



The NanoReg2 Grouping Approach

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& all NanoReg2 WP1 Members

Legislation	Nano-Definition	Grouping mentioned	Read-across possible	Grouping Guidance	Guidance NM grouping	Risk Assessment	
						HH	ENV
REACH (1907/2006/EC)	No (*)	Annex XI	Yes	Yes	Yes	Yes	Yes
CLP (1272/2008/EC)	No	No	Yes	Yes	No	Yes	Yes

(*)

The definition of a „substance“ implicitly covers all forms/ sizes.

Amendments of REACH annexes have been adopted by EC (28.04.2018), those contain a definition.

(Expected to come into force 01.01.2020)

Reference:

Mech A, Rasmussen K, Jantunen P et al. (2018)

Insights into possibilities for Grouping and Read-Across for Nanomaterials in EU Chemicals Legislation. *Nanotoxicology* (in press), DOI: 10.1080/17435390.2018.1513092

<https://www.tandfonline.com/doi/full/10.1080/17435390.2018.1513092>

Legislation	Nano-Definition	Grouping mentioned	Read-across possible	Grouping Guidance	Guidance NM grouping	Risk Assessment	
						HH	ENV
Biocidal (528/2012/EC)	Yes	Yes	Yes	Yes	No	Yes	Yes
Plant Protection Products (1107/2009/EC)	No	No	Yes (for food residues)	Yes (for some aspects)	No	Yes	Yes
Cosmetics (1223/2009/EC)	Yes	No	Yes	No (reference to OECD guidance)	No	Yes	(Yes) (under REACH)
FCM (1935/2004/EC)	No	No	Yes (but rarely applied)	Yes	No	Yes	N.A.
Plastic FCM (10/2011/EC)	No (mentioned)	No	Yes (but excluded for NMs)	No	No	Yes	N.A.
Novel Food (258/97/EC)	Yes	No	No	No	No	Yes	N.A.
Food additives	No	No	Yes	No	No	Yes	N.A.
Food enzymes	No	No	No	No	No	Yes	N.A.

Guidance under REACH recommends **registration of nanomaterials** in a **joint dossier with the non-nano form** of that substance (if existing).

In this case the nanomaterial is called a **nanofom** of that substance.

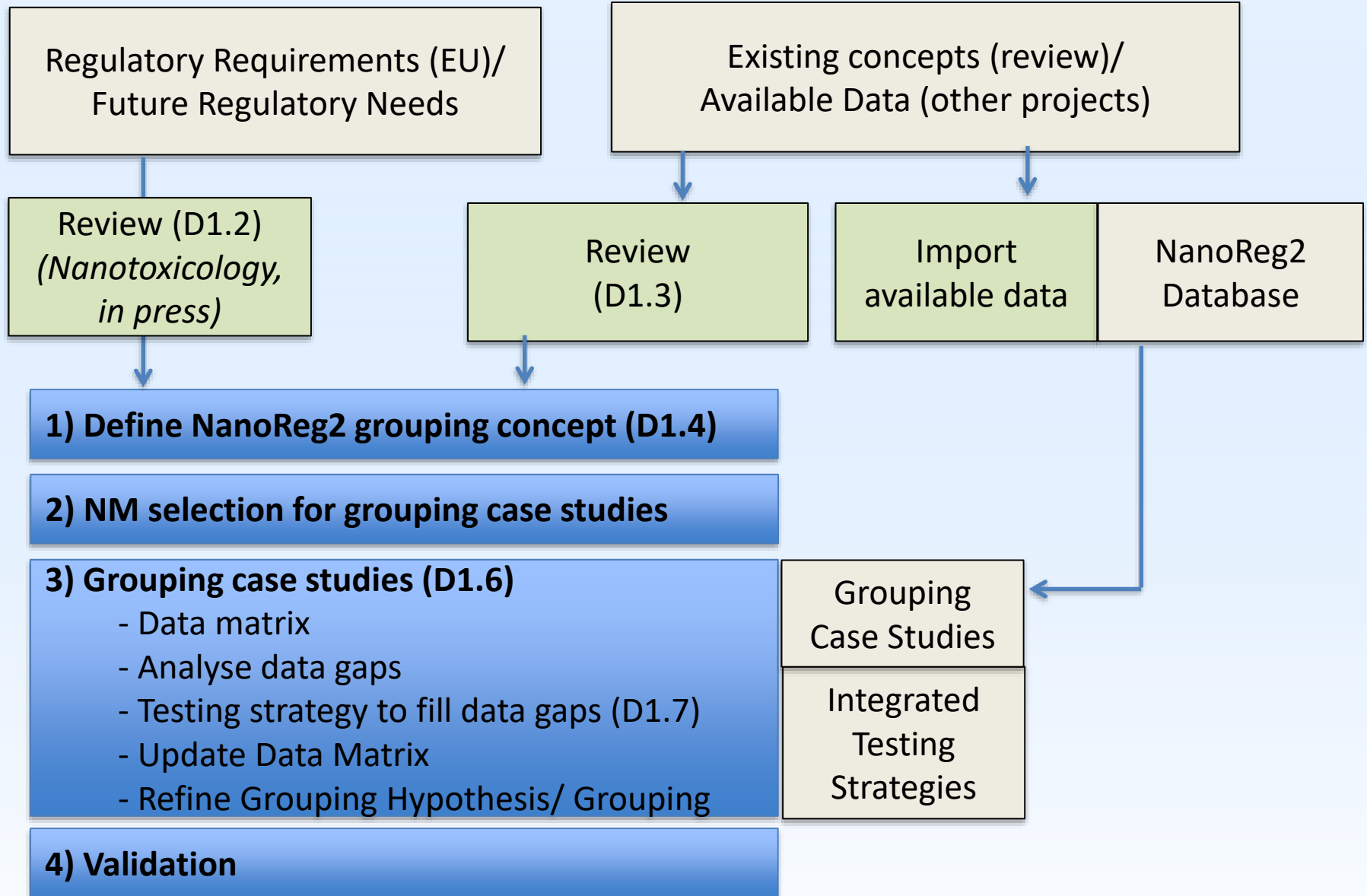
Grouping of chemicals always means grouping of different substances.

