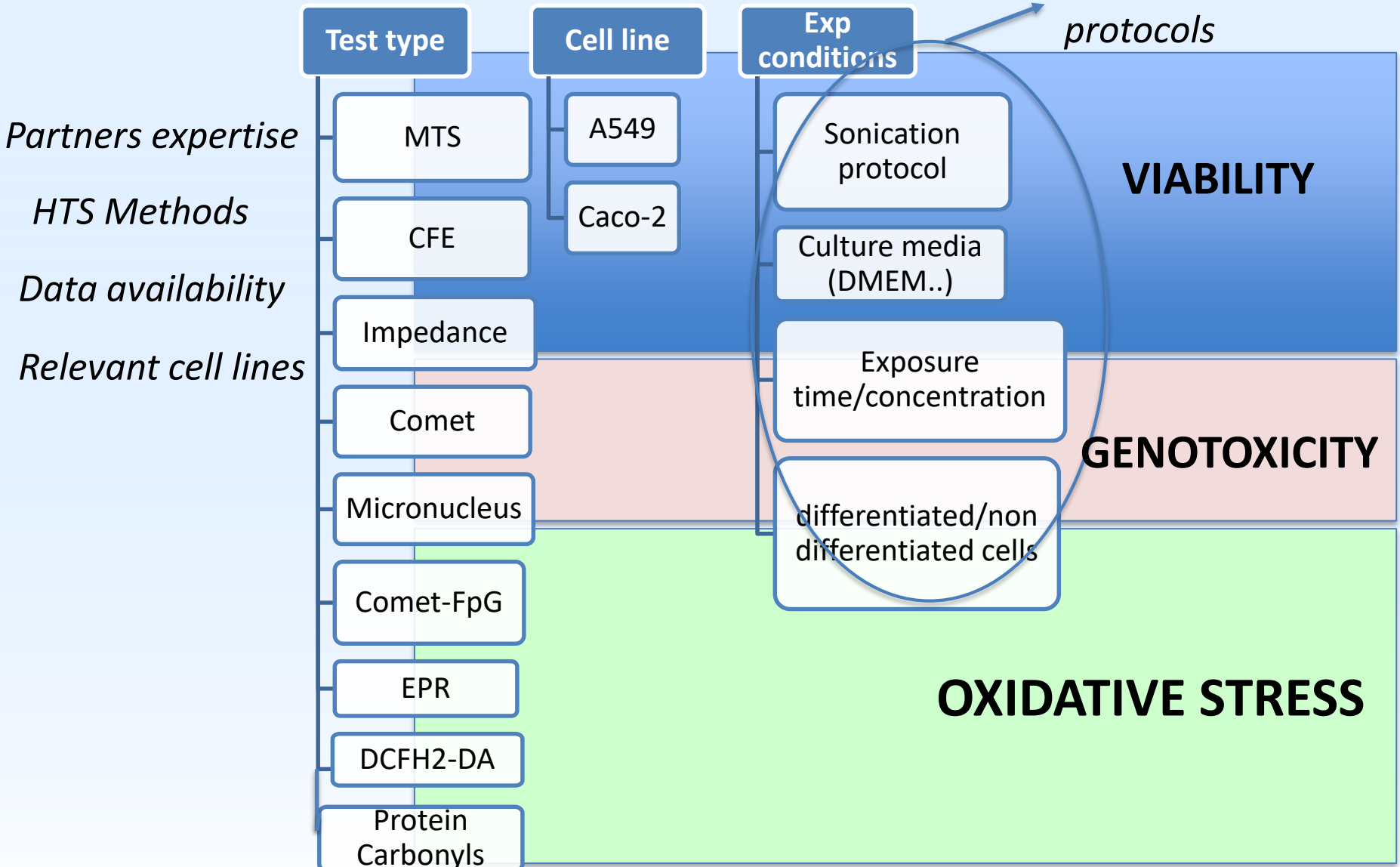
NanoReg2 Database

■ Data Gaps

**80% GAPS!!!**

Follow NANoREG protocols

# Additional data collection



**15 nanomaterials plus 6 materials from NanoReg2!!!**

# Data gap filling

Assays	Viability						Genotoxicity					Oxidative Damage		Oxidative Stress				
	CFE		Impedance		MTS		Micronucleus			Comet		Comet FpG		Protein carbonyl		ESR/EPR	DCFH2-DA	
NM	A549	Caco-2	A549	Caco-2	A549	Caco-2	A549/24	A549/48	Caco-2	A549	Caco-2	A549	Caco-2	Lit/BfR NRK-52E	A549	acellular	A549	Caco-2
TiO <sub>2</sub>	NM100																	
	NM101																	
	NM102																	
	NM103																	
	NM104																	
	NM105																	
ZnO	NM110																	
	NM111																	
	NM112																	
	NM113																	
SiO <sub>2</sub>	NM200																	
	NM201																	
	NM202																	
	NM203																	
	NM204																	

