SCIENCE-BASED RISK GOVERNANCE
OF NANO-TECHNOLOGY

RISK GONE

End-User Training Materials for the Users and the Research Community

DELIVERABLE 6.7_v2

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Abstract

This document represents the updated version of the RiskGONE deliverable 6.7, End-User Training Materials for the Users and the Research Community (WP6, environmental hazard assessment). Training materials include all types of materials that give practical directions (i.e., theoretical and practical aspects of the developed methods, either written or through video or other multi-media formats, Standard Operating Procedures (SOPs), guidelines developed within the RiskGONE project etc.) in the assessment of the environmental impact of nanomaterials utilizing pre-defined tests or assays. The current deliverable report collects information about the planned types and topics the training activities being, or to be, developed (e.g., presentations, factsheets, videos), their targeted audience(s) (e.g., the scientific community, contract research organizations performing standardized testing, regulators), any proposed cooperation between EU projects in development of the training materials, as well as the strategy for dissemination (i.e., social media, YouTube, Vimeo channel, RiskGONE website, protocols repositories etc.). The dissemination of the training materials will be linked to WP7, and therefore the materials will reach the widest possible public audience, including industry, the scientific and contract research communities as well as other stakeholders.

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List of Abbreviations

ENMs – Engineered nanomaterials

ERA – Environmental Risk Assessment

EUON - EU Observatory for Nanomaterials

GA – General Assemblies

QSAR - Quantitative Structure-Activity Relationship

RG – Risk Governance

SOP(s) – Standard Operating Procedure(s)

WP – Work package

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1. Introduction

The aim of WP6 is to support Risk Governance (RG) of Engineered Nanomaterials (ENMs) by delivering a more efficient, reliable, and user-friendly Environmental Risk Assessment (ERA) safety testing strategy through the development of an improved nano-specific predictive framework of harmonized and pre-validated regulatory-oriented ERA tools. During the project, project partners are focused on searching, improving, and developing strategies for evaluating the environmental impacts of several ENMs selected as case studies to cover a diverse range of ENM properties and application areas. After careful internal validation of the assay protocols through interlaboratory testing or round robin tests, adjusted and/or developed methodologies will be fully documented into Standard Operating Procedures (SOPs) and implemented and integrated into the RiskGONE Risk Governance (RG) framework, thereby enabling them to be used by a wide range of users. Since the methods have been internally validated for a specific set of ENMs, potential application for different ENMs, beyond those for which the method has been internally validated, will require particular care and consideration, as laid out in the training materials accompanying test method, which will highlight any points of concern or areas for specific confirmation of the assay validity to the specific ENM.

The objective of this deliverable is to collect all developed within WP6 end-user training materials. These training materials are based on the knowledge gained within WP6 and will be widely communicated and disseminated to ensure the sustainability of the RiskGONE project outputs (in cooperation with WP7).

From the perspective of WP6, developed methodologies will be reflected in written SOPs. The final test methods will also be documented as test guidelines and supported with the wide range of training materials (e.g., live and recorded workshops focusing on biological assays relevant to environmental hazard assessment, training videos, webinars, as well as factsheet dedicated to SOPs and guidance documents). This deliverable also assumes the developed before plan to reach the widest possible audience of RiskGONE training, in collaboration with WP7. All RiskGONE end-user activities will be disseminated through social media channels such as YouTube and Vimeo.

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End-user training materials activities

Different type of activities, that have been developed within WP6 to describe, document and train users in applications of the WP6 test methods, have been collected. In the final list we have included the following activities:

- Written documents related to the SOPs and guidance documents
- Webinars presentations on the methods and approaches
- Short training videos presenting the specific ERA methods
- PowerPoint presentations dedicated to specific ERA methods

All end-user training materials developed within WP6 will be made publicly available on the RiskGONE website. Moreover, the training videos and presentations will be also uploaded to the RiskGONE YouTube and Vimeo.

