WP4
ENMs CHARACTERISATION, *IN VITRO* DOSIMETRY AND ENVIRONMENTAL FATE

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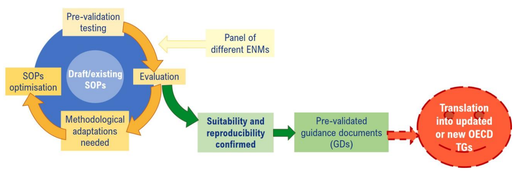
BACKGROUND, MOTIVATION AND OBJECTIVE

WP4 activities aimed to support and expand the work of the "Malta initiative" by developing and pre-validating draft GDs for ENMs

- **Characterization** WHAT THEY ARE
- **Fate** (OECD TG318) WHERE THEY GO
- ***In vitro* dosimetry** WHAT THEY DO



through verification and implementation of existing (or under development) TGs, in order to be aligned with activities of OECD and other international risk assessment and standardization bodies.

METHODS

Compounds selected: 1) Metal and metal oxides, 2) PLGA-coated ENMs, 3) MWCNTs.

Approach: Round Robins (RRs)

Criteria considered for methods' selection:

- Implementation capability
- Accessibility of the technique for SMEs (reduced cost and simple applicability)
- Robustness of the method

RESULTS / DISCUSSION

The RRs allowed to prepare internally pre-validated draft guidance documents for characterisation of P-CHEM properties, environmental fate, and dosimetry of ENMs including dispersibility, determination of surface charge, endotoxin content, particle number in suspension, hydrodynamic diameter and size distribution in water as well as in relevant biological media. Potential integrations/modifications to the existing TGs have been proposed by considering the relevance of specific parameters that were confirmed as key players with a view to a broader HARMONIZATION and STANDARDIZATION of the protocols.

T4.1 ENMs physicochemical characterization

Box 3. Statistical comparison of P-CHEM parameters from different labs after protocol optimization (from RR3)
Red: F and t values higher than the critical F and t statistics and p<0.05.
Green: F and t values lower than the critical F and t statistics and p>0.05.

		CSIC-IMI			CSIC-LIST			LIST-IMI			CSIC-INIC			LIST-INIC			IMI-INIC		
		Z-average hydrodynamic diameter	PDI	Zeta potential	Z-average hydrodynamic diameter	PDI	Zeta potential	Z-average hydrodynamic diameter	PDI	Zeta potential	Z-average hydrodynamic diameter	PDI	Zeta potential	Z-average hydrodynamic diameter	PDI	Zeta potential	Z-average hydrodynamic diameter	PDI	Zeta potential
ERM00000064	F statistic												n.d.			n.d.			n.d.
	Test statistic																		
	p value																		
ERM00000088	F statistic																		
	Test statistic																		
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ERM00000089	F statistic																		
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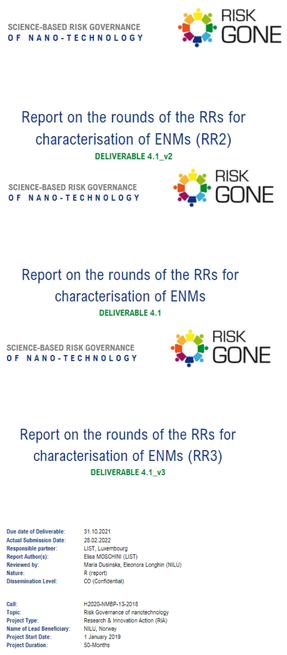
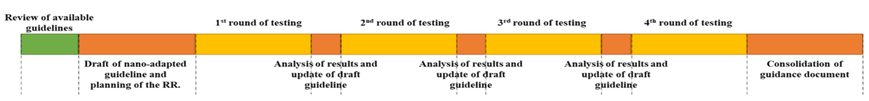




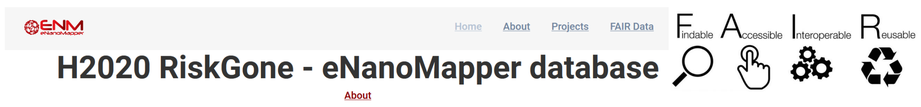
Box 4. available physicochemical data for the selected ENMs (MS TEAMS). *DMEM + 10% FBS; RPMI + 10% FBS

