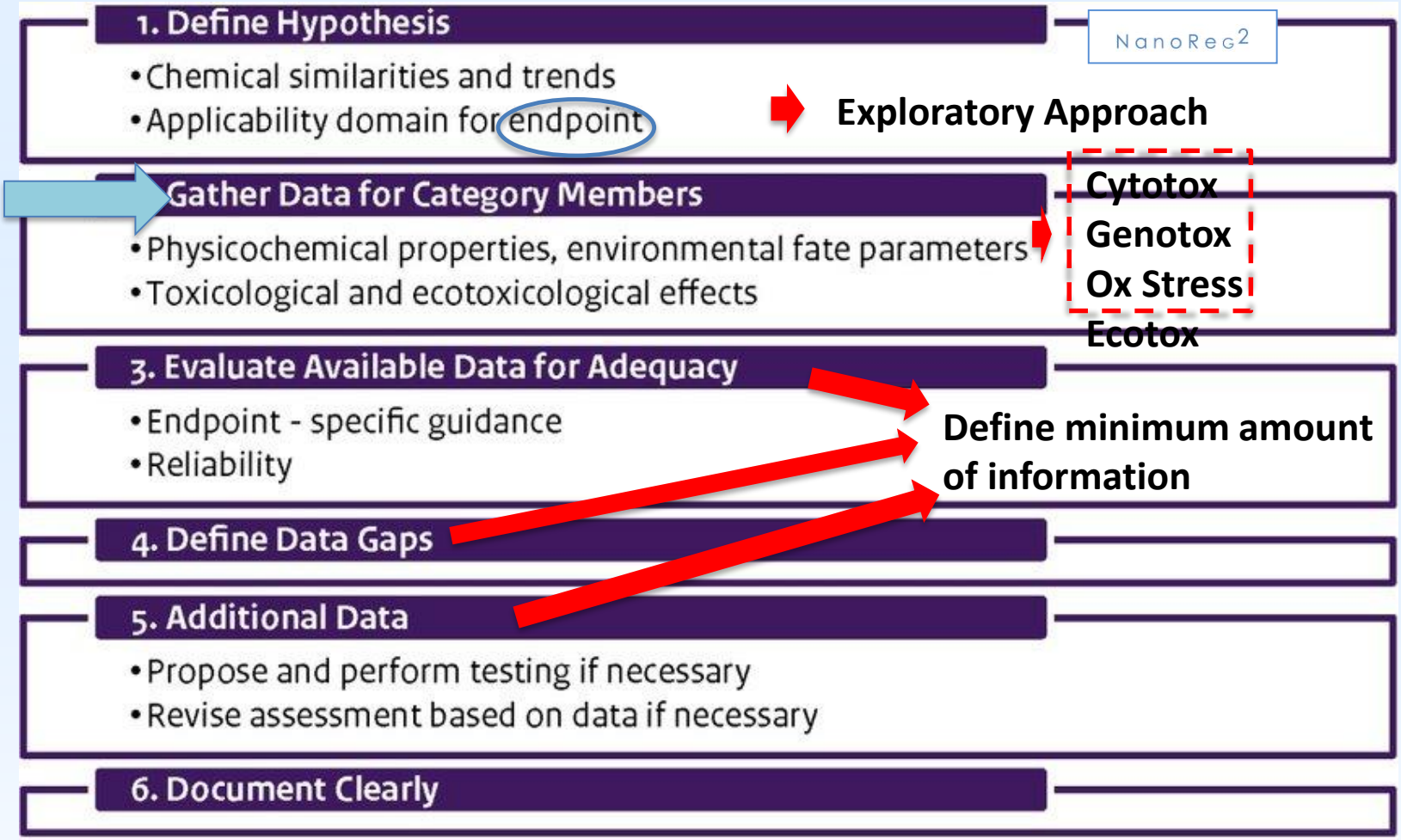
NanoReg2 grouping case studies
Human tox

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.

