Risk Assessment in Nanomedicine

Needs for harmonisation, standardisation and validation

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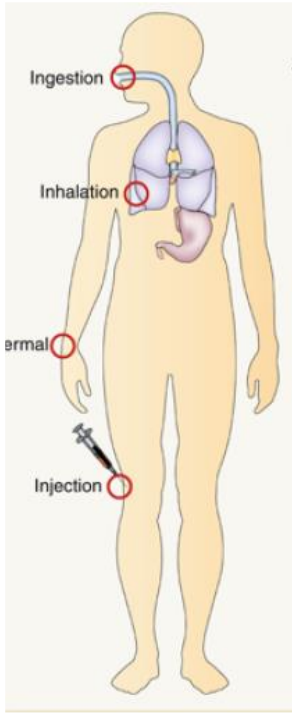
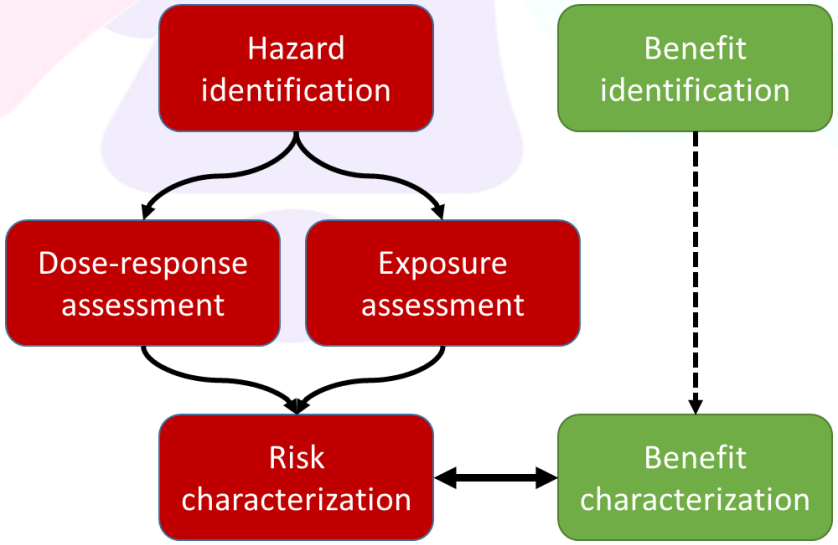
This project has received funding from the European Union's Horizon 2020 programme: grant agreement 814425.

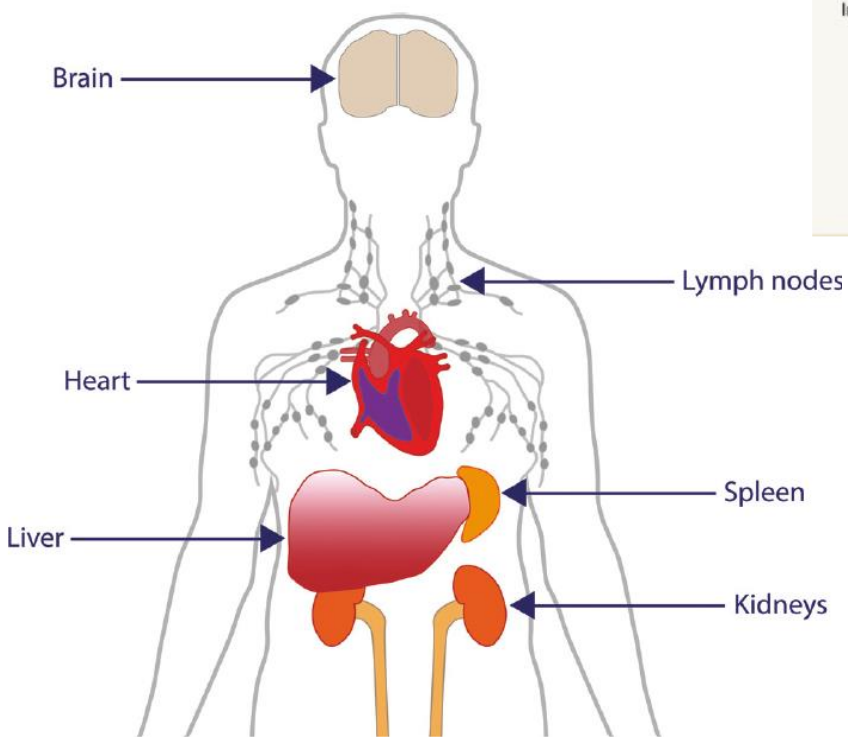
Risk Assessment

The traditional risk assessment methodology comprises the following stages:

- **Hazard identification**
- **Hazard characterization including dose-response assessment**
- **Exposure assessment**
- **Risk characterization**







From Foulkes et al., Biomater. Sci., 2020, 8, 4653–4664

This project has received funding from the European Union's Horizon 2020 programme: grant agreement 814425.

Regulation of NM, risk assessment of NM

Starting point REACH, 1907/2006

Harmonisation Governance

2008 — **EFSA**
Food additive Regulation 1333/2008, **OECD** Program established

2009 — NanoTEST
1223/2009, a definition of "NM and requires premarket notification
EMA workshop

2009 — Biocidal products regulation 528/2012

2011 — 2011/696/EU Definition, Labelling

2012 — **ECHA, SCCS, 2012**
Guidance on safety assessment of NMs in cosmetics (SCCS/1484/12)
Medical devices 2012

2014 — **FDA, SCCS, 2014**
Memorandum (SCCS/1524/13

2015 — **SCENIHR**
Guidance on medical devices
EFSA
Definition of NM in Novel Food Regulation (EU) 2015/2283.

2017 — **ECHA, 2017**
Registration dossiers that cover nanoforms:, best practices

2018 — **REACH 2018/1881 amending regulation 1907/2006**
EFSA, 2018
Guidance on RA of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health,

2019 — **SCCS, 2019**
GUIDANCE ON THE SAFETY ASSESSMENT OF NMs IN COSMETICS

2020 — **ECHA/NR/19/44 2020**
Updated guidance for registering substances in nanoform

2022 — **SCCS, 2021**
SCIENTIFIC ADVICE on the safety of nanomaterials in cosmetics

2022 — **EFSA, 2021**
Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles

2023 — **OECD, 2022**
Important issues on risk assessment of manufactured nanomaterials

