

# **LIST ACTIVITIES IN THE FRAMEWORK OF RISKGONE – H2020-NMBP-13-2018 SCIENCE-BASED RISK GOVERNANCE OF NANO-TECHNOLOGY**

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### WP3. GUIDELINES FOR RISK-BENEFIT ASSESSMENT

Box1. D3.2 Draft guidelines regarding the quantification of lifecycle environmental and human health risk indicators



# WP4. CHARACTERISATION, IN VITRO DOSIMETRY AND **ENVIRONMENTAL FATE**

**T4.1 ENMs physicochemical characterization** Box2. Particle delivery among RiskGONE partners

**Box3.** Template wizard for P-CHEM on eNanomapper interface



methodology is still a quite novel discipline and its implementation is also affected by the specific characteristics of the materials.

Depending on LCA competences and data availability, different approaches might be suggested, from the most qualitative one to the most comprehensive one (e.g. including characterization factors for the (eco)toxic effects of nanoparticles emissions).

To contribute in filling this gap LIST worked on the development of a decision tree to guide users in the choice of the most suitable LCA methodology based on their needs.

LIST contribute also to the preparation of "D3.1 Draft guidelines for risk assessment"

# **WP5. HUMAN HAZARD ASSESSMENT**

Task 5.3 Evaluation and refinement of innovative in vitro models and mechanistically relevant assays for human hazard assessment

**Box8.** Key search criteria for critical review of the literature on 2D

LIST participated to the literature review exercise under SU lead, aimed at the identification of high quality papers targeting in vivo, simple in vitro, complex *in vitro* models applied for ENMs human hazard assessment studies.

SCIENCE-BASED RISK GOVERNANCE OF NANO-TECHNOLOGY

Due date of Deliverable Actual Submission Date Responsible partner: Report Author(s):

Reviewed by: Nature: Dissemination Level

Topic: Risk Governance of nanotechnolog Project Type: Research & Innovation Action (RIA) Name of Lead Beneficiary: NILU, Norway Project Start Date: 1 January 2019 Project Duration: 50-Months

Draft guidelines for risk assessment



LIST was responsible for ENM delivery to other partners  $\rightarrow$  same batch provided

#### **Box4.** LAL protocol adaptation for application to ENMs



LIST worked together with NILU and CSIC (2RRs) on the development of a protocol for endotoxin determination. The resulting SOP has been incorporated in "D4.4 Pre-validate methods and guidance documents for the characterisation of

GONE	Home Proje	ct Search Templa	te Wizard 🔹 Help 👻	[elisa.moschini] Log out				
Template Wizard : physche	em			0				
H2020 RiskGone - eNanoMapper database								
Assay		Excel template download						
Particle size distribution by TEM, DLS,NTA (F	tiskGone) ~	Download templa	ate					
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Project	Partner/organisation		Work Package					
RISKGONE LIST - Luxemb		tute of Science and $~\sim~$	WP4-CHARACTERISATION, IN VITRO DOSII					
Materials table - all materials will be available for selection in the Excel file								
Show 10 🗢 entries								
Adaption of	ENM identifiers in a	aroomont with		Previous 1 2 Next				
ERM ID Name		plier Supplier code	Batch	chemistry $\uparrow \downarrow$ CAS $\uparrow \downarrow$				
ERM00000062 ERM00000062 (Oirman Al	lioxide CHEBI_51050 Sigr	na Aldrich 637254	MKCK4358 TiO2	2 1317-70-0				

LIST worked together with IDEA Consult and with the other WP4 partners to improve existing P-CHEM Data Collection Templates, to make them harmonized and more user-friendly for users with various backgrounds.

#### **Box5.** Protocol development for particle counting (by NTA)

Parameter	Script
Equilibration of the temperature	SETTEMP 25
Start of the camera interface	
Start the loop of acquisition	REPEATSTART
Pump a little volume	PUMPADV
5 sec stabilization	DELAY 5 Consolidated pre-validated guidance document on nanoparticle counting
60 sec of recording	CAPTURE 60 DELVERABLE 4.6
1 sec break	DELAY 1
Restart the loop 2 times	REPEAT 2 Let faithments bit 1 2020 Bit 1 202
Switch off temperature control	TEMPERATURECONTROI
Start the data processing	PROCESSSINGLESETTIN set of a function of the set of th
Open the export window	EXPORTRESULTS



LIST worked on the development of an easy implementable protocol for particle counting by NTA. Particle number is a key parameter for applicability of OECD TG318 (Environmental fate). As result of this work, the "D4.6 Pre-validate methods and guidance documents for the characterisation of physicochemical properties (particle counting) of ENMs" has been prepared.