ECHA Strategy in NanoReg2

NanoReg2

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

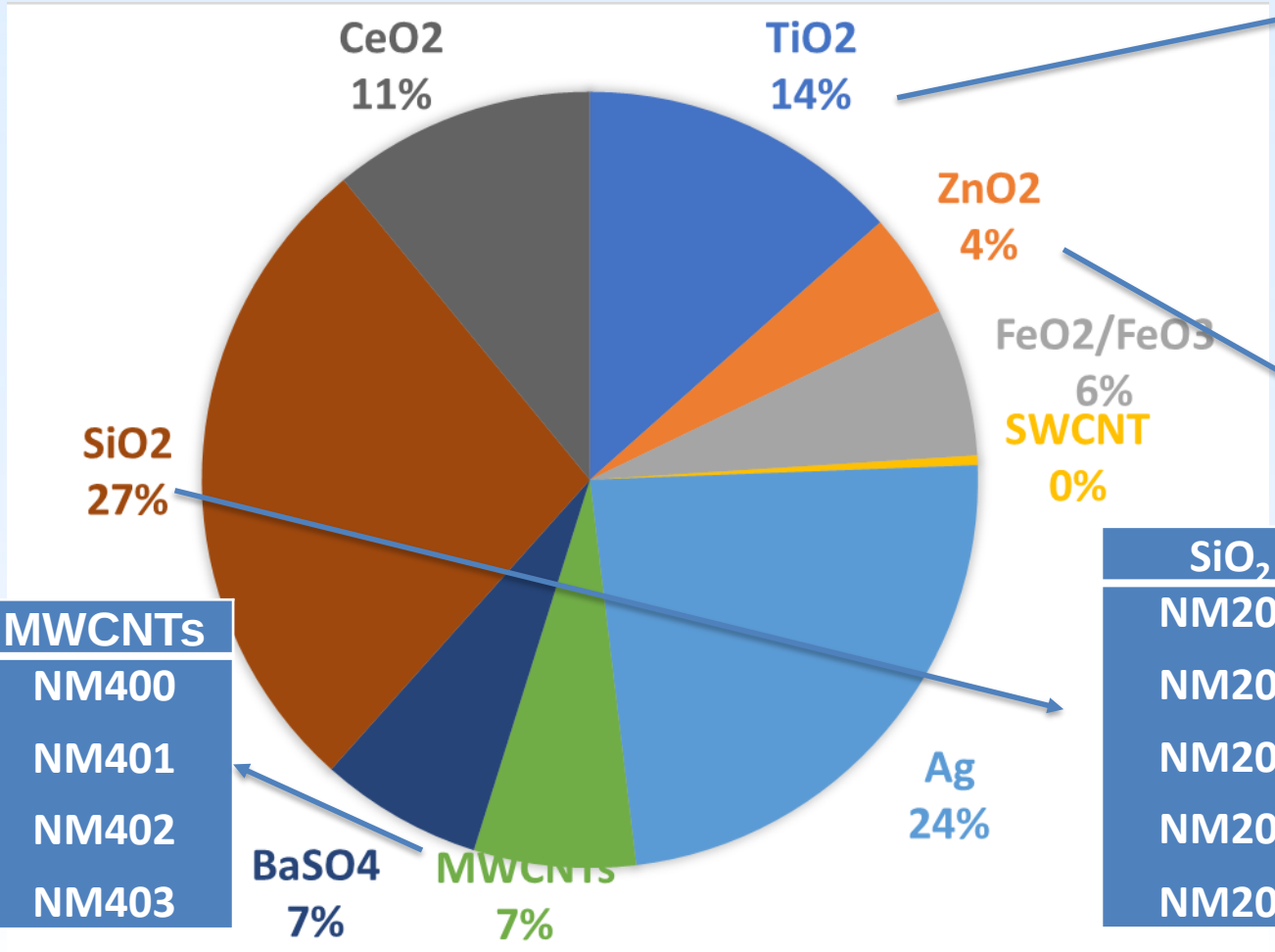
Data gathering for category members

NanoReg2

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

Industrially relevant materials

Data retrieved from the NanoReg2 database



TiO_2
NM100
NM101
NM102
NM103
NM104
NM105

ZnO
NM110
NM111
NM112
NM113

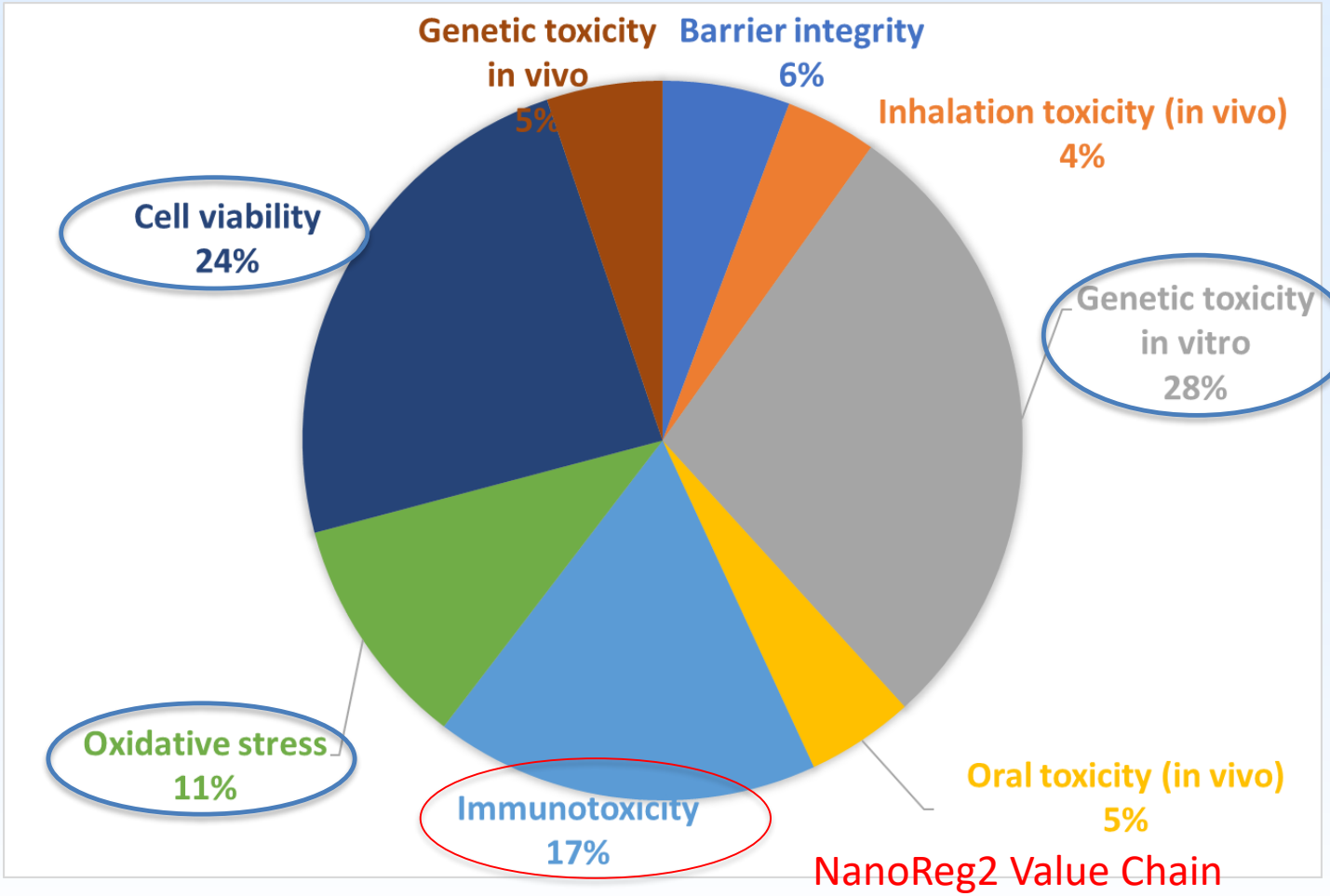
SiO_2
NM200
NM201
NM202
NM203
NM204

MWCNTs
NM400
NM401
NM402