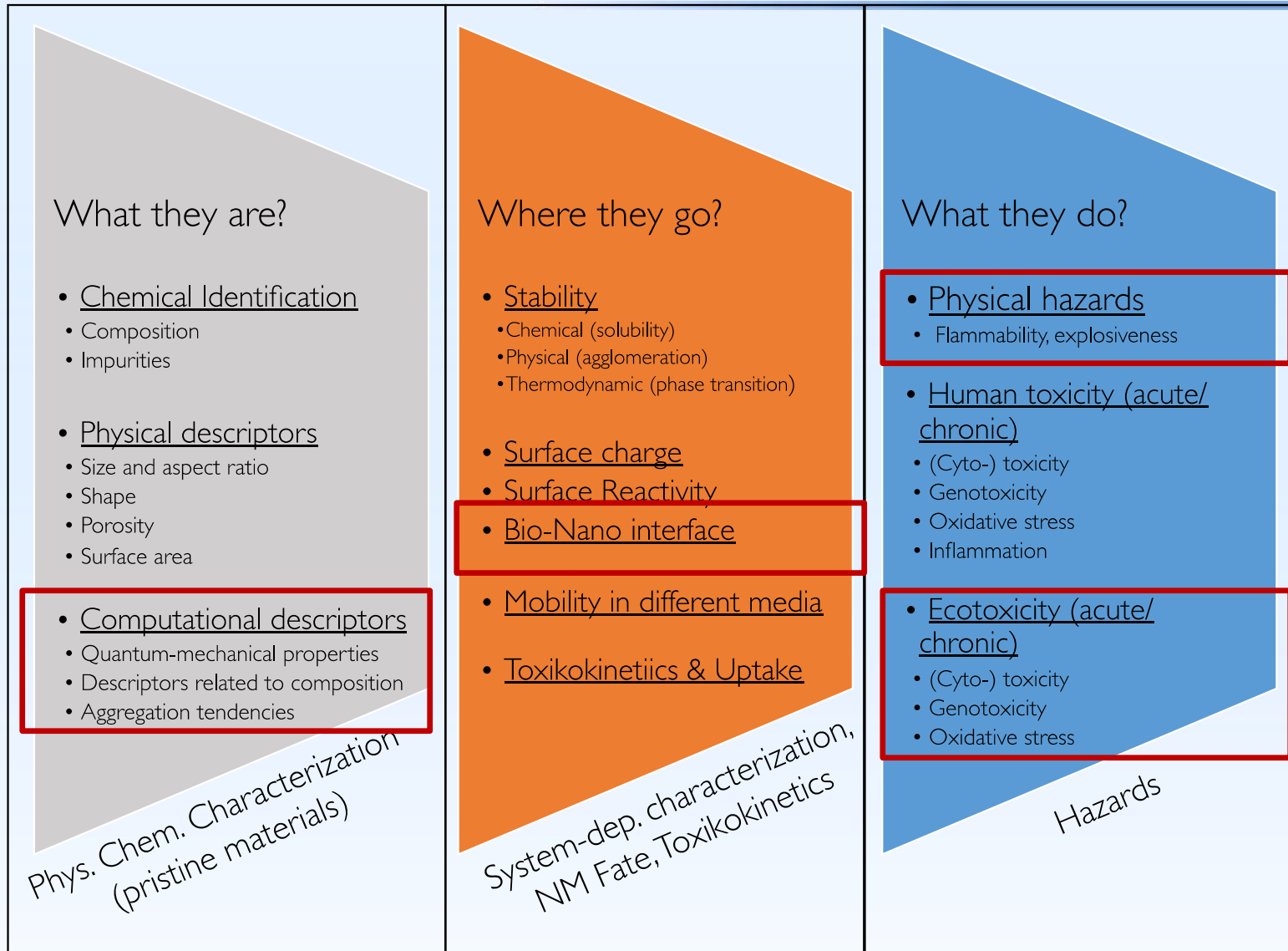
Grouping of nanomaterials could mean

- a) Grouping of **different nanofoms of one substance**
- b) Grouping of **nanofom(s) and the non-nanofom of one substance**
- c) Grouping **across substances**