SCCS Nano Guidance

RISK GONE

Regulatory landscape in nanomedicine for RA

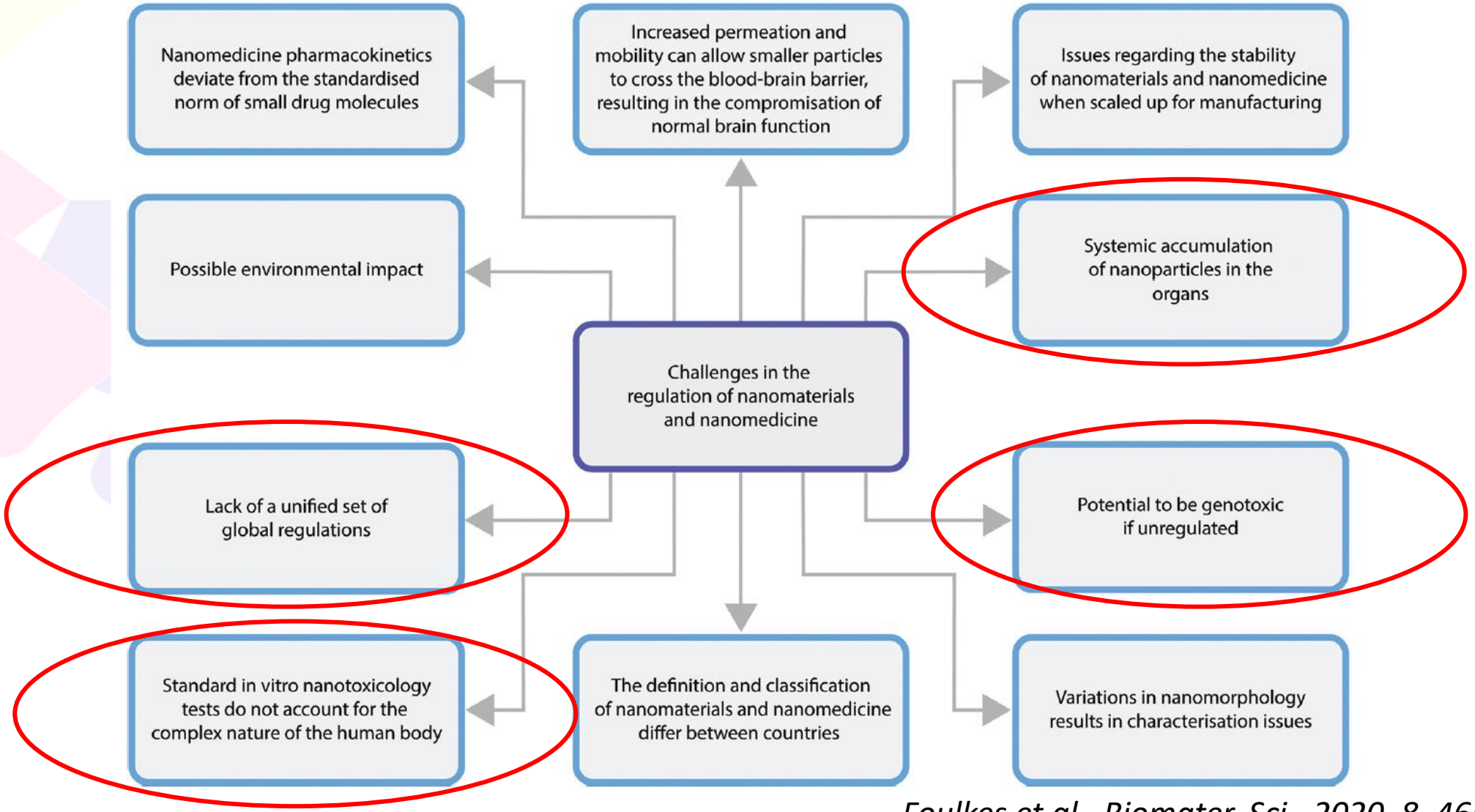
European Medicines Agency - EMA

- General Medicinal Product legislation on regulating nanomedicines using current risk/benefit-analysis principles- no specific regulatory framework
- Regulated either as medicinal products or medical devices.
- Limited guidance on regulatory information needs
- Several guidance documents- a range of specific preliminary guidelines for a range of nanomedicine preparation standards.
- Definition of nanomedicine
- Multidisciplinary expertise to evaluate nanomedicines
- Expert groups established
- EMA-FDA communication of experts

US Food and Drug Administration - FDA

- Produced a draft guidance on drug products, including nanomaterials
- Evaluation case by case

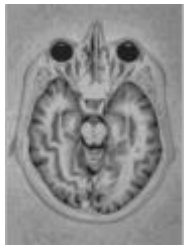
Challanges in regulation of nanomedicinale products

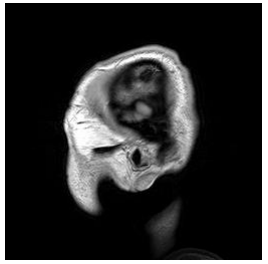


Foulkes et al., Biomater. Sci., 2020, 8, 4653–4664

NanoTEST

http://www.nanotest-fp7.eu

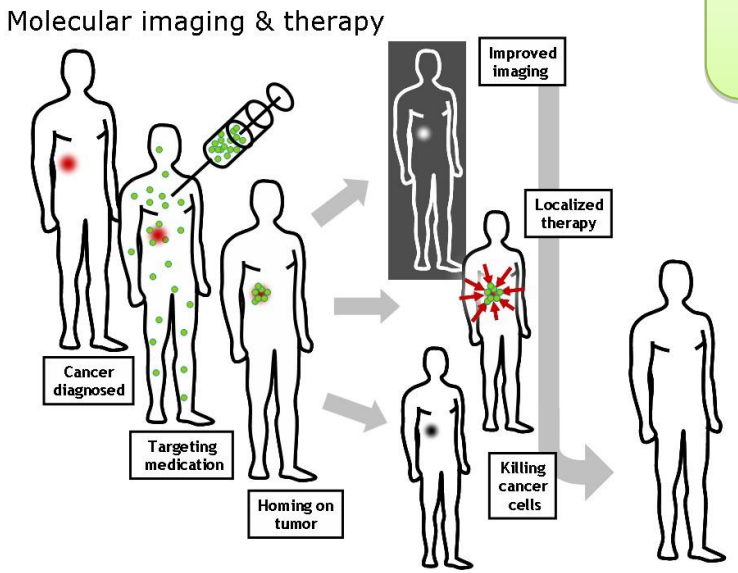




Work programme topic:
HEALTH-2007-1.3-4 "Alternative testing strategies for the assessment of the toxicological profile of nanoparticles used in medical diagnostics".

Starting date: April 1st, 2008,
Length: 42 months
EC contribution: 2,994,383 Euro

Interaction of medical nanoparticles with biological systems



Aim is to develop testing strategies and high-throughput toxicity-testing protocols using *in vitro* and *in silico* methods essential for the risk assessment of NPs used in medical diagnostics and compare them with *in vivo*

Over 15 years of nanosafety research and development of NAMs

NanoTEST

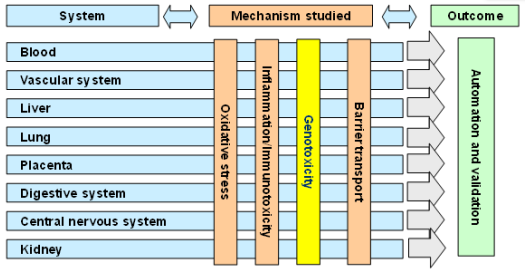
In vitro ⟷ *in vivo* ⟷ *ex vivo*

Blood: human blood cells- leucocytes, granulocytes, monocytes, etc. TK6

Vascular: HCEC, EC219, ECp23, HL1

Liver: hepatocytes, kupffer cells and liver sinusoidal endothelial cells (LSEC), HepG2

Kidney: monkey kidney Cos-1 cells, human HEC

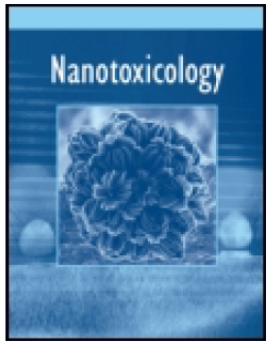


Placenta: Placenta perfusion, BeWo cells

Central nervous system : HCEC, EC219, Murine N11 micro

Digestive system :

Liung: bronchial epithelial

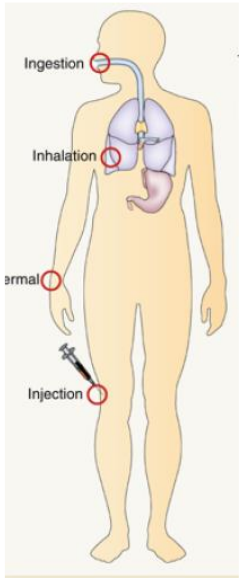


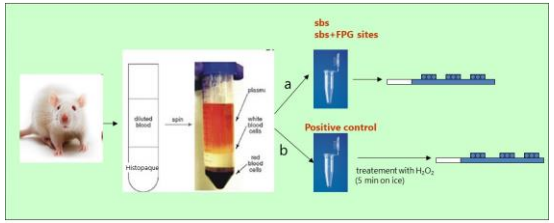
Nanotoxicology

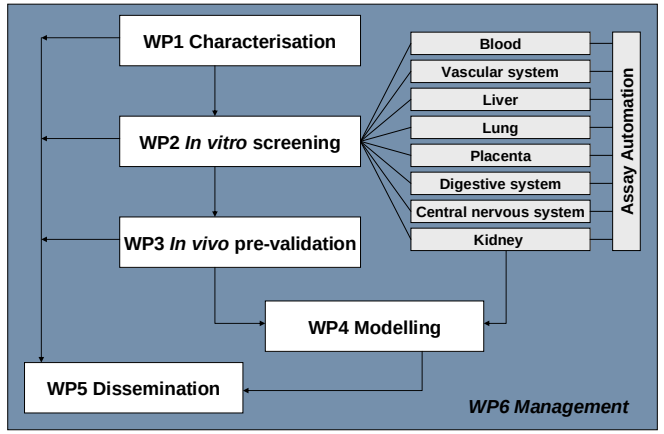
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/inan20

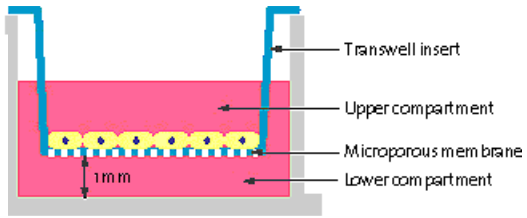
NanoTEST in a Nutshell

Maria Dusinska[a] & Lang Tran[b]
[a] Norwegian Institute for Air Research, Kjeller, Norway and
[b] Lang Tran, IOM, Edinbourg, UK
Published online: 06 May 2015.