NM403

Data gathering for category members

Data availability per end point

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



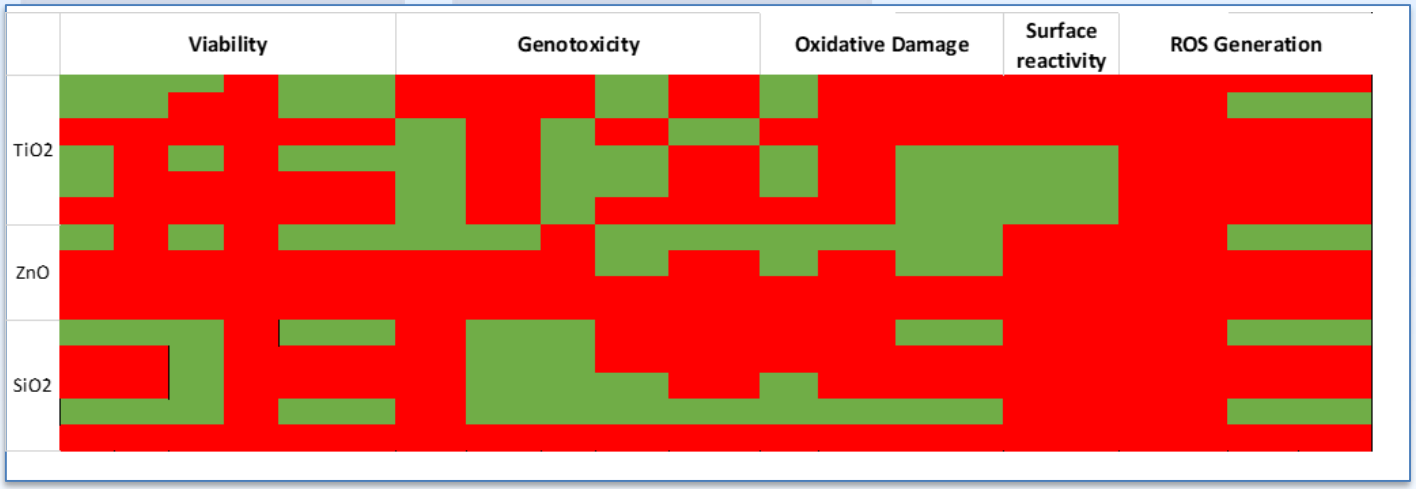
NanoReg2 Value Chain Demonstrators

Initial Approach – Data gathering and identification of gaps

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

Cytotox	Genotox	Ox Stress
• MTT • AB • CFE • NRU • LDH • Impedance	• MN • Comet • Comet Fpg	• DCFH2-DA

19 materials!



NanoReg2 Database

Data Gaps

80% GAPS!!!