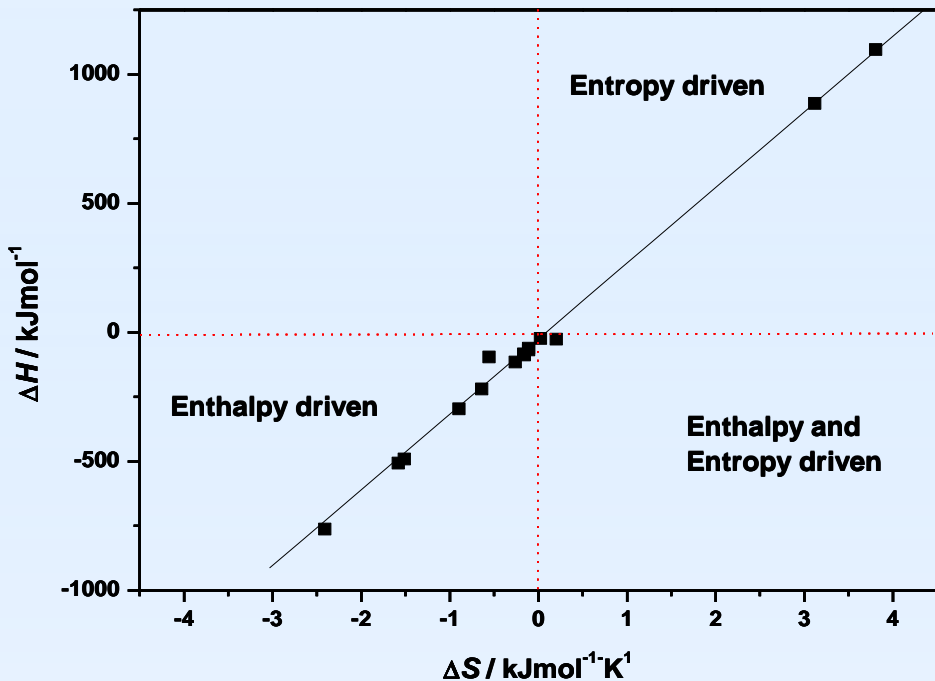
Regulatory Requirements:

- Grouping of **nanofoms of one substance** currently considered most important.
- **NanoReg2 followed an exploratory approach to establish grouping of nanofoms of one substance.**