 Data Gaps

**Only 10% Data gaps!**



# Data scoring (NanoSolutions adapted to NanoReg2) NanoReg<sup>2</sup>

		Points
Toxicity reaches	20%	+1
	50%	+1
	80%	+1
IC50	≤100 µg/mL	+2
	≤ 60 µg/mL	+2
	≤ 20 µg/mL	+2

Points	Category	Score
0 - 1	1	non toxic
2 - 5	2	slightly toxic
6 - 9	3	toxic

(Too many categories for modelling)



**Cytotoxicity**

## Genotoxicity

Category	Score	Criteria
Negative	1	No significant effect
Equivocal	2	Dose response/one concentrations significantly different from control
Positive	3	Dose response and one concentration significantly different from control

# Data scoring (NanoSolutions adapted to NanoReg2) NanoReg<sup>2</sup>

		Points	Points	Category
<b>Toxicity reaches</b>	20%	+1	0	1
	50%	+1	1	2
	80%	+1	2-5	3
<b>IC50</b>	≤100 µg/mL	+2	6,7	4
	≤ 60 µg/mL	+2	8,9	5
	≤ 20 µg/mL	+2		

Points	Category	Score
0 - 1	1	non toxic
2 - 5	2	slightly toxic
6 - 9	3	toxic



## Cytotoxicity

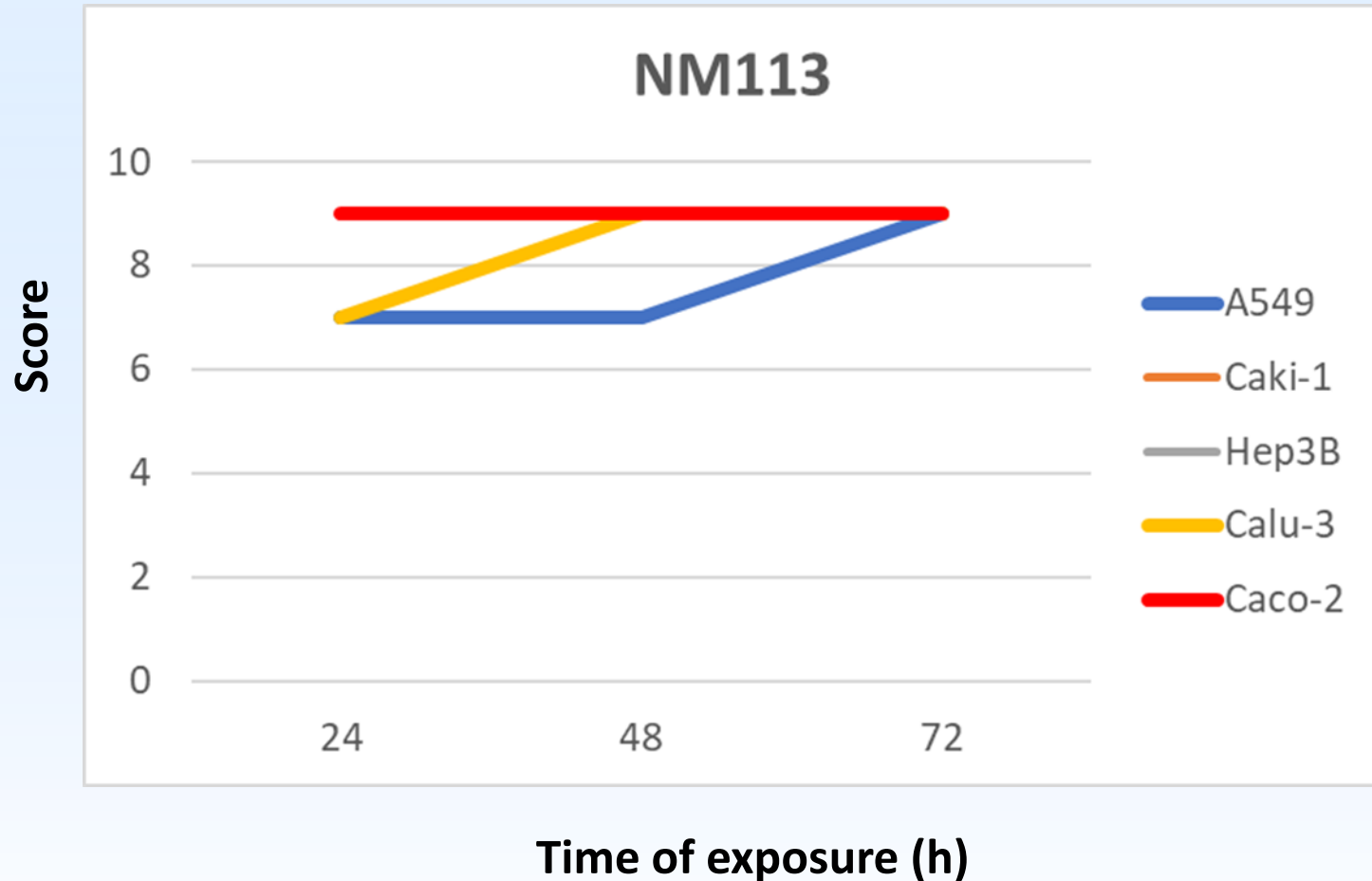
### Oxidative Stress

Response (%) compared to -ve	Score	Oxidative Stress
100 +/- 15	1	Negative
115- 150	2	Weak
150- 200	3	Medium
>200	4	High