Aditional data collection

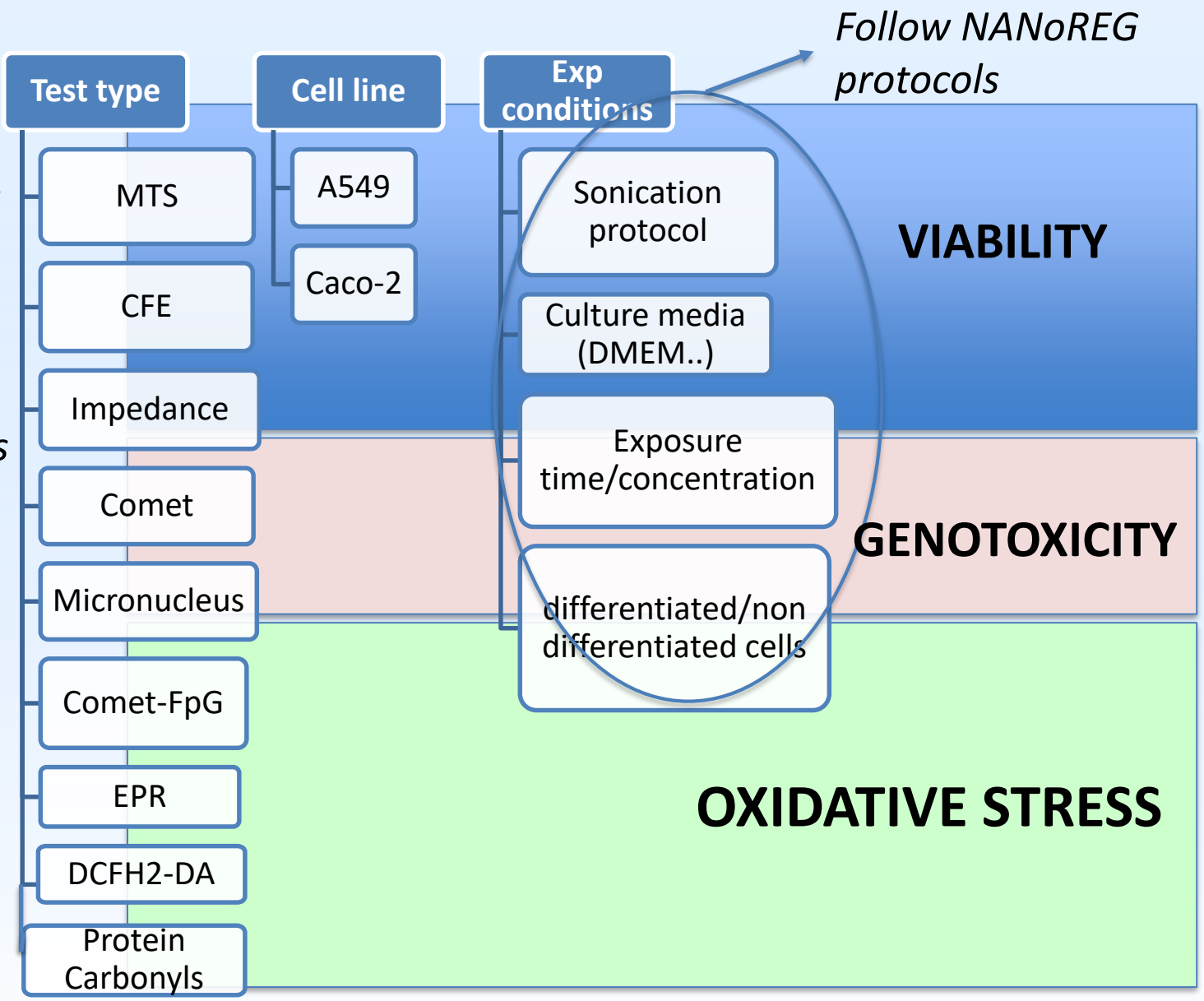
NanoReg2

Partners expertise

HTS Methods

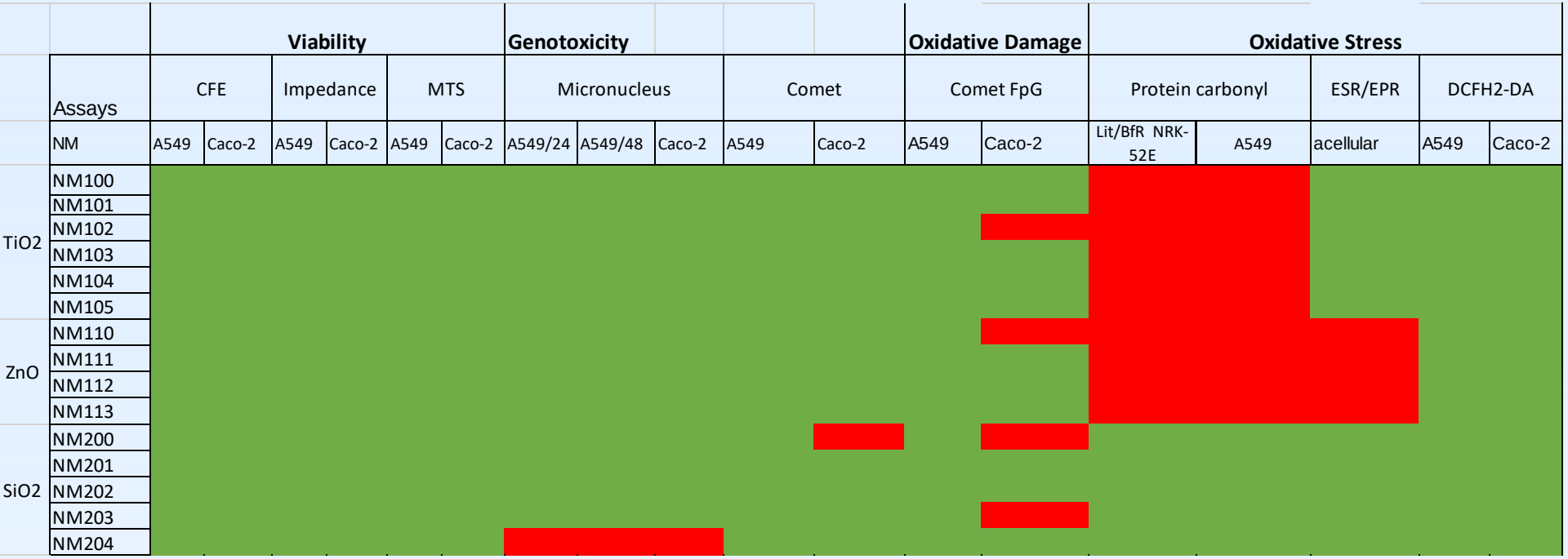
Data availability

Relevant cell lines



15 nanomaterials plus 6 materials from NanoReg2!!!