Based on approach suggested by EU FP7 MARINA, with modifications



Bio-Nano-Interface

Interactions of NMs with selected proteins are analyzed (ITC). This allows for classification based on thermodynamic parameters such as

- binding affinity,
- binding stoichiometry,
- binding enthalpy,
- Entropy, and
- binding energy

I. $\Delta H < 0$, $\Delta S < 0$: Predominantly enthalpy driven (van der Waals and/or electrostatic interactions)

II. $\Delta H > 0$, $\Delta S > 0$: Predominantly entropy driven (hydrophobic forces)

III. $\Delta H < 0$, $\Delta S > 0$: Favorable enthalpy ΔH AND favorable entropy ΔS .

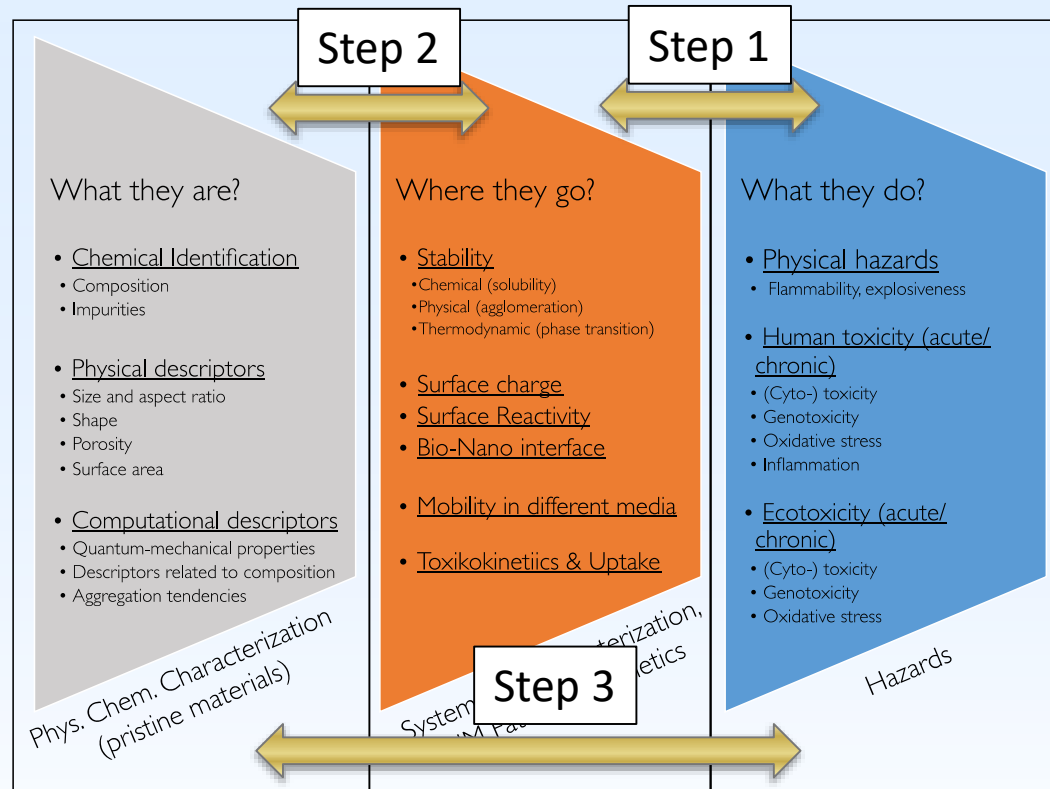
(suggests several bonds, predominantly hydrophobic with some electrostatic interactions)

I: Establish „similarity“ based on physico-chemical parameters considering

- chemical identity (composition, impurities...)
- physical identity: measured parameters, intrinsic & extrinsic parameters
- calculated (in silico) or semi-calculated (based on in silico analysis of measured parameters)

II: Establish correlations between physico-chemical information & toxicity in 3 steps