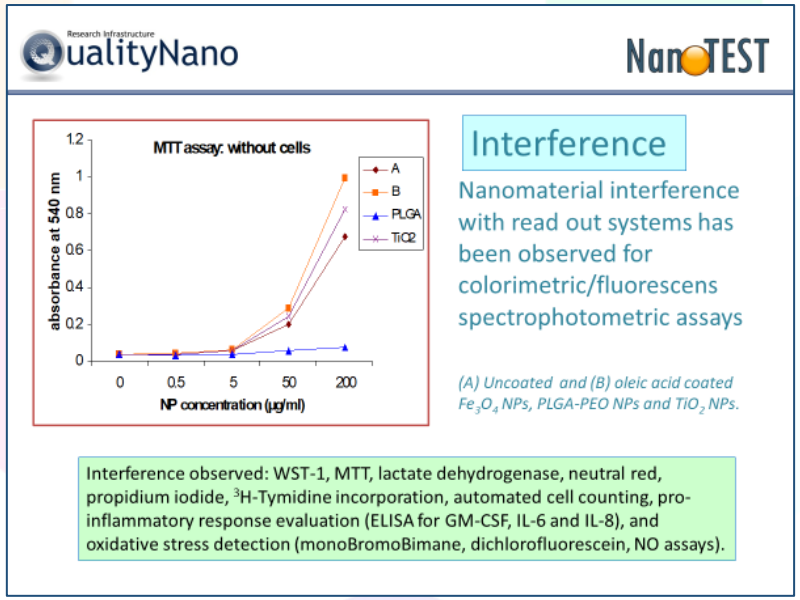


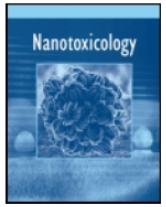




3-D structure, spheroids, more representative of tissue architecture and allows to study drug diffusion inside the « tissue »

Interference of nanomaterials with assay components or detection system





Nanotoxicology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/inan20

Toxicity screenings of nanomaterials: challenges due to interference with assay processes and components of classic in vitro tests

Rina Guadagnini[a], Blanka Halamoda Kenzaoui[b], Laura Walker[c], Giulio Pojana[df], Zuzana Magdolenova[e], Dagmar Bilanicova[df], Margaret Saunders[c], Lucienne Juillerat-Jeanneret[c], Antonio Marcomini[df], Anna Huk[e], Maria Dusinska[e], Lise M Fjellsbø[e], Francelyne Marano[a] & Sonja Boland[a]

[a] Univ Paris Diderot, (Sorbonne Paris Cité), UMR 8251 CNRS, Unit of Functional and Adaptive Biology (BFA), Laboratory of Molecular and Cellular Responses to Xenobiotics (RMCX), 75205 Paris cedex 13, France,

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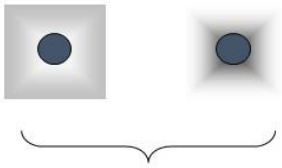
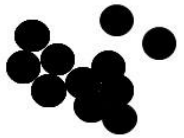
[e] NILU-Norwegian Institute for Air Research, Health Effects Laboratory, Department of Environmental Chemistry, P.O. Box 100, 2027, Kjeller, Norway and

[f] DFBC-Department of Phylosophy and Cultural Heritage, Dorsoduro 3484/D, 30123 Venice, Italy

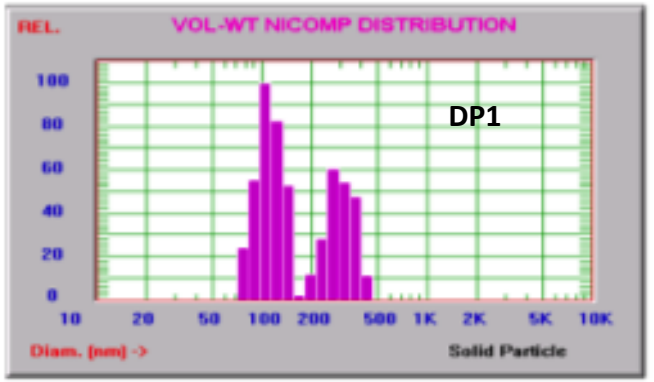
Published online: 06 May 2015.

Interference control needs to be included for all tests methods

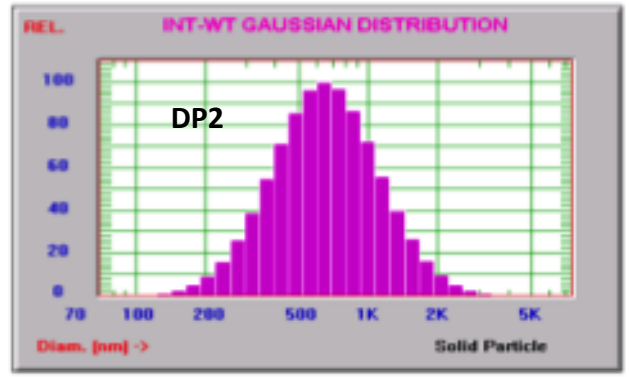
NP properties:	**Light adsorption**	**Light scattering**	**Aggregation/ Agglomeration**	**Surface reactivity**	**Dissolution**
				 Adsorption/Reaction with: Assay reagents; Biomolecules	
Problematic techniques:	**Spectrophotometry Spectrofluorometry**		**Flow cytometry Cell counting**	**Chemistry Biochemistry Immunochemistry**	**Chemistry Biochemistry**
Problematic assays:	WST-1, MTT, NR, ^{3}H-T, mBBr, DCF, Griess reagent…		Cell proliferation, PI uptake…	MTT, ^{3}H-T…	ELISA, LDH…	MTT, LDH…

Dispersion of NMs is important

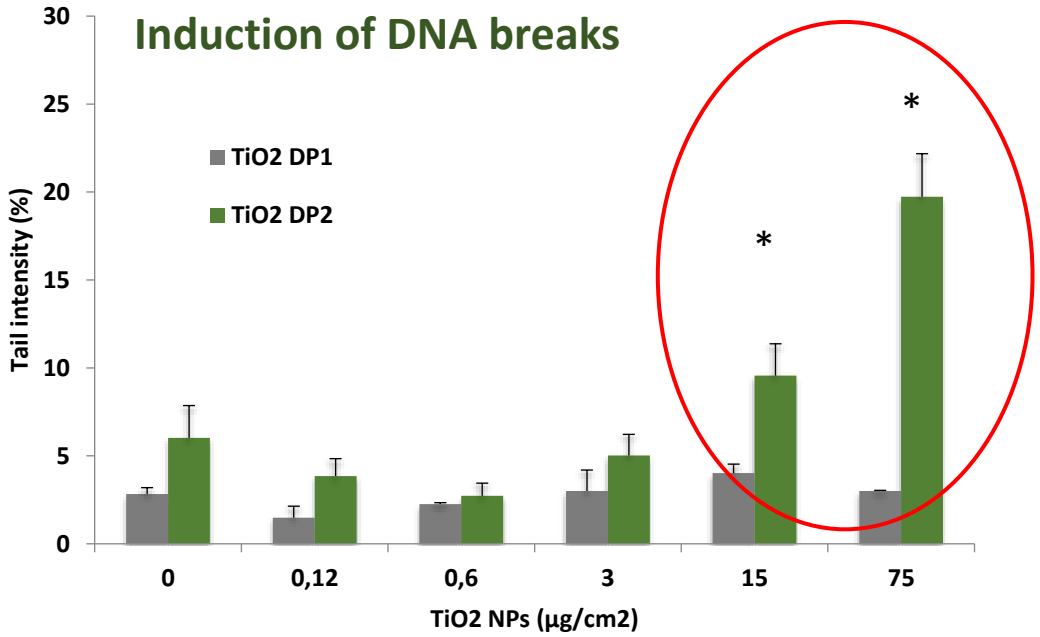
- The stability of a dispersion; two dispersion protocols
- Same TiO_2 - protein corona formation
- Different results
- Concentration can be affected by agglomeration, sedimentation, binding with other moieties in the medium, or adhesion to glass/plastic



DP 1 with serum



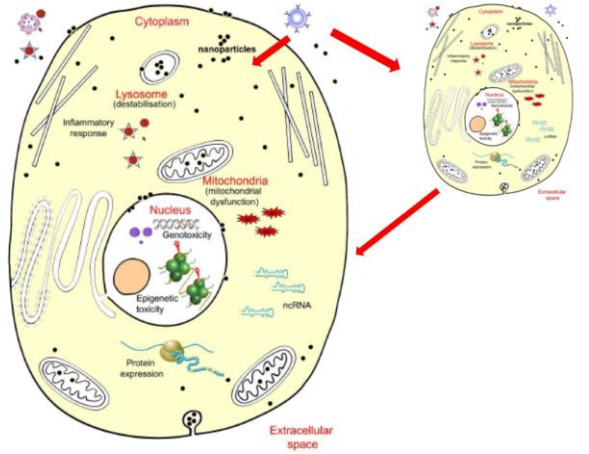
DP2: without serum



Magdolenova et al, Impact of agglomeration and different dispersions of titanium dioxide nanoparticles on the human related in vitro cytotoxicity and genotoxicity. J Environ Monit., 2012, 14(2):455-64.

This project has received funding from the European Union's Horizon 2020 programme: grant agreement 814425.