ERM identifiers	Name	Supplier	Stock suspension (in water)	Concentration Analysed suspension (water or CCM)	Time points	DLS water and CCM* Z-average = HMD	PDI	Z-potential	N. particles (NTA)	shape/size (TEM) (water and CCM)	Effective density (CCM)	Endotoxin content (water)
ERM00000062	TiO2 sigma	Sigma-Aldrich	1; 5 mg/mL	10; 100 ug/mL	0, 3, 24h	X	X	X	X	X	X	X
ERM00000063	ZnO sigma	Sigma-Aldrich	240 mg/mL	10; 100 ug/mL	0, 3, 24h	X	X	X	X	X	X	X
ERM00000064	TiO2 JRC	JRC - *in kind*	1; 5 mg/mL	10; 100 ug/mL	0, 3, 24h	X	X	X	X	X	X	X
ERM00000065	ZnO JRC	JRC - *in kind*	1; 5 mg/mL	10; 100 ug/mL	0, 3, 24h	X	X	X	X	X	X	X
ERM00000083	PLGA-AuNPs-WOW	MyBiotech - *in kind*	1; 5 mg/mL	10; 100 ug/mL	0, 24h	X	X	X	X	X	X	X
ERM00000084	PLGA-AuNPs-NP	MyBiotech - *in kind*	40 ug/mL	10; 100 ug/mL	0, 24h	X	X	X	X	X	X	X
ERM00000085	AuNPs-1 (15nm)	MyBiotech - *in kind*	300 ug/mL	10; 100 ug/mL	0, 24h	X	X	X	X	X	X	X
ERM00000086	AuNPs-2 (50nm)	MyBiotech - *in kind*	300 ug/mL	10; 100 ug/mL	0, 24h	X	X	X	X	X	X	X
ERM00000088	Pl-CuO (CuO 40nm)	PlasmaChem	1; 5 mg/mL	10; 100 ug/mL	0, 3, 24h	X	X	X	X	X	X	X
ERM00000089	WC/Co	NanoAmor	1; 5 mg/mL	10; 100 ug/mL	0, 3, 24h	X	X	X	X	X	X	X
ERM00000325	MWCNT 3wt%	Nanocyl	30 mg/mL	10; 100 ug/mL	0, 3, 24h	X	X	X	X	X	X	X

- ✓ **D4.2 Pre-validate methods and guidance documents for the characterisation of physicochemical properties (hydrodynamic diameter and size distribution) of ENMs**
- ✓ **D4.3 Pre-validate methods and guidance documents for the characterisation of physicochemical properties (dispersability) of ENMs.**
- ✓ **D4.4 Pre-validate methods and guidance documents for the characterisation of physicochemical properties (endotoxins content) of ENMs**
- ✓ **D4.5 Pre-validate methods and guidance documents for the characterisation of physicochemical properties (zeta potential) of ENMs**
- ✓ **D4.6 Pre-validate methods and guidance documents for the characterisation of physicochemical properties (particle counting) of ENMs.**

Box 1. Methods selected for ENMs characterization and reference protocols

Property	Proposed method	TG / ECHA guidance	Partner*
Dispersability of ENMs	DLS	R.7.1.1 ISO13318	**CSIC**, CID, LIST, UNIVE, INIC
Hydrodynamic Diameter and size distribution	DLS / NTA	R.7.1.14 OECD TG110	**CID**, LIST, NILU, UNIVE, INIC
Zeta potential	ZetaSizer		**CSIC**, LIST, UNIVE, CID
Endotoxins	Limulus amoebocyte lysate (LAL) test or Endotoxin Detection Assay based on ELISA technology (EndoLISA)	FutureNanoNeeds WP6	**NILU**, CSIC, LIST
Particle counting	NTA		NILU, **LIST**, INIC
Effective Density of suspended ENMs	Volumetric centrifugation method (VCM)[46]		**CID**, LIST