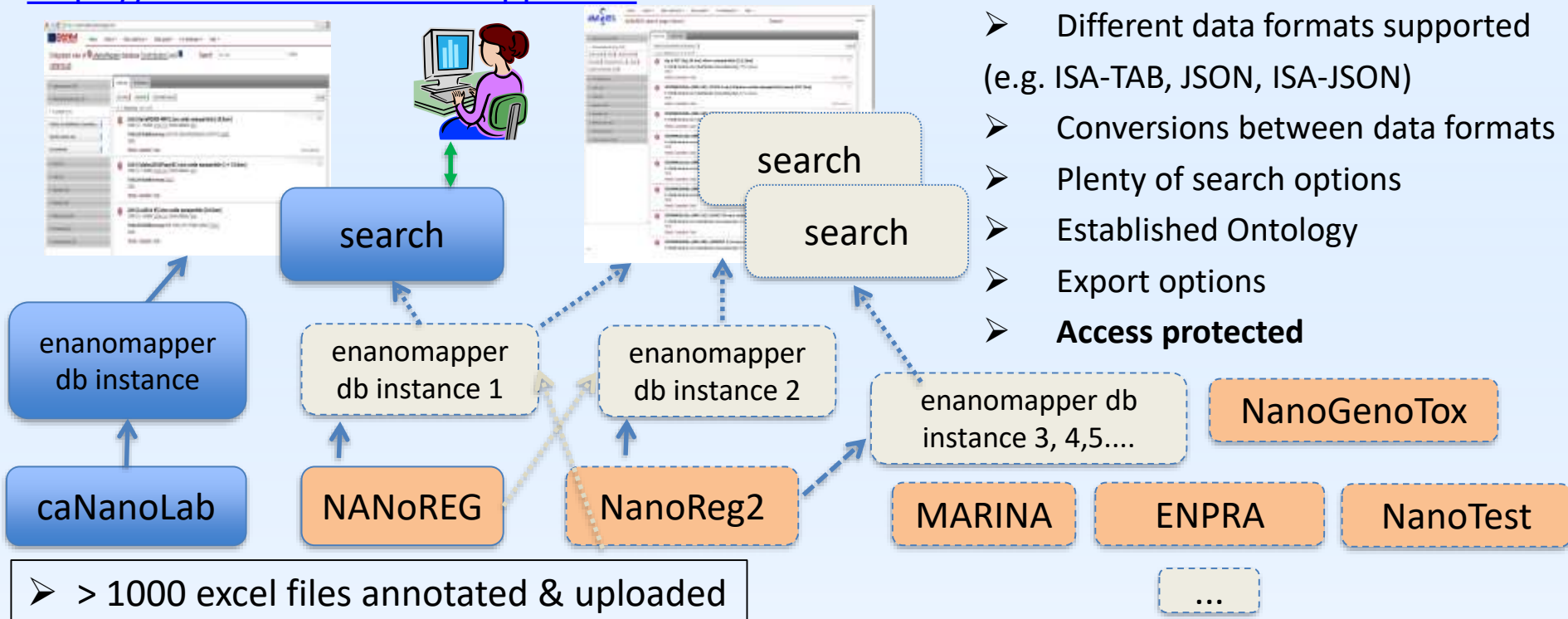
- use of in silico methods to establish these correlations



	ECHA guidance	NanoReg2	RIVM (Sellers et al. 2015)	DF4nano (Arts et al. 2015)	MARINA (Oomen et al. 2015)	NANoREG (Dekkers et al. 2016)
Chemical composition	X	X	X	X	X	X
Impurities	X	X	X	X	X	X
Crystalline structure	X	X	X	X	X	
Surface characteristics (coating, functionalization, capping)	X	X	X	X	X	
Surface charge	X	X	X	X	X	X
Particle size and range	X	X	X	X	X	X
Shape/aspect ratio	X	X	X	X	X	X
Surface area/Porosity	X	X	X	X	X	X
Aggregation/agglomeration	X	X				X
Water solubility	X	(X)	X	X	X	X
Dissolution/ Dissolution rate (in relevant media)	X	X	X	X	X	X
Hamaker constant			X			

Based on eNanoMapper

<https://search.data.enanmapper.net>



- Automated data import (e.g. excel, OECD HT, ISA-TAB, IUCLIDS)
- Different data formats supported (e.g. ISA-TAB, JSON, ISA-JSON)
- Conversions between data formats
- Plenty of search options
- Established Ontology
- Export options
- **Access protected**