ENM genotoxicity (AOP and MoA)



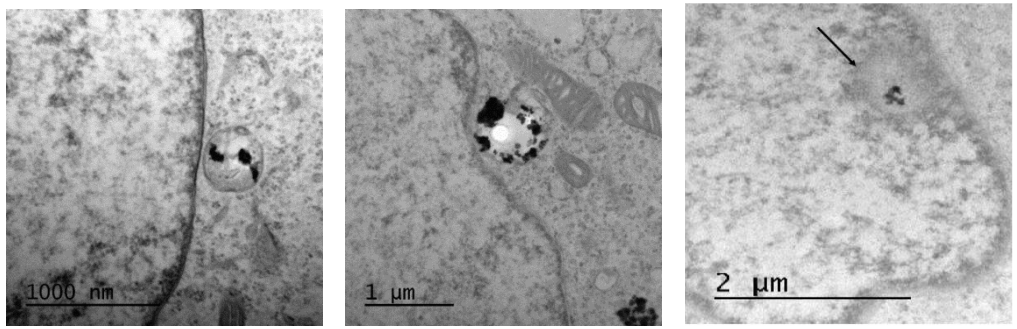
Primary genotoxicity:

- **Direct interaction with DNA**
- **Indirectly:** via oxidative stress, cell membrane damage, lipid peroxidation, mitochondrial disfunction, antioxidant depletion, damage to spindle apparatus, damage or interaction with cell signalling, via epigenetic changes, or intermediate molecules or proteins involved in cell cycle, DNA replication, DNA repair or normal cell function

Secondary genotoxicity:

Oxidative DNA attack by ROS via activated phagocytes (neutrophils, macrophages) during ENM-induced inflammation

ENM uptake by cells – important especially when negative results are obtained



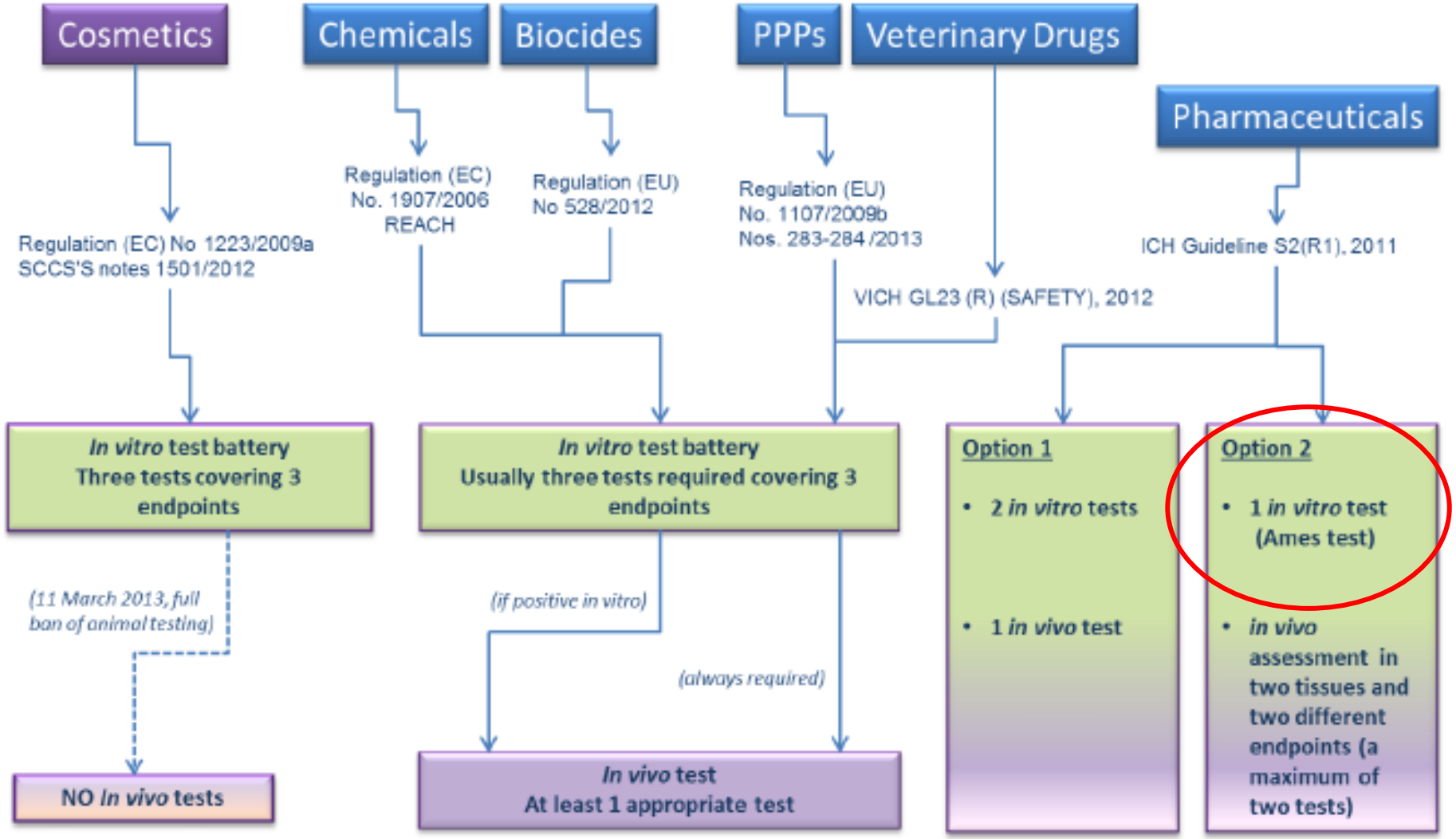
This project has received fu
programme: grant agreement 8

Doak et al., 2012, Magdolenova et al., 2014, Dusinska et al., 2015, 2017, 2019

Previous and current EU Regulations for Genotoxicity testing

Previous regulation: three tests battery currently - two tests battery

Consumer safety Committee no *in vivo* tests!



Requirements for genotoxicity testing (SCCS):

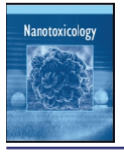
- Characterisation in culture medium; information on size and size distribution and stability of the test suspension
- Uptake of NMs by cells

Genotoxicity tests:

- Mammalian gene mutation (OECD TG 476, 490)
- Clastogenicity & aneugenicity - micronucelus (OECD TG 487)
- Weight of evidence: DNA damage (alkaline and enzyme-linked comet assay)

Follow up studies for dermally applied cosmetics (WoE):

- 3D skin model for micronucleus and comet assay
- Gene expression (ToxTracker)



Nanotoxicology

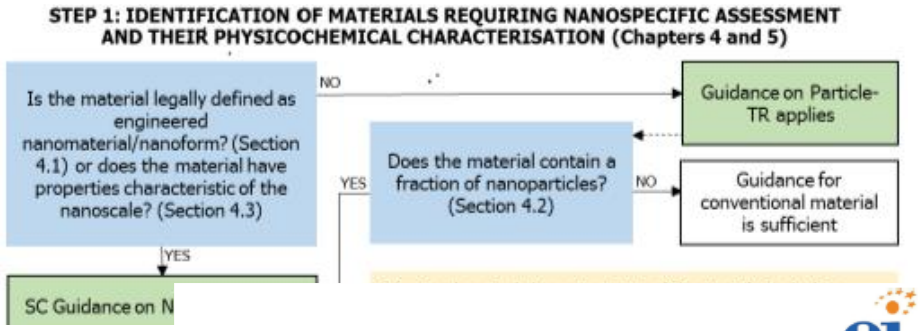
ISSN: 1743-5390 (Print) 1743-5404 (Online) Journal homepage: http://www.tandfonline.com/loi/inan20

Towards an alternative testing strategy for nanomaterials used in nanomedicine: Lessons from NanoTEST

M. Dusinska, S. Boland, M. Saunders, L. Juillerat-Jeanneret, L. Tran, G. Pojana, A. Marcomini, K. Volkovova, J. Tulinska, L. E. Knudsen, L. Gombau, M. Whelan, A. R. Collins, F. Marano, C. Housiadas, D. Bilanicova, B. Halamoda Kenzaoui, S. Correia Carreira, Z. Magdolenova, L. M. Fjellsbø, A. Huk, R. Handy, L. Walker, M. Barancokova, A. Bartonova, E. Burello, J. Castell, H. Cowie, M. Drlickova, R. Guadagnini, G. Harris, M. Harju, E. S. Heimstad, M. Hurbankova, A. Kazimirova, Z. Kovacikova, M. Kuricova, A. Liskova, A. Milcamps, E. Neubauerova, T. Palosaari, P. Papazafiri, M. Pilou, M. S. Poulsen, B. Ross, E. Runden-Pran, K. Sebekova, M. Staruchova, D. Vallotto & A. Worth

This project has received funding from the European Union's Horizon 2020 programme: grant agreement 814425.

Implementation of the SC Guidance on nano-RA. Tiered approach



Based on testing ENMs in four di

Step 1, Characterisation of test m
degradation of the nanomaterial t
conditions representative of the
Step 2 In vitro digestion
Step 3 in vivo
Stpe 3 Specific studies

Blue questions to address;
Green: nano risk assessmen
Yellow: testing (nanospecifi

Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: Part 1, human and animal health

Abstract

The European Food Safety Authority (EFSA) has updated the Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health. It covers the application areas within EFSA's remit, including novel foods, food contact materials, food/feed additives and pesticides. The updated guidance, now Scientific Committee Guidance on nano risk assessment (SC Guidance on Nano-RA), has taken account of relevant scientific studies that provide insights to physicochemical properties, exposure assessment and hazard characterisation of nanomaterials and areas of applicability. Together with the accompanying EFSA Guidance on Technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles (Guidance on Particle-TR), the SC Guidance on Nano-RA specifically elaborates on physicochemical characterisation, key parameters that should be measured, methods and techniques that can be used for characterisation of nanomaterials and their determination in complex matrices. The SC Guidance on Nano-RA also details aspects relating to exposure assessment and hazard identification and characterisation. In particular, nanospecific considerations relating to *in vitro*/*in vivo* toxicological studies are discussed and a tiered framework for toxicological testing is outlined. Furthermore, *in vitro* degradation, toxicokinetics, genotoxicity, local and systemic toxicity as well as general issues relating to testing of nanomaterials are described. Depending on the initial tier results, additional studies may be needed to investigate reproductive and developmental toxicity, chronic toxicity and carcinogenicity, immunotoxicity and allergenicity, neurotoxicity, effects on gut microbiome and endocrine activity. The possible use of read-across to fill data gaps as well as the potential use of integrated testing strategies and the knowledge of modes or mechanisms of action are also discussed. The Guidance proposes approaches to risk characterisation and uncertainty analysis.