Target audience for training materials

This section defines the target audience for the RiskGONE end-user training material. After detailed discussion with the WP6 partners (and with collaboration with WPs 4, 5 and 7 partners), the following target audience groups have been established as being the most relevant:

- Research scientists (from academia to industry)
- Regulatory scientists including those working in contract research organizations charged with generating data for regulatory dossiers
- Toxicology-related industry sectors which regularly incorporate ENMs into toxicology-based assays and biological testing
- Governmental bodies
- Non-governmental bodies

Collaboration with other European projects

The main aim of the RiskGONE project is to provide procedures and methods for risk governance of ENMs. The knowledge gained within the RiskGONE will be communicated to the wide scientific community, and the training materials (e.g., SOPs, practical guidance videos, etc.) will be targeted to the ongoing EU projects and especially to the standardization organisations, contract research organisations and the EU Observatory for Nanomaterials (EUON). The idea of close collaboration with other EU projects is to harmonize all templates,

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SOPs development, methodologies, procedures, and training that are related to ERA of ENMs relevant to the activities in WP6 within RiskGONE. RiskGONE (WP6) already closely collaborates with PATROLS, NanoSolveIT, and NanoCommons projects where the methods for assessing the environmental risk of ENMs are a common ground of activities (including e.g., the development of the strategy for the AOP approach for *Daphnia magna* reproduction). Existing collaboration with NanoHarmony is also being utilized to support the transfer of the updated test guidelines for *Daphnia magna* chronic (reproduction) and multi-generational testing into regulation.

The list of other highly relevant projects dedicated to ENMs, with which RiskGONE collaborates or would like to collaborate in future is presented below, although not all have dedicated ecotoxicity activity:

- Gov4Nano
- NANORIGO
- NanoInformaTIX
- CompSafeNano
- Diagonal
- DaNa4.0
- NanoFabNet
- SbD4Nano
- SABYDOMA
- NanoSolveIT
- NanoHarmony

2. Scientific progress

The training materials generated within WP6 include developed SOPs which were internally validated and adjusted in the round robin exercises. The developed training materials focus attention on the key adaptions of existing tests (such as the OECD 202 and 211 tests for Daphnia) needed for assessment of pristine and aged ENMs and provide detailed explanations of all areas of the assays which are the most important/critical and required extensive training. We have focused on development of a set of harmonized SOPs for the following assays relevant to ecotoxicology testing of ENMs (Table 1):

- Non-invasive impedance-based methods for nanotoxicity testing on Red Trout Gut cells and zebrafish embryo cells (no OECD test as yet but impedance included in OECD Series on the Safety of Manufactured Nanomaterials No. 85);

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- Impedance-based flow cytometry method for single cell-based nanotoxicity testing on daphnia resting eggs (note as with zebrafish embryo where removal of the chorion is needed to allow access of the ENM, for resting eggs the ephippium will need to be removed and methods to optimize this are under development at UoB). (no OECD test as yet but impedance included in OECD Series on the Safety of Manufactured Nanomaterials No. 85);
- Microfluidic impedance-based methods for eco-nanotoxicity testing (no OECD test as yet but impedance included in OECD Series on the Safety of Manufactured Nanomaterials No. 85);
- Comet assay with Alamar Blue assays for cytotoxicity applied to fish cells using rainbow trout gill cells and Zebrafish embryo cells (OECD Series on the Safety of Manufactured Nanomaterials No. 85);
- Comet assay applied on hemolymph extracted from whole *Daphnia magna* exposed using the RiskGONE adaptions to the OECD 202 test methodology.

In addition to development of training videos dedicated to the experimental measurements, we have focused also on developing videos and factsheets that inform users about *in silico* methods that could be useful as alternative/support approaches to experimental testing. Based on the guideline developed in RiskGONE for NanoQSAR (Quantitative Structure-Activity Relationship (QSAR)) (submitted in WP3), we have delivered a series of short informative videos dedicated to the QSAR methodology and its application in ERA.

Table 1 summarizes the most current end-user training materials which have been – or will be developed within WP6 – eco-toxicological hazard assessment. It presents the subjects and forms of the training materials that were developed by contributing partners and indicates the location of the training materials. All developed trainings are available internally to project partners through Teams (Communication & Dissemination Materials canal). However, these will be made publicly available through the RiskGONE website and the RiskGONE YouTube and Vimeo channels once the methods are finalized so they are accessible to broad audiences.