Data gap filling



Only 10% Data gaps!

Data scoring (NanoSolutions adapted to NanoReg2)

NanoReg2

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

(Too many categories for modelling)

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

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)

		Points	Points	Category
Toxicity reaches	20%	+1	0	1
	50%	+1	1	2
	80%	+1	2-5	3
IC50	≤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

Project #646221, Funded by the Horizon 2020
Framework Programme of the European Union

Case Studies
- Preliminary results-

Case Study: TiO2

NanoReg2

Cytotoxicity (A549, Caco-2)

		MTS		Impedance		CFE		AB (A549)	
		A549	Caco-2	A549	Caco-2	A549	Caco-2	3h	24h
TiO_2	NM-100	0	0	0	1	0	0	1	1
	NM-101	0	0	0	2	0	0	0	0
	NM-102	0	0	0	4	0	4	0	0
	NM-103	0	0	0	1	0	1	1	0
	NM-104	0	0	0	1	0	1	1	0
	NM-105	0	0	0	3	0	1	0	0

Genotoxicity (A549)

		3h		24h		24h	48h
		CA	+Fpg	CA	+FPG	MN	MN
TiO_2	NM-100	3	3	2	2	1	1
	NM-101	2	2	2	1	1	1
	NM-102	1	1	1	1	2	2
	NM-103	1	1	2	2	1 | 2	2
	NM-104	1	1	1	2	2	2
	NM-105	1	1	1	1	2	2

Ox Stress (NRK-52E)

		NRK-52E	
		Carbonyls	
		IB	ELISA
TiO_2	NM-100	Weak-Medium	Medium-Strong
	NM-101	Strong	Strong
	NM-102	Negative	Negative-Weak
	NM-103	Negative	Negative-Weak
	NM-104	Weak	Negative
	NM-105	Weak-Medium	Medium-Strong

0-1 non tox
2-5 slightly tox
6-9 High tox

Cytotox (A549, CaCo-2)

All TiO2 — negative/ weak (Caco-2 more sensitive)

Genotoxicity (A549)

TiO2 NM-100 — positive

Other TiO2 — negative/ equivocal

Ox Stress (NRK-52E)

TiO2 NM-100, NM-101, NM-105 — positive

Other — negative/weak

Case Study: TiO2

NanoReg2

Cytotoxicity (A549, Caco-2)

		MTS		Impedance		CFE		AB (A549)	
		A549	Caco-2	A549	Caco-2	A549	Caco-2	3h	24h
TiO_2	NM-100	0	0	0	1	0	0	1	1
	NM-101	0	0	0	2	0	0	0	0
	NM-102	0	0	0	4	0	4	0	0
	NM-103	0	0	0	1	0	1	1	0
	NM-104	0	0	0	1	0	1	1	0
	NM-105	0	0	0	3	0	1	0	0

Genotoxicity (A549)

		3h		24h		24h	48h
		CA	+Fpg	CA	+FPG	MN	MN
TiO_2	NM-100	3	3	2	2	1	1
	NM-101	2	2	2	1	1	1
	NM-102	1	1	1	1	2	2
	NM-103	1	1	2	2	1 / 2	2
	NM-104	1	1	1	2	2	2
	NM-105	1	1	1	1	2	2

Ox Stress (NRK-52E)

		NRK-52E	
		Carbonyls	
		IB	ELISA
TiO_2	NM-100	Weak-Medium	Medium-Strong
	NM-101	Strong	Strong
	NM-102	Negative	Negative-Weak
	NM-103	Negative	Negative-Weak
	NM-104	Weak	Negative
	NM-105	Weak-Medium	Medium-Strong

1 negative
2 Equivocal
3 Positive

Cytotox (A549, CaCo-2)

All TiO2 — negative/ weak (Caco-2 more sensitive)

Genotoxicity (A549)

TiO2 NM-100 — positive

Other TiO2 — negative/ equivocal

Ox Stress (NRK-52E)

TiO2 NM-100, NM-101, NM-105 — positive

Other — negative/weak

Case Study: TiO2

NanoReg2

Cytotoxicity (A549, Caco-2)

		MTS		Impedance		CFE		AB (A549)	
		A549	Caco-2	A549	Caco-2	A549	Caco-2	3h	24h
TiO_2	NM-100	0	0	0	1	0	0	1	1
	NM-101	0	0	0	2	0	0	0	0
	NM-102	0	0	0	4	0	4	0	0
	NM-103	0	0	0	1	0	1	1	0
	NM-104	0	0	0	1	0	1	1	0
	NM-105	0	0	0	3	0	1	0	0