Keywords

GUIDANCE

EFSA Journal

ADOPTED: 30 June 2021

doi: 10.2903/j.efsa.2021.6769

Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles

EFSA Scientific Committee,
Simon More, Vasileios Bampidis, Diane Benford, Claude Bragard, Thorhallur Halldorsson, Antonio Hernández-Jerez, Susanne Hougaard Bennekou, Kostas Koutsoumanis, Claude Lambré, Kyriaki Machera, Hanspeter Naegeli, Søren Nielsen, Josef Schlatter, Dieter Schrenk, Vittorio Silano (deceased), Dominique Turck, Maged Younes, Jacqueline Castenmiller, Qasim Chaudhry, Francesco Cubadda, Roland Franz, David Gott, Jan Mast, Alicja Mortensen, Agnes G. Oomen, Stefan Weigel, Eric Barthelemy, Ana Rincon, Jose Tarazona and Reinhilde Schoonjans

Abstract

Following a mandate from the European Commission, EFSA has developed a Guidance on Technical Requirements (Guidance on Particle-TR), defining the criteria for assessing the presence of a fraction of small particles, and setting out information requirements for applications in the regulated food and feed product areas (e.g. novel food, food/feed additives, food contact materials and pesticides). These requirements apply to particles requiring specific assessment at the nanoscale in conventional materials that do not meet the definition of engineered nanomaterial as set out in the Novel Food Regulation (EU) 2015/2283. The guidance outlines appraisal criteria grouped in three sections, to confirm whether or not the conventional risk assessment should be complemented with nanospecific considerations. The first group addresses solubility and dissolution rate as key physicochemical properties to assess whether consumers will be exposed to particles. The second group establishes the information requirements for assessing whether the conventional material contains a fraction or consists of small particles, and its characterisation. The third group describes the information to be presented for existing safety studies to demonstrate that the fraction of small particles, including particles at the nanoscale, has been properly evaluated. In addition, in order to guide the appraisal of existing safety studies, recommendations for closing the data gaps while minimising the need for conducting new animal studies are provided. This Guidance on Particle-TR complements the Guidance on risk assessment of nanomaterials to be applied in the food and feed chain, human and animal health updated by the EFSA Scientific Committee as co-published with this Guidance. Applicants are advised to consult both guidance documents before conducting new studies.

Keywords: nanomaterial, nanofraction, solubility, dissolution/degradation rate, sample dispersion protocol, electron microscopy, particle size distribution

This project has received funding programme: grant agreement 814425.

EFSA Genotoxicity testing strategy for nanomaterials

Genotoxicity testing of ENMs should follow the general indications of the EFSA genotoxicity testing strategy (EFSA Scientific Committee, 2011a) addressing three critical endpoints:

In vivo genotoxicity testing is required when at least one of the *in vitro* tests indicates genotoxic activity, or if it is not appropriate to test the nanomaterial *in vitro*.

A follow-up *in vivo* study should be carried out, unless it can be demonstrated by other means that the positive *in vitro* findings are not relevant for the *in vivo* situation.

Expert judgement should be used to select and justify one or more of the available *in vivo* tests e.g.

- *in vivo* mammalian erythrocyte micronucleus test (OECD TG 474 (OECD, 2016a));
- *in vivo* mammalian alkaline comet assay (OECD TG 489 (OECD, 2016d));
- transgenic rodent somatic and germ cell gene mutation assay (OECD TG 488 (OECD, 2013)).

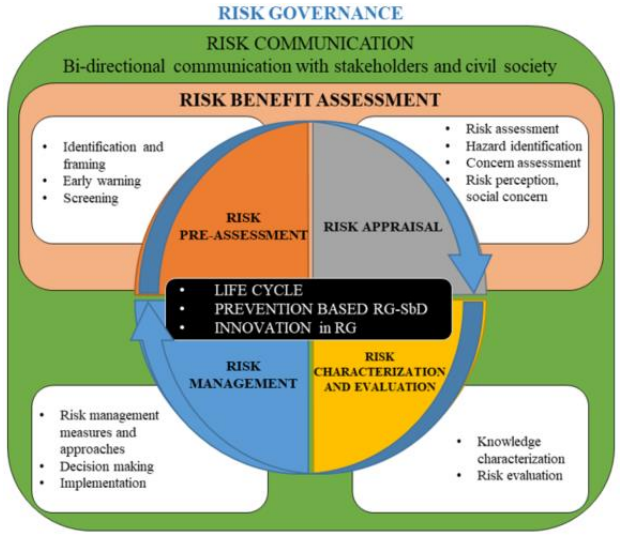
programme: grant agreement 814425.

RiskGONE - one of the main objectives

- To develop risk assessment and risk governance framework and cloud platform as digital tool to provide information and guide to scientists, regulators industry and other stakeholders

- To contribute to the standardisation and validation process for ENM by evaluating, optimizing and pre-validating SOPs, and produce nano-specific test guidelines (TGs) and guidance documents (GDs)

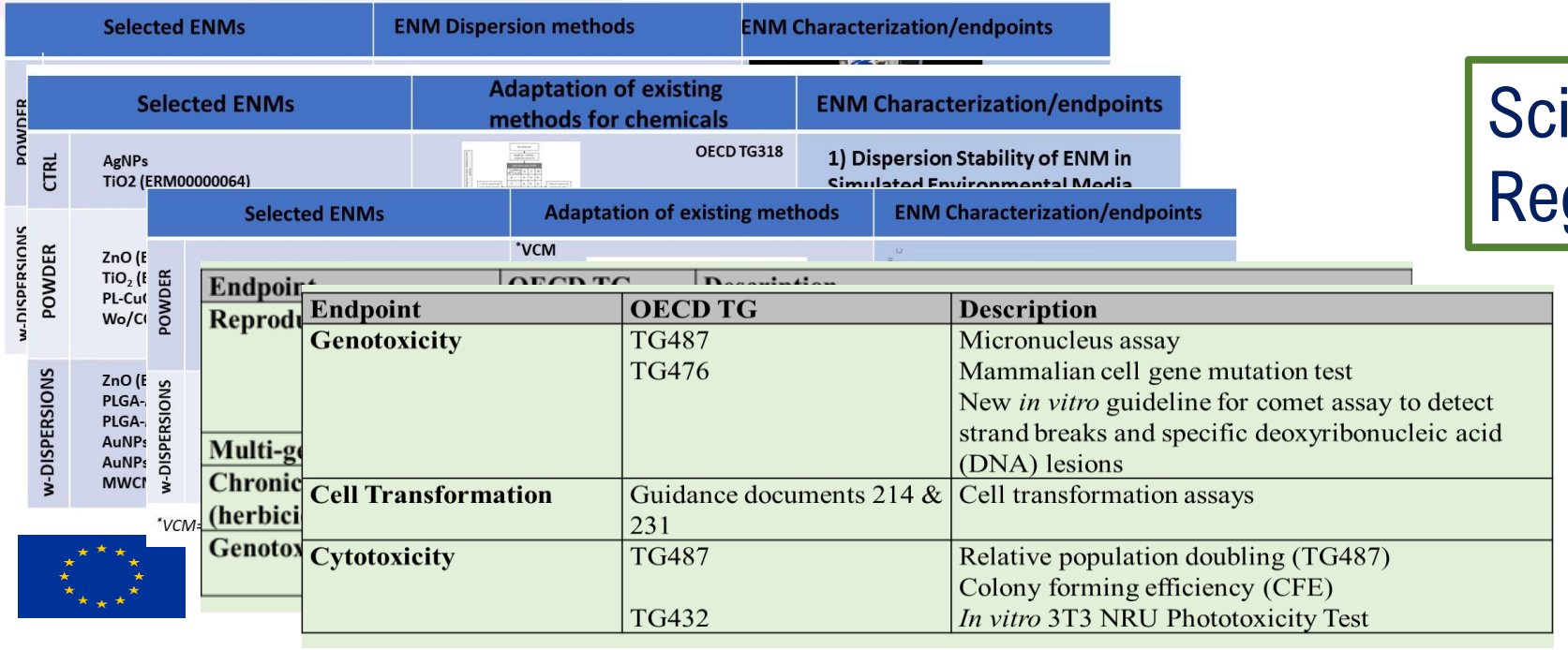
- To provide trainings and training material



This project has received funding from the European Union's Horizon 2020 programme: grant agreement 814425.