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Table 1. Catalogue of end-user training materials developed from activities within WP6 – Environmental Risk Assessment

Partner	Assay	End-user training material		RiskGONE File Location	Public Location
		Planned/Achieved	Available		
NILU	Alamar Blue assay applicable to fish cells	• Alamar Blue SOP for RR2. No changes required from RR1 SOP.	• Short PowerPoint presentation on Alamar Blue. • Alamar Blue SOP. • Data capture template for Alamar Blue assay	• Short PowerPoint presentation on Alamar Blue: Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training. • Alamar Blue SOP for RR1: Microsoft TEAMS > Task 5.1 SOPs.	• Will be made available on the RiskGONE website
NILU	Comet assay applicable to fish cells	• Comet assay SOP updated version for RR2. • Comet assay Practical online course/video learning	• Comet assay SOP for RR1. • PowerPoint presentation on comet assay. • Data capture template for Comet assay adapted for fish cells	• Short PowerPoint presentation on Comet assay: Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training. • Comet assay SOP for RR1: Microsoft TEAMS > Task 5.1 SOPs.	• Video available on the RiskGONE website> Resources
QSARLab	NanoQSAR AOP	• Webinar on AOPs for risk assessment of nanomaterials – Section 3: NanoQSAR-AOPs	• Recorded presentation	• This could be available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training	• RiskGONE website > Resources > Webinars
IMI	AOP	• Webinar on AOPs for risk assessment of nanomaterials – Section 1: Introduction	• Recorded presentation	• This could be available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training	• RiskGONE website > Resources > Webinars

IMI	Sex-related response to NMs	• Factsheet on sex-related response to NMs	• Pdf (three pages) facts on the sex-related response to NMs	• This could be available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training	• This will be available on the RiskGONE website
QSARLab	Alternatives for animal testing	• Factsheet on alternative models for toxicity testing (in collaboration with PATROLS)	• Pdf (one page) facts on alternative methods	• PDF on QSAR: Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training	• This will be available on the RiskGONE website
QSARLab	QSAR for nanomaterials	• Factsheet on QSAR modelling for nanomaterials	• Pdf (one page) facts on alternative methods	• This could be available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training	• This will be available on the RiskGONE website
QSARLab	QSAR for nanomaterials	• Short training video	• Recorded video	• This could be available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training	• This will be available on the RiskGONE website
QSARLab	NanoQSAR-AOP for ecotox	• Factsheet on NanoQSAR-AOP for ecotox	• Under development	• This could be available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training	• This will be available on the RiskGONE website
QSARLab	QSAR methodology	• Short training video	• Recorded video	• This could be available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training	• This will be available on the RiskGONE website
UiB	Non-invasive impedance-	• PowerPoint	• Under development	• This will be made available on	• This will be made

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	based methods for nanotoxicity testing on Red Trout Gut cells	presentation		Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training	available on the RiskGONE website
UiB	Non-invasive impedance-based methods for nanotoxicity testing on Red Trout Gut cells	• Video of key stages	• Under development	• This will be made available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training & in WPs 5 and 6 folders	• This will be made available on the RiskGONE website
UiB	Microfluidic impedance-based methods for eco-nanotoxicity testing	• PowerPoint presentation	• Under development	• This will be made available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training	• This will be made available on the RiskGONE website
UiB	Microfluidic impedance-based methods for biomimetic eco-nanotoxicity testing	• Video of key stages	• Under development	• This will be made available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training & in WPs 5 and 6 folders	• This will be made available on the RiskGONE website
UiB	Impedance-based flow cytometry method for nanotoxicity testing on daphnia eggs	• PowerPoint presentation	• Under development	• This will be made available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training & in WPs 5 and 6 folders	• This will be made available on the RiskGONE website
UiB	Impedance-based flow cytometry method for single cell-based	• Video of key stages	• Under development	• This will be made available on Microsoft TEAMS > Project RiskGONE > Communication &	• This will be made available on the RiskGONE website