Genotoxicity (A549)

		3h		24h		24h	48h
		CA	+Fpg	CA	+FPG	MN	MN
TiO_2	NM-100	3	3	2	2	1	1
	NM-101	2	2	2	1	1	1
	NM-102	1	1	1	1	2	2
	NM-103	1	1	2	2	1 / 2	2
	NM-104	1	1	1	2	2	2
	NM-105	1	1	1	1	2	2

Ox Stress (NRK-52E)

		NRK-52E	
		Carbonyls	
		IB	ELISA
TiO_2	NM-100	Weak-Medium	Medium-Strong
	NM-101	Strong	Strong
	NM-102	Negative	Negative-Weak
	NM-103	Negative	Negative-Weak
	NM-104	Weak	Negative
	NM-105	Weak-Medium	Medium-Strong

1 Neg/weak
2 weak/medium
3 Strong

Cytotox (A549, CaCo-2)

All TiO2 — negative/ weak (Caco-2 more sensitive)

Genotoxicity (A549)

TiO2 NM-100 — positive

Other TiO2 — negative/ equivocal

Ox Stress (NRK-52E)

TiO2 NM-100, NM-101, NM-105 — positive

Other — negative/weak

Case Study: TiO2

	Genotoxicity (A549)		Ox. Stress (NRK-52E)		Zeta Potential (mV)	Total non-TiO2 content	Coating
	Positive	Negative/ Equivocal	Positive	Negative/ Weak		(wt%)	
NM-100	x		x		-40.6	2.3	No
NM-101		x	x		-27.5	1.9	Yes (unstable? Incomplete ?)
NM-102		x		x	30.3	0.4	No
NM-103		x		x	39.1	8.7	Yes
NM-104		x		x	4.39	7.3	Yes
NM-105		x	x		-32.6	0.2	No

Suggestions for Key Toxicity Drivers (TiO2)

1) Absence of coating
2) Impurities
3) Highly Negative zeta Potential

Negative/Medium
6-9 Strong

Case Study: SiO2

NanoReg2

Cytotoxicity (A549, Caco-2)

		MTS		Impedance		CFE		AB (A549)	
		A549	Caco2	A549	Caco2	A549	Caco2	3h	24h
SiO_2	NM-200	0	0	1	3	0	0	0	0
	NM-201	0	0	0	1	0	3	0	0
	NM-202	0	0	1	1	2	3	0	0
	NM-203	0	0	0	0	0	0	1	0
	NM-204	0	0	0	0	0	1	0	0

Genotoxicity (A549)

		3h		24h		24h	48h	
		CA	+Fpg	CA	+FPG	MN	MN	
SiO_2	NM-200	2	2	2	1	2	1	2
	NM-201	1	1	1	1	2	2	
	NM-202	1	1	1	1	2	2	
	NM-203	1	1	2	1	1	2	
	NM-204	1	1	1	1			

Ox Stress (NRK-52E)

		Carbonyls	
		A549	NRK-52E
SiO_2	NM-200	Weak	Strong
	NM-201	Negative	Negative
	NM-202	Weak	Strong
	NM-203	Negative	Negative
	NM-204	Negative	Weak

0-1/weak
2-5 Medium
6-9 Strong

Cytotox (A549, CaCo-2)

All SiO2 — negative/ weak (CaCo-2 more sensitive)

Genotoxicity (A549)

All SiO2 — negative/ equivocal

Ox Stress (NRK-52E)

SiO2 NM-200, NM-202 — positive (NRK-52E more sensitive)

Other — negative/weak

Case Study: SiO2

	Ox. Stress (NRK-52E)		Purity (wt%)	Porosity (m27g)	Dissolution in Gambles	Dustiness (mg/Kg)
	Positive	Negative/ Weak				
NM-200	x		95,3	30,044	18	293
NM-201		x	95,5	23,144	4,9	218
NM-202	x		99,4	8,268	7,5	91
NM-203		x	99,6	5,332	8,8	218
NM-204		x	98,5	17,485	5,9	1058