Development of Guidance documents and Test guidelines

- **Standardisation and validation process** by evaluating, optimizing and pre-validating SOPs, TGs and integrating them into RG framework (support & expand Malta project and NanoHARMONY)
- **Harmonisation** – same approach, same NMs, characterization, dispersion,
- **Interlaboratory comparisons** to standardize and pre-validate methods, etc.
- Develop and deliver at least **12 pre-validated draft guidance documents for characterisation, dosimetry, fate, human and environmental hazard assessment.**



Endpoint	OECD TG	Description
Genotoxicity	TG487 TG476	Micronucleus assay Mammalian cell gene mutation test New *in vitro* guideline for comet assay to detect strand breaks and specific deoxyribonucleic acid (DNA) lesions
Cell Transformation	Guidance documents 214 & 231	Cell transformation assays
Cytotoxicity	TG487 TG432	Relative population doubling (TG487) Colony forming efficiency (CFE) *In vitro* 3T3 NRU Phototoxicity Test

Scientific acceptance
Regulatory acceptance

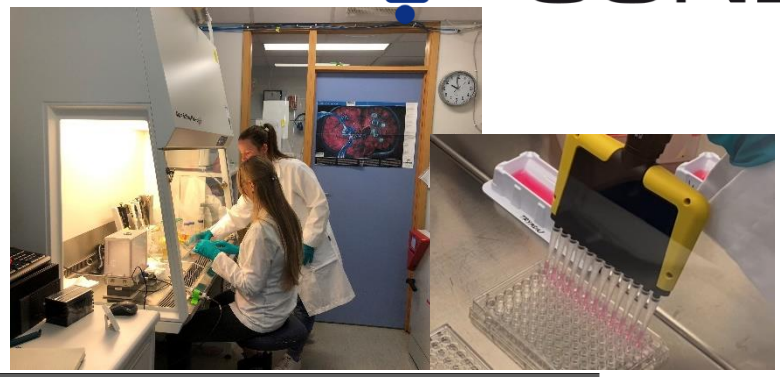
RiskGONE approach

Testing the selected methods (SOPs) for their nano-specific applicability

RiskGONE partners

Series of interlaboratory comparison studies

Malta project, NanoHarmony



ERM identifiers	Name	RR1	RR2	RR3
ERM00000062	Titanium dioxide	x	x	
ERM00000063	Zinc oxide	x	x	
ERM00000064	Titanium dioxide	x	x	x
ERM00000065	Zinc oxide	x	x	
ERM00000067	Ag nanowires	x		
ERM00000083	PLGA-AuNPs-WOW	x	x	
ERM00000084	PLGA-AuNPs-NP	x	x	
ERM00000085	AuNPs-1 (nominal 15nm)	x	x	
ERM00000086	AuNPs-2 (nominal 50nm)	x	x	
ERM00000088	CuO 40nm		x	x
ERM00000089	Nano Tungsten Carbide/Cobalt Powder		x	x
ERM00000325	MWCNT 3wt%		x	

Pre-validation testing

Panel of different ENMs

SOPs optimisation

Draft/existing SOPs

Evaluation

Methodological adaptations needed

Suitability and reproducibility confirmed

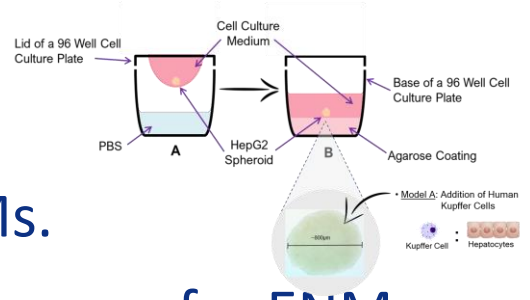
Scientific documents to support development of TG/GD

Translation into SPSF

This project has received funding from the European Union's Horizon 2020 programme: grant agreement 814425.

Human hazard assessment

Aim: support ENMs risk governance by delivering a more reliable ENM-tailored safety testing strategy, to improve and enhance the tools supporting risk decision making.



Objectives:

1. Evaluate & adapt *in vitro* human hazard assessment OECD TGs for ENMs.
2. Evaluate & adapt high-throughput and high content, interference-free assays for ENM.
3. Evaluate & adapt novel mechanism-based *in vitro* test systems for ENM.
4. Evaluation and verification of AOPs specific for ENMs.
5. Developed and generated a variety of training materials on all Round Robin methods

- **RR1 and RR2 experimental work finalised with 6 ENMs - Report for CFE, Comet assay, HPRT and Micronucleus assay**
- **One paper for each method - CFE, HPRT, MN, CA, impedance & cytotoxicity assays.**
- **Catalogue of training materials – RR, refined and/or modified SOPs, coupled to laboratory-based training videos**

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TYPE Methods
PUBLISHED 28 September 2022
DOI 10.3389/ftox.2022.981701

Check for updates

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Nanotoxicology,
a section of the journal Frontiers in Toxicology

RECEIVED 29 June 2022
ACCEPTED 24 August 2022
PUBLISHED 28 September 2022

CITATION
Longhin EM, El Yamani N,
Rundén-Pran E and Dusin...
The alamar blue assay in t...
safety testing of nanomate...
Front. Toxicol. 4:981701.
doi: 10.3389/ftox.2022.98...

The alamar blue assay in the context of safety testing of nanomaterials

Eleonora Marta Longhin*, Naouale El Yamani,
Elise Rundén-Pran and Maria Dusinska

Health Effects Laboratory, Department for Environmental Chemistry, NILU-Norwegian Institute for Air Research, Kjeller, Norway

The Alamar Blue (AB) assay is widely used to investigate cytotoxicity, cell proliferation and cellular metabolic activity within different fields of toxicology. The use of the assay with nanomaterials (NMs) entails specific aspects including the potential interference of NMs with the test. The procedure of the AB assay applied for testing NMs is described in detail and step-by-step, from NM preparation, cell exposure, inclusion of interference controls, to the analysis and interpretation of the results. Provided that the

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TYPE Methods
PUBLISHED 07 September 2022
DOI 10.3389/ftox.2022.983316

Check for updates

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Nanotoxicology,

The colony forming efficiency assay for toxicity testing of nanomaterials—Modifications for higher-throughput

Elise Rundén-Pran[1]*, Espen Mariussen[1,2], Naouale El Yamani[1],
Elisabeth Elje[1,3], Eleonora Marta Longhin[1] and Maria Dusinska[1]

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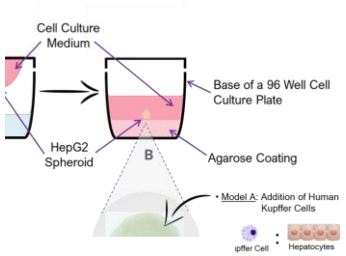

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TYPE Methods
PUBLISHED 12 October 2022
DOI 10.3389/ftox.2022.986318

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OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Nanotoxicology,
a section of the journal Frontiers in Toxicology

RECEIVED 04 July 2022
ACCEPTED 20 September 2022
PUBLISHED 12 October 2022

The miniaturized enzyme-modified comet assay for genotoxicity testing of nanomaterials

N. El Yamani[1]*, E. Rundén-Pran[1], A. R. Collins[2], E. M. Longhin[1], E. Elje[1], P. Hoet[3], I. Vinković Vrček[4], S. H. Doak[5], V. Fessard[6] and M. Dusinska[1]

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METHODS
published: 08 June 2022
doi: 10.3389/ftox.2022.864753

Check for updates

Thymidine Kinase$^{+/-}$ Mammalian Cell Mutagenicity Assays for Assessment of Nanomaterials

*Tao Chen[1], Maria Dusinska[2] and Rosalie Elespuru[3]**

[1]*Division of Genetic and Molecular Toxicology, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, AR, United States,* [2]*Health Effects Laboratory, NILU-Norwegian Institute for Air Research, Kjeller, Norway,* [3]*Division of Biology, Chemistry and Materials Science, US Food and Drug Administration, CDRH/OSEL, Silver Spring, MD, United States*

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Edited by:

The methods outlined here are part of a series of papers designed specifically for genotoxicity assessment of nanomaterials (NM). Common Considerations such as NM characterization, sample preparation and dose selection, relevant to all genotoxicity assays, are found in an accompanying paper. The present paper describes methods for evaluation of mutagenicity in the mammalian (mouse) *thymidine kinase* (*Tk*) gene occurring in L5178Y mouse lymphoma (ML) cells and in the designated *TK* gene in human lymphoblastoid TK6 cells. Mutations change the functional genotype from $TK^{+/-}$ to $TK^{-/-}$, detectable as cells surviving on media selective for the lack of thymidine kinase (TK) function. Unlike cells with TK enzyme function, the $TK^{-/-}$ cells are unable to integrate the toxic selection agent, allowing these cells to survive as rare mutant colonies. The ML assay has been shown to detect a broad spectrum of genetic damage, including both small scale