➤ > 1000 excel files annotated & uploaded

Study Type	NANOREG	MARINA	NanoGenoTox	NanoTest	SUM
Phys.Chem.	2793	134	220	61	3208
Tox	2313	235	1308	238	4094
Ecotox	62	49	-		111
SUM	5168	418	1528	299	

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Cytotoxicity

Assays:

Alamar blue (AB)
Colony Forming Efficiency (CFE)
Impedance
MTS
Neutral Red Uptake (NRU)
LDH

Selected cell models:

A549, BEAS-2B, Caco-2

Genotoxicity

Assays:

Comet Assay
(+/- Fpg)

Micronucleus Assay
(+/- Cytochalasin B)

Selected cell models:

A549, BEAS-2B, Caco-2

Ox. Stress

Assays:

Several acellular assays

- EPR (2 spin traps)
- Spin trapping + MS
- Acellular DCFDA

DNA ox. damage (Comet +/-Fpg)
Protein ox. damage (carbonyls)

Selected cell models:

A549, NRK-52E

A scoring system was developed for each endpoint to facilitate categorization

- to transform data into a form that can be easier analysed
- render different assays comparable

(individual read-outs can be different: shape of dose/response curve & scale)

in vitro (mussel hemocyte cells and fish cell lines)

- 1) Alterations on cell metabolism using different assays (e.g. MTT, Alamar Blue)
- 2) Lysosomal functioning (e.g. Neutral Red Assay)
- 3) Plasma membrane integrity (e.g. CFDA-AM)

In vivo

- 4) Freshwater microalgae (*Pseudokirchneriella subcapitata*) growth inhibition test (OECD TG 201)
- 5) *Daphnia magna* acute immobilization (OECD TG 202)

Again, a scoring system was developed for each endpoint to facilitate categorization (inspired by CLP classification)

	JRC ID (old code)	JRC ID (new code)	Primary particle size nm	Size Distribution	Specific Surface Area m ² /g	Other information
TiO₂	NM-100	JRCNM01000 a	50- 150	<100 (27%) <50 (12%)	9	Anatase
	NM-101	JRCNM01001 a	5-6	<100 (95%) <50 (77%) <10 (11%)	170/316	Anatase
	NM-102	JRCNM01002 a	21-22	N/A	66/78	Anatase
	NM-103	JRCNM01003 a	22-26	<100 (52%) <50 (13%)	51	Rutile Al-coated
	NM-104	JRCNM01004 a	23-27	<100 (53%) <50 (12%)	52/56	Rutile Al-coated
	NM-105	JRCNM01005 a	15-24	N/A	46	Rutile/ Anatase

	JRC ID (old code)	JRC ID (new code)	Primary particle size nm	Size Distribution	Specific Surface Area m ² /g	Other information
SiO₂	NM-200	JRCNM02000 a	14-23	<100 (89%) <50 (70%) <10 (2%)	189	Synthetic amorphous, precipitated
	NM-201	JRCNM02001 a	17-19	<100 (82%) <50 (55%) <10 (1%)	140	Synthetic amorphous, Precipitated
	NM-202	JRCNM02002 a	15-20	<100 (80%) <50 (55%) <10 (1%)	204	Synthetic amorphous, Thermal
	NM-203	JRCNM02003 a	13-45	<100 (78%) <50 (48%)	203	Synthetic amorphous, Thermal
	NM-204	JRCNM02004 a	10-19	<100 (71%) <50 (36%)	137	Synthetic amorphous, precipitated