Lessons learnt

2) For SiO2 it might be useful to conside other parameters

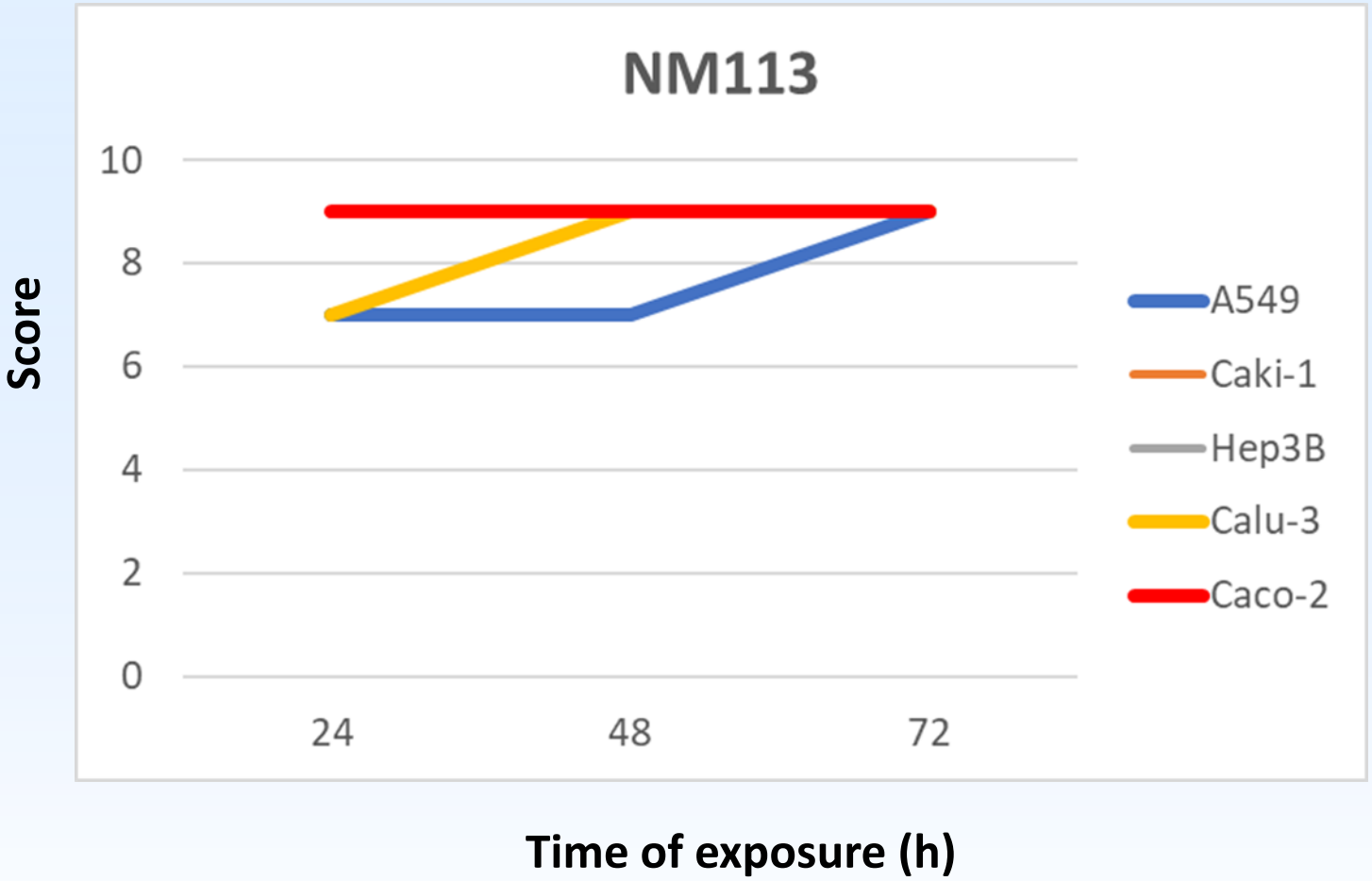
- 0-1/weak
- 2-5 Medium
- 6-9 Strong

3) Differences in phys. chem. parameters (e.g. zeta-potential, bulk density) do not give an obvious idea for a grouping hypothesis