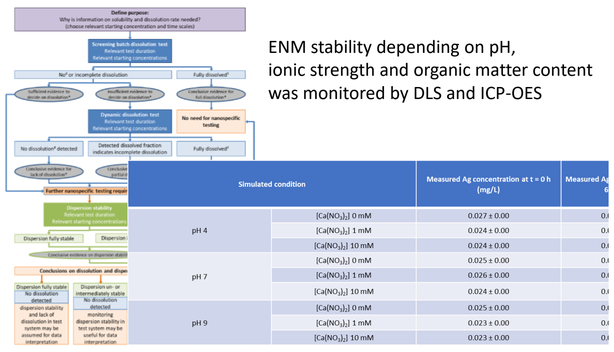


Box 2. List of ENMs selected for verification and implementation of existing protocols and pre-validation of SOPs

ERM identifiers	Name	CAS	Supplier	Supplier code	Core
ERM00000062	Titanium dioxide	1317-70-0	Sigma-Aldrich	637254	TiO2
ERM00000063	Zinc oxide	1314-13-2	Sigma-Aldrich	721077	ZnO
ERM00000064	Titanium dioxide	13463-67-7	JRC	JRCNM01005a	TiO2
ERM00000065	Zinc oxide	1314-13-2	JRC	JRCNM01101a	ZnO
ERM00000067	Ag nanowires	7440-22-4	Plasmachem	PL-AgW50	Ag
ERM00000083	PLGA-AuNPs-WOW	7440-57-5	MyBiotech GmbH	MJR-20190919-2	Au
ERM00000084	PLGA-AuNPs-NP	7440-57-5	MyBiotech GmbH	MJR-20190919-1	Au
ERM00000085	AuNPs-1 (nominal 15nm)	7440-57-5	MyBiotech GmbH	MJR-20190920-1	Au
ERM00000086	AuNPs-2 (nominal 50nm)	7440-57-5	MyBiotech GmbH	MJR-20190918-1	Au
ERM00000088	CuO 40nm	1317-38-0	PlasmaChem	PL-CuO	[Cu]=O
ERM00000089	Nano Tungsten Carbide/Cobalt Powder	12718-69-3	NanoAmor	5561HW	[WC]-[Co]
ERM00000325	MWCNT 3wt%	7782-42-5	Nanocyl	AQUACYL 0303-NC7000	Carbon

T4.2 Environmental fate (based on OECD TG318)

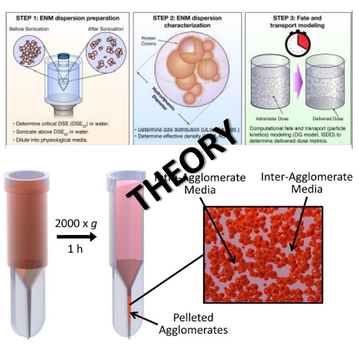
Box 5. OECD decision tree for choice of the experimental conditions for testing



ENM stability depending on pH, ionic strength and organic matter content was monitored by DLS and ICP-OES

T4.3 ENM *In vitro* dosimetry

Box 6. Optimization of protocol for Effective Density determination



DeLoid et al., 2017. *Nature Protocols*

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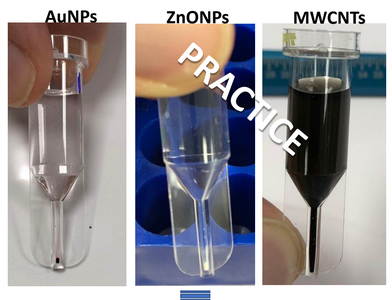

Need of further optimization For some ENMs

- ✓ **D4.7 Pre-validate methods and guidance documents for the characterisation of physicochemical properties (effective density) of ENMs.**
- ✓ **D4.9 Harmonised SOP for the resuspension of ENMs in biological media and in vitro dosimetry**

T4.4 Preparation of training material

Box 7. Video-training for Particle counting (NTA) and Particle size, Z-pot determination (DLS) www.riskgone.eu

CONCLUSIONS

- 6 pre-validation methods and guidance documents prepared
- 2 reports (on applicability of OECD TGs and ENMs dispersion for *in vitro* dosimetry) delivered
- Harmonization of data collection templates for P-CHEM
- Participation to "fairification" process by exploiting eNanomapper tools

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