Human hazard assessment

RISK GONE

OECD
Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2022)15

Unclassified

English - Or. English

21 September 2022

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

Study Report and Preliminary Guidance on the Adaptation of the In Vitro micronucleus assay (OECD TG 487) for Testing of Manufactured Nanomaterials

Series on Testing and Assessment
No. 359

This project has received funding from the European Union's Horizon 2020 programme: grant agreement 814425.

frontiers | Frontiers in Toxicology

METHODS
published: 24 May 2022
doi: 10.3389/ftox.2022.859122

Check for updates

Common Considerations for Genotoxicity Assessment of Nanomaterials

Rosalie K. Elespuru[1], Shareen H. Doak[2], Andrew R. Collins[3], Maria Dusinska[4], Stefan Pfuhler[5], Mugimane Manjanatha[6], Renato Cardoso[7] and Connie L. Chen[8]*

[1]Division of Biology, Chemistry and Materials Science, Office of Science and Engineering Laboratories, Center for Devices and Radiological Health, U.S. Food and Drug Administration, Silver Spring, MD, United States, [2]Institute of Life Science, Swansea University Medical School, Swansea, United Kingdom, [3]Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Blindern, Norway, [4]Health Effects Laboratory, Department of Environmental Chemistry, NILU-Norwegian Institute for Air Research, Kjeller, Norway, [5]Global Product Stewardship, Human Safety, Procter & Gamble Mason Business Centre, Mason, OH, United States, [6]Division of Genetic and Molecular Toxicology, Food and Drug Administration, National Center for Toxicological Research, Jefferson, AR, United States, [7]Millipore Sigma, Rockville, MD, United States, [8]Health and Environmental Sciences Institute, Washington, DC, MD, United States

Genotoxicity testing is performed to determine potential hazard of a chemical or agent for direct or indirect DNA interaction. Testing may be a surrogate for assessment of heritable genetic risk or carcinogenic risk. Testing of nanomaterials (NM) for hazard identification is generally understood to require a departure from normal testing procedures found in international standards and guidelines. A critique of the genotoxicity literature in Elespuru et al., 2018, reinforced evidence of problems with genotoxicity assessment of nanomaterials (NM) noted by many previously. A follow-up to the critique of problems (what is wrong) is a series of methods papers in this journal designed to provide practical information on what is appropriate (right) in the performance of genotoxicity assays altered for NM assessment. In this "Common Considerations" paper, general considerations are addressed, including NM characterization, sample preparation, dosing choice, exposure assessment (uptake) and data analysis that are applicable to any NM genotoxicity assessment. Recommended methods for *specific assays* are presented in a series of additional papers in this special issue of the journal devoted to toxicology methods for assessment of nanomaterials: the *In vitro* Micronucleus Assay, TK Mutagenicity assays, and the *In vivo* Comet Assay. In this context, NM are considered generally as insoluble particles or test articles in the nanometer size range that present difficulties in assessment using techniques described in standards such as OECD guidelines.

Keywords: nanomaterials, genotoxicity, methods, mutagenicity, clastogenicity, biocompatibility

OPEN ACCESS

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Specialty section:
This article was submitted to Nanotoxicology, a section of the journal Frontiers in Toxicology

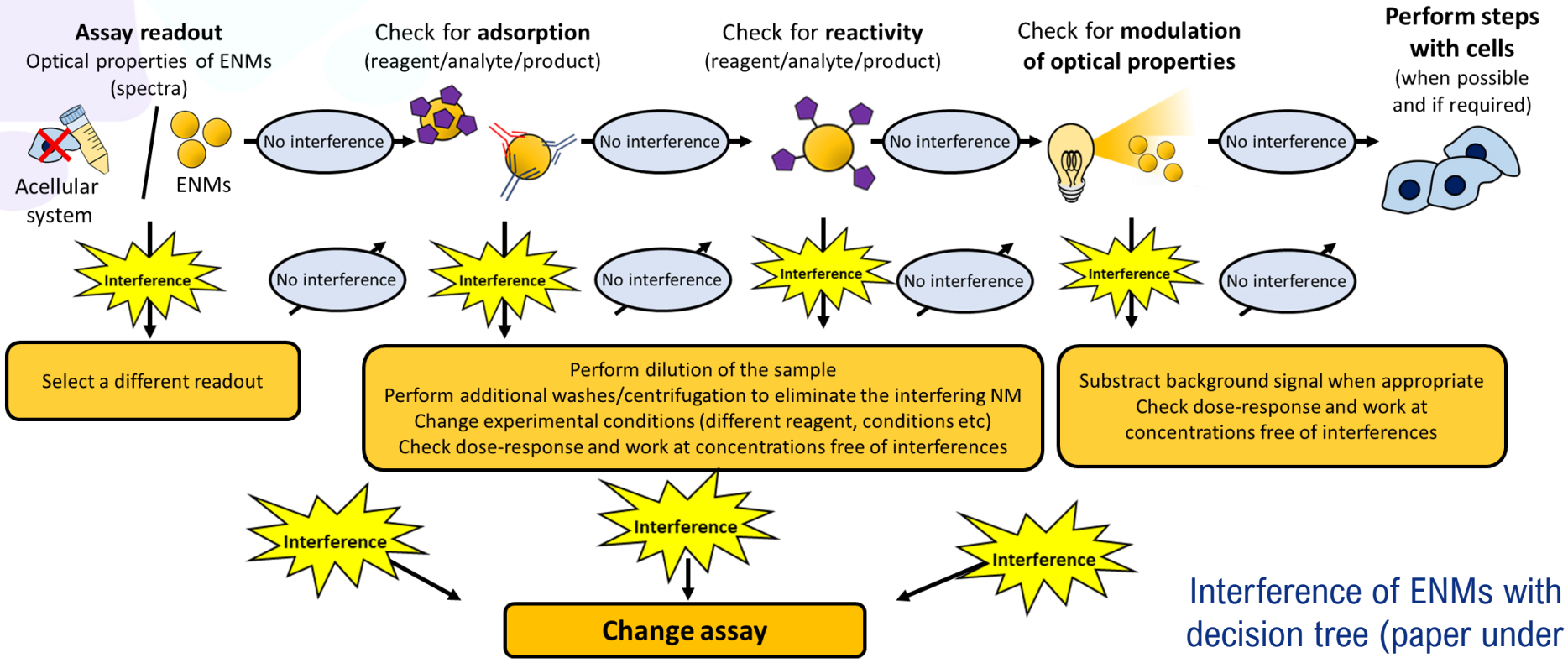
Received: *20 January 2022*
Accepted: *02 May 2022*
Published: *24 May 2022*

Citation:

Guidance on controlling interference

Testing selected hazard assessment in vitro methods for their nano-specific applicability

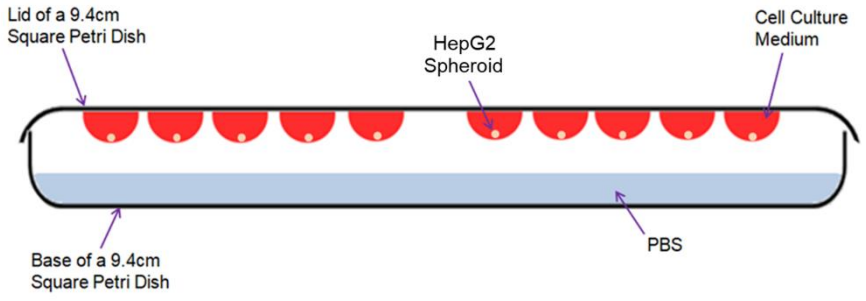
Nano-specific challenges such as potential interference of ENMs with test methods and inclusion of interference controls have been addressed



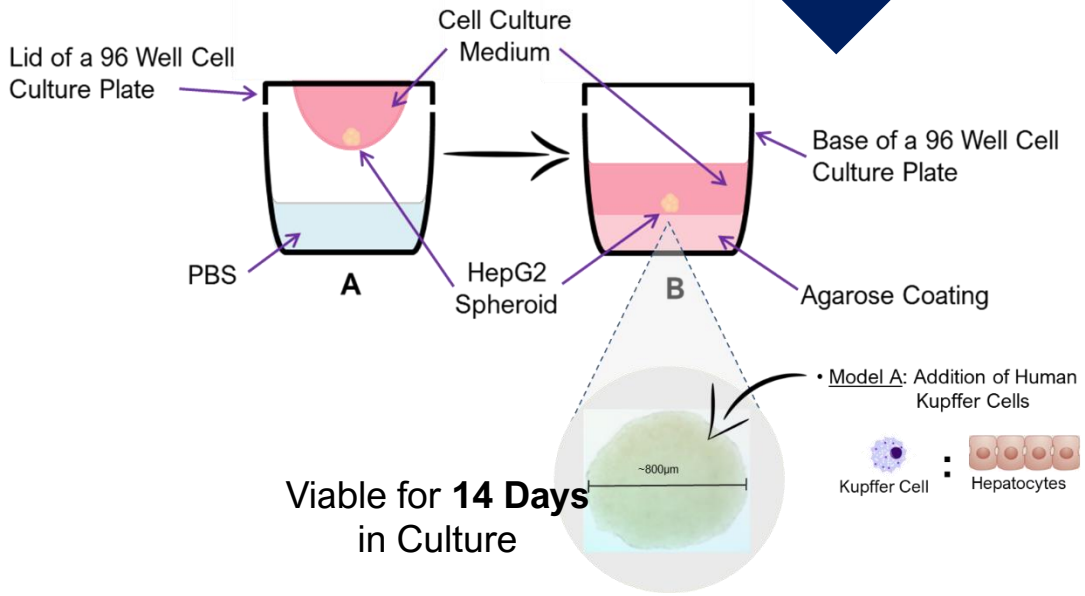
Interference of ENMs with test method decision tree (paper under preparation)

This project has received funding from the European Union's Horizon 2020 programme: grant agreement 814425.