Further Issues

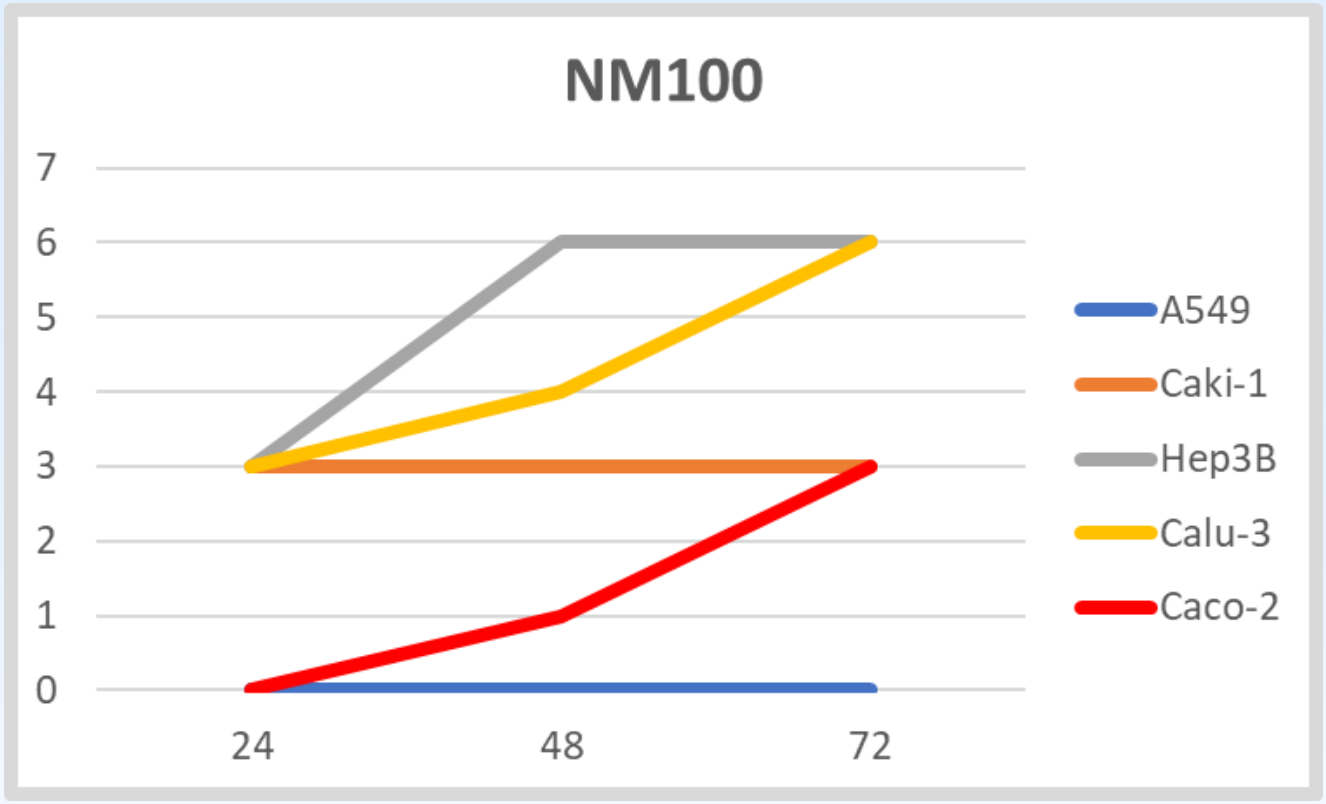
Results Impedance - cell line choice

Homogeneous response when toxic



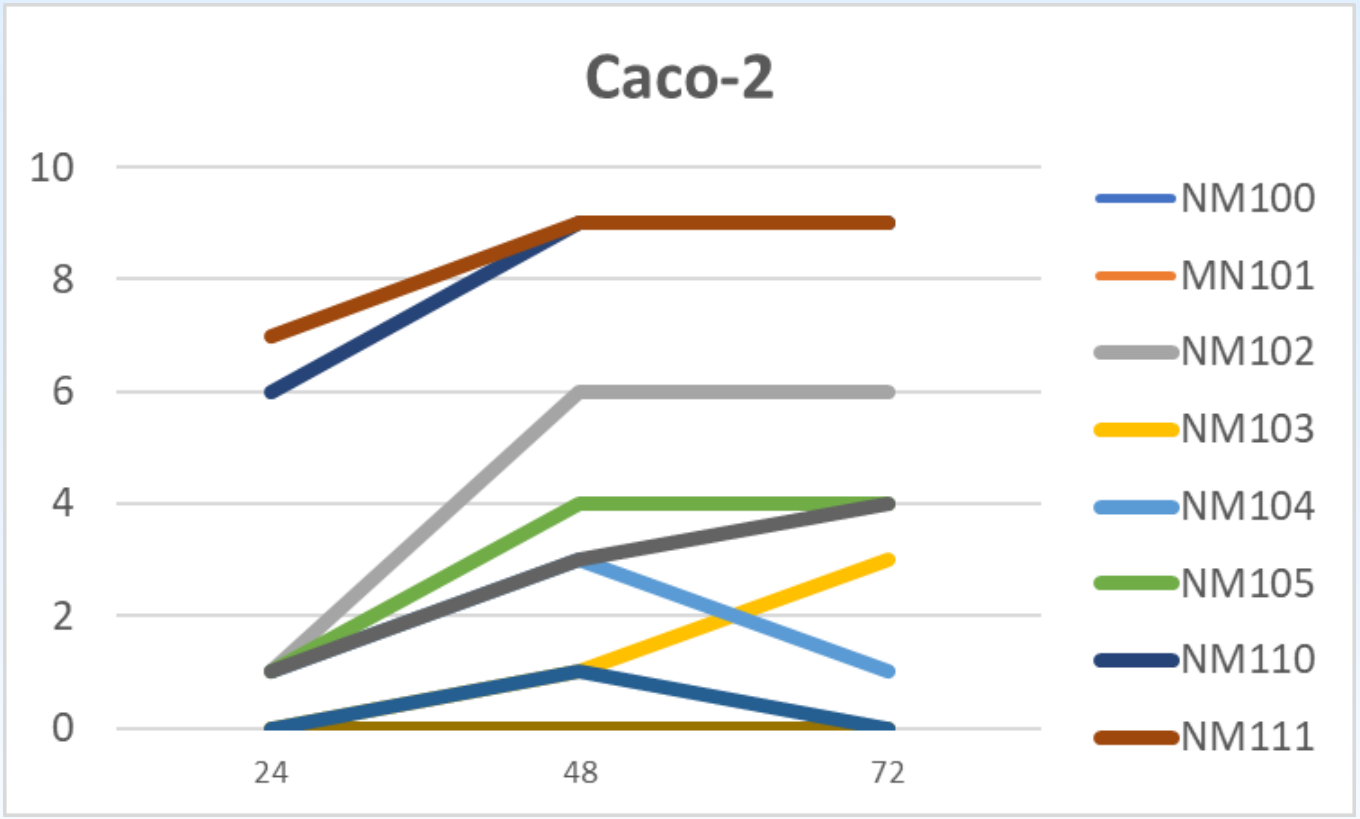
Results Impedance - cell line choice

"Medium toxicity» depends on cell line



Results Impedance – time of exposure

Results depend on exposure time



Issues to take into account for data collection

NanoReg2

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 (µg/cm^2 or µg/ml)

Exposure

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

THANK YOU!