#### *in vitro* monoculture models.

Exposure Condition

Monoculture Cell Line	Search Terms	Endpoints (derived from advanced model results)	Partner Responsib
A549	A549 AND ZnO nanoparticles	Cytotoxicity, genotoxicity, (pro)-inflammatory	NILU & LIST
	A549 AND TiO <sub>2</sub> nanoparticles	response	
	A549 AND Ag nanoparticles		
	A549 AND CuO nanoparticles		
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#### **Box9.** Experimental design for interlaboratory comparison study

Tissue being modelled Lung	Advanced Model LIST Lung Model: A549/EA.hy926/THP-1	Endpoint & Assay Employed Cytotoxicity (Alamar Blue) Inflammatory Response (IL- 8 ELISA)	Partner(s) SU LIST NILU	Nanomaterials PlasmaChem (PL-CuO) CuO (ERM00000088) NM105 TiO <sub>2</sub> (erm:ERM00000064) Triton X-100 Positive control for cytotoxicity. LPS Positive inflammatory marker	Exposure Time (h) 24	Concentrations Negative control (Saline (0.9% NaCl) CuO, 1000 ng/cm <sup>2</sup> TiO <sub>2</sub> , 1000 ng/cm <sup>2</sup> Triton X-100, 1% LPS, 1ug/ml	Sonication LIST SOP	with the selection applied f	internal of inn of ENM <i>itro</i> alvec
Deposition (ng/cm <sup>2</sup> ) 00 01 00	0 0 0 0 0 NC TiO <sub>2</sub> Material	La La La Cuo	b 1 Depositi b 2 Depositi		CON <sup>ICCO</sup> purmont	Participation of the second	CCM CCM+TIO2 CCM+CuO AlamarBlue AlamarBlue+TiO2 AlamarBlue+CuO	Alveolus	on-a-trans
Viability (%)	A 200 - Lab 1 V 150 - 100 - 50 - José 0 - V (S <sup>1</sup> ) (S <sup>1</sup> ) (S <sup>1</sup> ) (S <sup>1</sup> )	iability =		bility 20000 15000- (Jwg6d) 10000- 5000- 5000- 0- 0- 0- 0- 0- 0- 0- 0-	Lab 1	IL6 🗖 Lab 2		Typ-I Epithelial cell	Alveolus Surfactan

The alveolar triculture model established at LIST has been used in an interlaboratory study to evaluate cytotoxic and inflammatory effects after exposure to TiO<sub>2</sub> and CuO through VitroCell Cloud-12.

The labs followed the same SOP, targeting the same deposited dose around 1000 ng/cm<sup>2</sup> No interference between the ENMs tested and the component of the assays have been observed The model set up in Lab 1 was more responsive to ENM exposure The results about IL-8 production looked comparable

The results of this exercise have been combined know-how of the partners for the novative in vitro models to be human hazard assessment.

olar triculture model (Klein et al., 2013)

# Air-Liquid Interface Surfactan Endothelial cells (EA.hy 926) Capillary : Alveolar type II epithelial cells (A549)

physicochemical properties (endotoxins content) of ENMs.

#### T4.3 ENM In vitro dosimetry

Box6. Effect of VCM application on representative ENMs



LIST joined **CIDETEC** in RR exercise for determination of Effective Density of selected ENMs

#### **T4.4 Preparation of training material**

#### **Box 7.** Video-training for Particle counting (NTA)



LIST contributed to the preparation of publicly available training material www.riskgone.eu

# WP6. ECO-TOXICOLOGICAL HAZARD ASSESSMENT

Box11. Models for ecotoxicological hazard assessment

LIST contributed to supervising the preparation of "D6.3 Documented protocols, data capture, and meta data templates for revised OECD tests, and pre-validated alternative test methods."

LIST contributed to the literature review exercise by selecting protocols, models and methods for ecotoxicological hazard assessment of ENM as potential endocrine disruptors.

LIST joined NanoHarmony activities direct to the evaluation and adaptation of OECD TG 201 and TG 203 to ENMs.



# WP7. COMMUNICATION AND LINKS TO INTERNATIONAL BODIES



#### Link to international bodies

#### LIST staff are members of:

- OECD Steering group on Advance Materials
- OECD Steering group on Sustainable Innovation
- OECD ESCA meeting
- OECD expert group on Biodistribution
- OECD expert group on Intestinal fate

#### LIST is participating annually to:

• OECD WPMN meetings • OECD WNT meetings



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