Development of advanced 3D liver models – cell line based spheroids



Transfer from hanging drop to submerged culture enhanced **viability** and **longevity** of the model



Viable for **14 Days** in Culture

Mutagenesis, 2020, 35, 319–329
doi:10.1093/mutage/geaa018
Original Manuscript
Advance Access publication 11 August 2020

OXFORD

Original Manuscript

Adaptation of the *in vitro* micronucleus assay for genotoxicity testing using 3D liver models supporting longer-term exposure durations

Gillian E. Conway[1,*], Ume-Kulsoom Shah[1], Samantha Llewellyn[1], Tereza Cervena[1,2], Stephen J. Evans[1], Abdullah S. Al Ali[1], Gareth J. Jenkins[1], Martin J. D. Clift[1] and Shareen H. Doak[1,*]

jove Journal of Visualized Experiments

Video Article

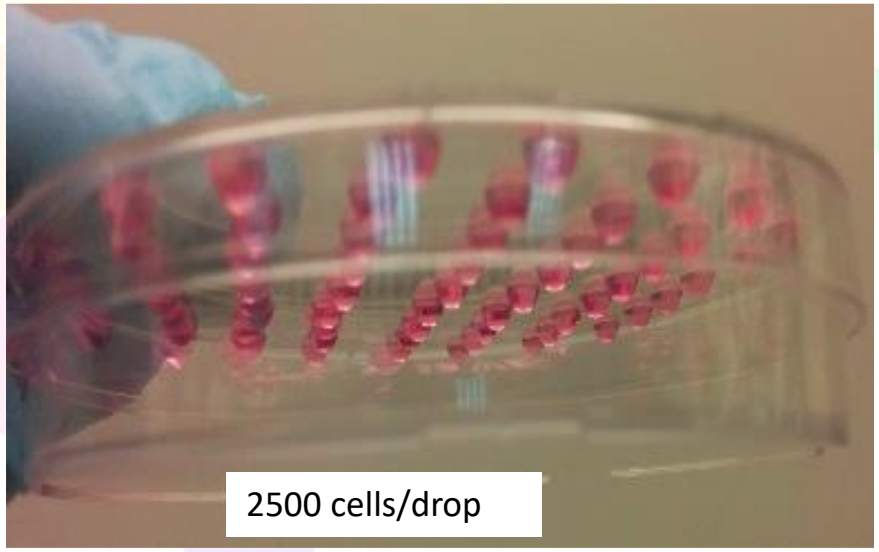
Advanced 3D Liver Models for In vitro Genotoxicity Testing Following Lon Term Nanomaterial Exposure

Samantha V. Llewellyn[1], Gillian E. Conway[1], Ume-Kulsoom Shah[1], Stephen J. Evans[1], Gareth J.S. Jenkins[1], Martin J.D. Clift[1], Shareen H. D

Assessing the Transferability and Reproducibility of 3D *In Vitro* Liver Models from Primary Human multi-cellular Microtissues to Cell-line based HepG2 Spheroids.

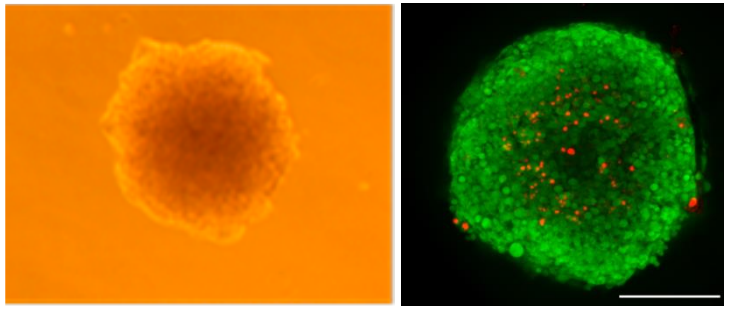
Samantha V. Llewellyn[1], Ali Kermanizadeh[2], Victor Ude[3], Nicklas Raun Jacobsen[4], Gillian E Conway[1], Ume-Kulsoom Shah[1], Marije Niemeijer[5], Martijn J. Moné[5], Bob van de Water[5], Shambhu Roy[6], Wolfgang Moritz[7], Vicki Stone[3], Gareth J.S. Jenkins[1] and Shareen H. Doak[1*].

3D liver model – HepG2 Spheroids



2500 cells/drop

Seeded as hanging drops



HepG2 spheroids

Cytotoxicity
by AlamarBlue

after 4 days

Cultured
for 1 week

Mutat Res Gen Tox En 845 (2019) 403033

Contents lists available at ScienceDirect

Mutat Res Gen Tox En

journal homepage: www.elsevier.com/locate/gentox

The comet assay applied to HepG2 liver spheroids

Elisabeth Elje[a], Michelle Hesler[b], Elise Rundén-Pran[a], Pascal Mann[b], Espen Mariussen[a], Sylvia Wagner[b], Maria Dusinska[a], Yvonne Kohl[b,*]

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ARTICLE INFO

Keywords:
Liver spheroids
Advanced *in vitro* model
Genotoxicity
Comet assay
2D and 3D culture

ABSTRACT

In accordance with the 3 Rs to reduce *in vivo* testing, more advanced *in vitro* models, moving from 2D monolayer to 3D cultures, should be developed for prediction of human toxicity of industrial chemicals and environmental pollutants. In this study we compared cytotoxic and genotoxic responses induced by chemicals in 2D and 3D spheroidal cultures of the human liver cancer cell line HepG2.

HepG2 spheroids were prepared by hanging drop technology. Both 3D spheroids and 2D monolayer cultures were exposed to different chemicals (colchicine, chlorpromazine hydrochloride or methyl methanesulfonate) for geno- and cytotoxicity studies. Cytotoxicity was investigated by alamarBlue assay, flow cytometry and confocal imaging. DNA damage was investigated by the comet assay with and without Fpg enzyme for detection of DNA

nanomaterials

MDPI

Article

Hepato(Geno)Toxicity Assessment of Nanoparticles in a HepG2 Liver Spheroid Model

Elisabeth Elje [1,2], Espen Mariussen [1], Oscar H. Moriones [3,4], Neus G. Bastús [3], Victor Puntes [3,5,6], Yvonne Kohl [7], Maria Dusinska [1] and Elise Rundén-Pran [1,*]

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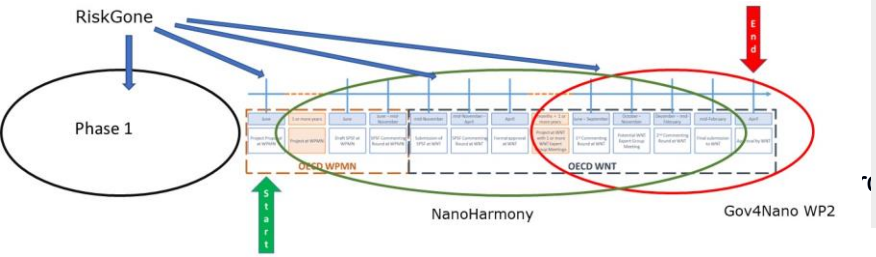
Elje E, Hesler M, Rundén-Pran E
The comet assay applied to Her

This project
programme.

Need for harmonisation of hazard and risk assessment of nanomaterials across legislations.
OECD TGs and standards needed on NAMs

- 30 standardized SOPs and protocols, more than 40 publications
- Database with all *in vitro* endpoints – transferred to e-Nanomaper
- Testing strategy suggested

- Cloud platform and decision support tool for RA and RG
- 8 GD on risk and benefit analysis, 12+ pre-validated methods - applications for OECD TGs
- FAIR data management, RiskGONE Database
- Harmonised template for data reporting
- 30 published paper, 20+ in preparation
- Priority list and plan to submit SPSF in 2023 – comet assay for strand breaks and oxidised DNA lesions, CFE,
- 3D liver model for combined MN and CA
- cell transformation assay (CTA) – link to project 4.145 (IATA)



Acknowledgement

Health Effects Laboratory, NILU:
Elise Rundén-Pran, Naouale El Yamani, Eleonora Marta Longhin, Alexandra Misci Hudecova, Elisabeth Elje, Erin McFadden, Tatiana Honza, Tanima Sengupta, Maria Dusinska

NanoTEST and RoiskGONE consortia

THANK YOU!

Maria DUSINSKA

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This project has received funding from the European Union's Horizon 2020 programme: grant agreement 814425.