## Further Issues

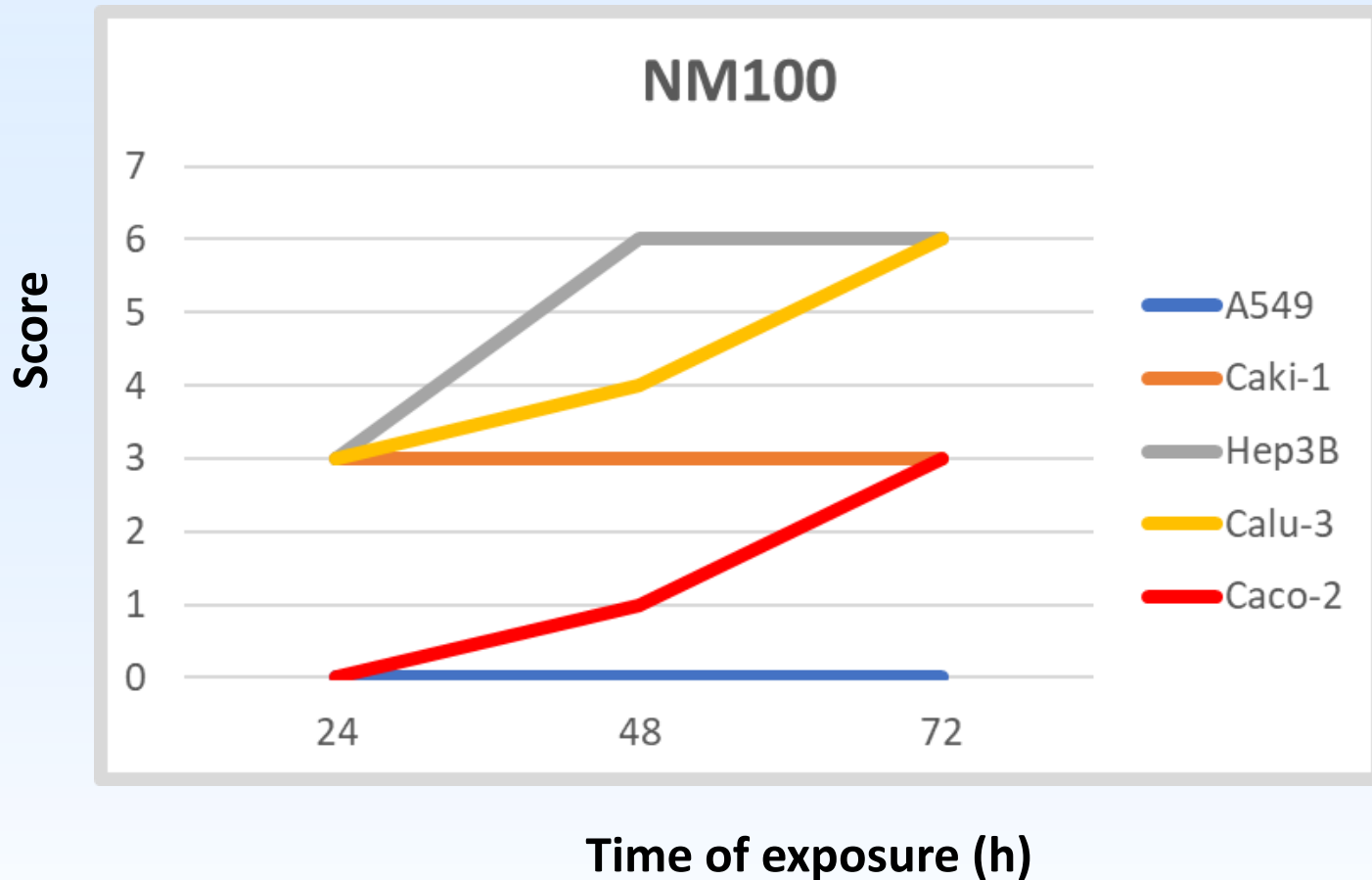
# Results Impedance - cell line choice

Homogeneous response when toxic



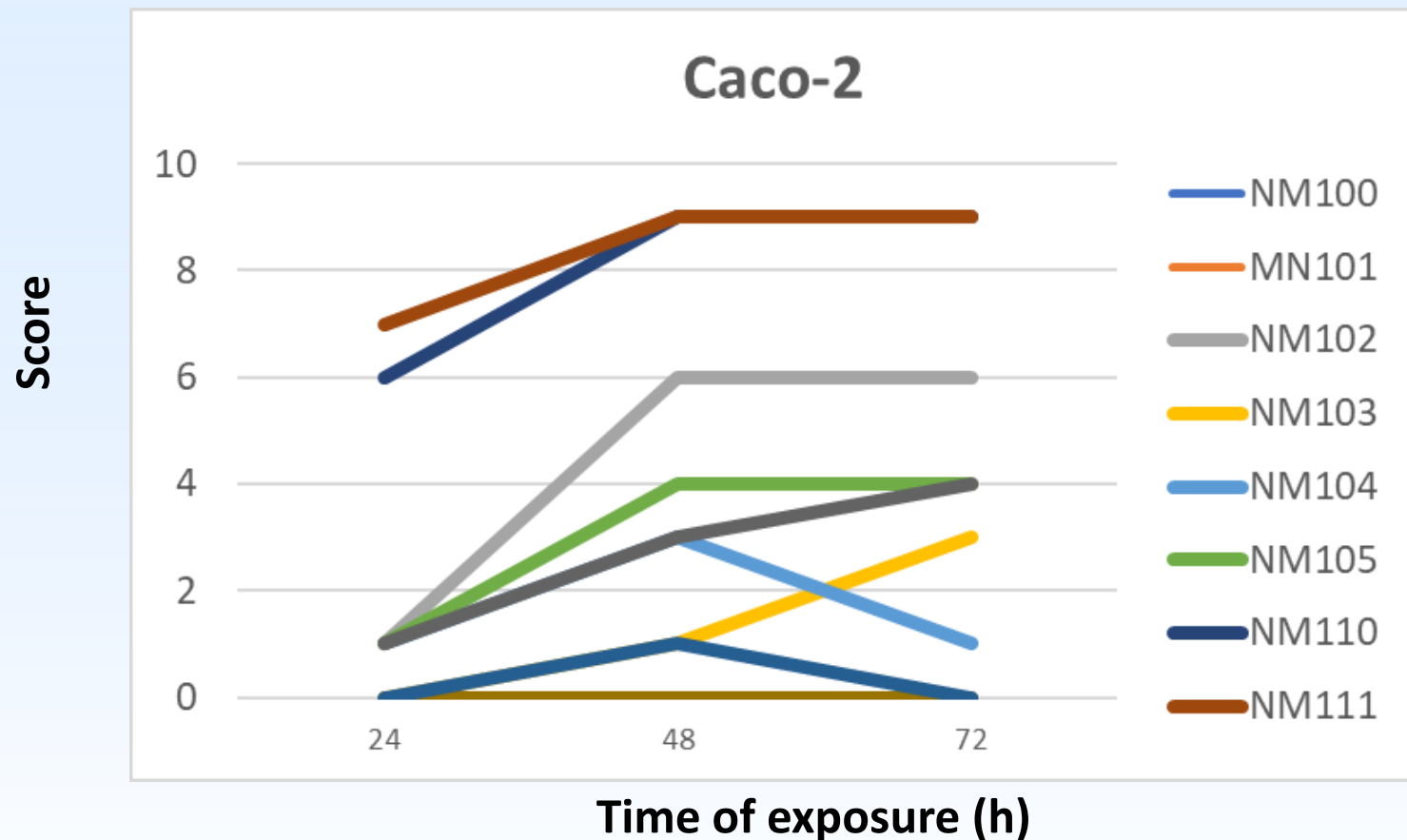
# Results Impedance - cell line choice

“Medium toxicity» depends on cell line



# Results Impedance – time of exposure

Results depend on exposure time



## Data gathering

Minimum amount of information needed for the grouping (quality data)

- References to standard operation protocols (SOP):
  - Standard data analysis and data processing protocols, dispersion protocols, Essential sample annotation ( e.g. technical or biological replicates)
  - Cell line information and number seeded
  - Exposure time
  - Concentrations and volumes used
- Need enough data points for a dose-response curve.
- Phys-chem of the particles as pristine and in media

## New Data production and data collection

Follow agreed SOP with all the minimum standards established.  
Group data according to experimental conditions:  
Allocate data to defined categories  
Phys-chem of the particles

## Metrics

- Harmonise units ( $\mu\text{g}/\text{cm}^2$  or  $\mu\text{g}/\text{ml}$ )

## Exposure

- Consider using in vitro dosimetry models (ISDD, VCM, ADRM, DG)

**THANK YOU!**