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	nanotoxicity testing on daphnia eggs			Dissemination Materials > Training & in the WP 6 folders	
UoB	Culturing and maintenance of immortalized zebrafish embryo (ZF4) cells	• Detailed SOP • Video protocol • Recorded presentation	• Under development	• This will be made available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training & in the WP6 folder	This will be made available on the RiskGONE website
UoB	Comet assay protocol for ZF4 cells coupled with quantification of NM uptake and localisation	• Detailed SOP • Video protocol • Recorded presentation • Data capture template	• Under development	• This will be made available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training & in the WP6 folder	• This will be made available on the RiskGONE website
UoB	Acute toxicity testing of freshly dispersed and aged NMs using *Daphnia magna* (OECD 202)	• Detailed SOP • Recorded presentation • Data capture template	• Under development	• This will be made available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training & in the WP6 folder	• This will be made available on the RiskGONE website
UoB	Extension of OECD 202 to include determination the NMs acquired biomolecule corona	• Detailed SOP • Recorded presentation • Data capture template • Video protocol	• Under development	• This will be made available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training & in the WP6 folder	• This will be made available on the RiskGONE website
UoB	Comet assay on hemolymph following whole daphnia exposure to fresh versus aged NMs	• Detailed SOP • Recorded presentation • Data capture template	• Under development	• This will be made available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training & in the WP6 folder	• This will be made available on the RiskGONE website

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		• Video protocol			
UoB	Chronic (reproductive) toxicity testing using *Daphnia magna* (OECD 211) using freshly dispersed and aged NMs	• Detailed SOP • Recorded presentation • Data capture template • Video protocol	• Under development	• This will be made available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training & in the WP6 folder	• This will be made available on the RiskGONE website
UoB	Extension of chronic (reproductive) toxicity testing using *Daphnia magna* (OECD 211) to include recovery of the NMs and evaluation of their acquired biomolecule corona at different timepoints	• Detailed SOP • Recorded presentation • Data capture template • Video protocol	• Under development	• This will be made available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training & in the WP6 folder	• This will be made available on the RiskGONE website
UoB	Extension of chronic (reproductive) toxicity testing using *Daphnia magna* (OECD 211) test to include multiple generations using a paired continuous exposure and recovery generations	• Detailed SOP • Recorded presentation • Data capture template • Video protocol	• Under development	• This will be made available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training & in the WP6 folder	• This will be made available on the RiskGONE website
UoB	Dynamic energy budget model integrated into AOP for NMs-reproductive impairment in *Daphnia magna*	• Recorded presentation • PowerPoint presentation	• Under development	• This will be made available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training & in the WP6 folder	• This will be made available on the RiskGONE website
NovaM &	QSAR model for prediction	• Training manual	• Recorded presentation	• This will be made available on	• This will be made

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UoB	of acute toxicity of freshly dispersed versus aged NMs to *Daphnia magna*	• Video demonstration • PowerPoint presentation • (In collaboration with NanoSolveIT)	• QMRF report form explaining the model (In collaboration with NanoSolveIT)	Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training & in the WP6 folder	available on the RiskGONE website
NovaM & UoB	QSAR model for prediction of epigenetic effects in daphnids exposed to freshly dispersed versus aged NMs	• Training manual • Video demonstration • PowerPoint presentation • Recorded presentation • (In collaboration with NanoSolveIT)	• Under development	• This will be made available on Microsoft TEAMS > Project RiskGONE > Communication & Dissemination Materials > Training & in the WP6 folder	• This will be made available on the RiskGONE website

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3. Deviations from description of action

Due to Covid-19 pandemic, face-to-face workshops and training events could not take place, and partners have been very flexible in their approaches and have used the time to focus on online-based events and preparation of training videos dedicated to developed SOPs and guidelines. All partners involved in this task participated in the strategic project meetings dedicated to training materials (i.e. joint meeting of WP4,5,6,7) where they could contribute and comment on the development of the activity plan and its dissemination. All ideas and comments related to this task were also discussed at general assemblies (GA) and regular consortium meetings.

4. Conclusions

The deliverable 6.7 presents the progress in the development and completion of end-user training materials related to the WP6 – eco-toxicological hazard assessment, as defined by Task 6.4. It includes types and topics of activity, targeted audiences, cooperation between EU projects as well as a dissemination strategy. All activities in the Task 6.4 have been regularly addressed and discussed during the WP6 and consortium meeting so all developed presentations/videos are the most robust. The training materials are the final products that present the proposed methods and developed SOPs/guidances. They were evaluated after series of round robins to be the most appropriate in the assessment of the environmental impacts of nanotechnologies. The trainings developed within WP6 are available to all RiskGONE partners in the relevant folders in TEAMS, and the final materials will be shared with all the community and main stakeholders through the website and social media.

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The publication reflects only the author's co-authors of the training materials view and the European Commission is not responsible for any use that may be made of the information it contains.

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