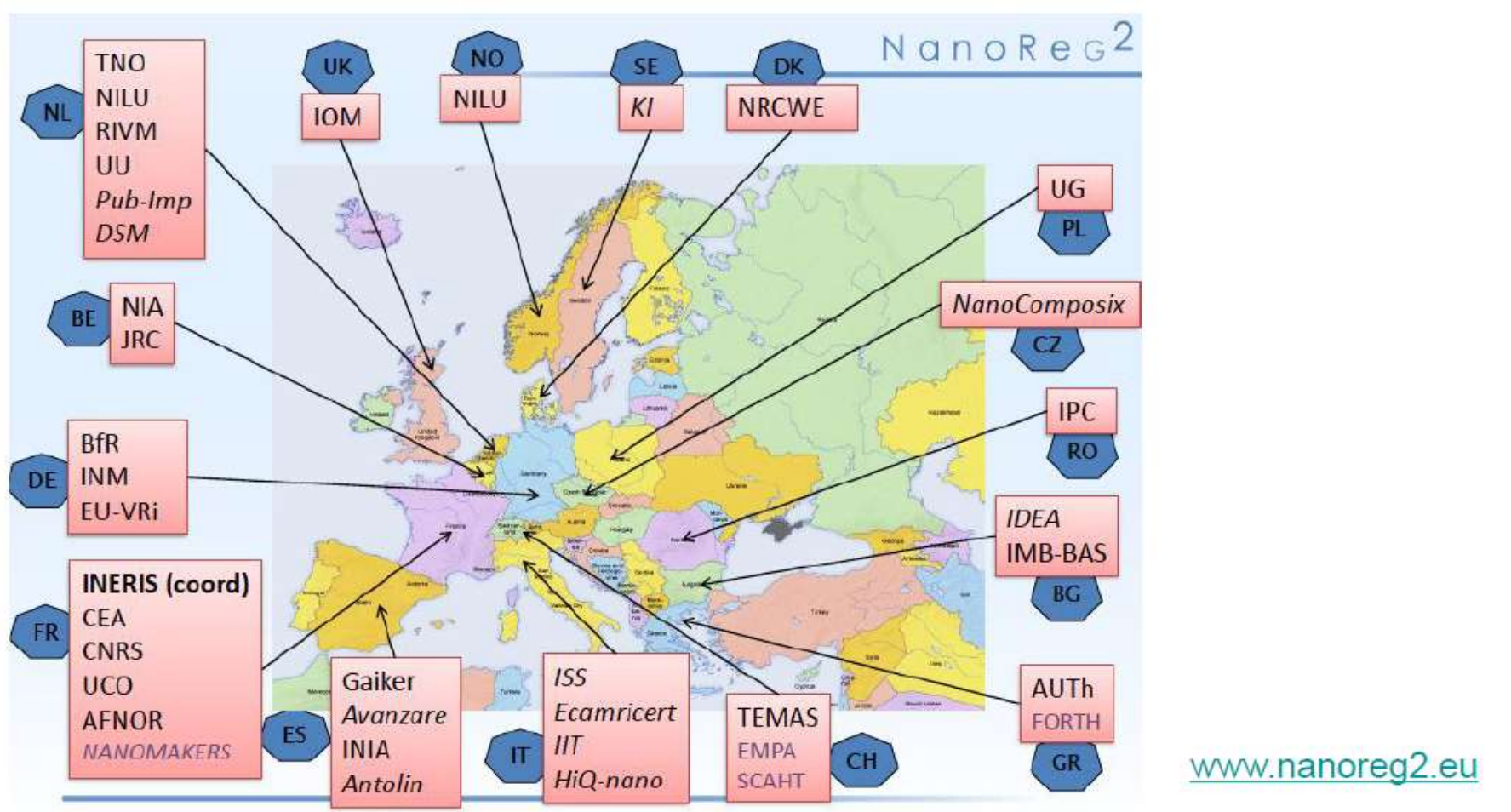
	JRC ID (old code)	JRC ID (new code)	Primary particle size, nm	Size Distribution	Specific Surface Area m ² /g	Other information
ZnO	NM-110	JRCNM01100a	158	N/A	12	uncoated
	NM-111	JRCNM01101a	152	N/A	15	Triethoxycaprylsilane coated
	(NM-112)	(NM-112)	(42.5+/-3.6)			from Fraunhofer ITEM, DE
	(NM-113)	(NM-113)	(891.8+/-800)			from Fraunhofer ITEM, DE

	JRC ID (old code)	JRC ID (new code)	Average Length nm	Average Diameter nm	Specific Surface Area m ² /g	Other information
MWCNT	NM-400	JRCNM4000a	846	11	254	
	NM-401	JRCNM4001a	4048	67	140	
	NM-402	JRCNM4002a	1372	11	226	
	NM-403	JRCNM4003a	443	12	189	
	(Mitsui-7)		5000	88		OECD WPMN material
	(Nikkiso)		940	48		OECD WPMN material

Thank you!



More information in the following talks!



www.nanoreg2.eu

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