

Risk Assessment in Nanomedicine

Needs for harmonisation, standardisation and validation

Maria DUSINSKA

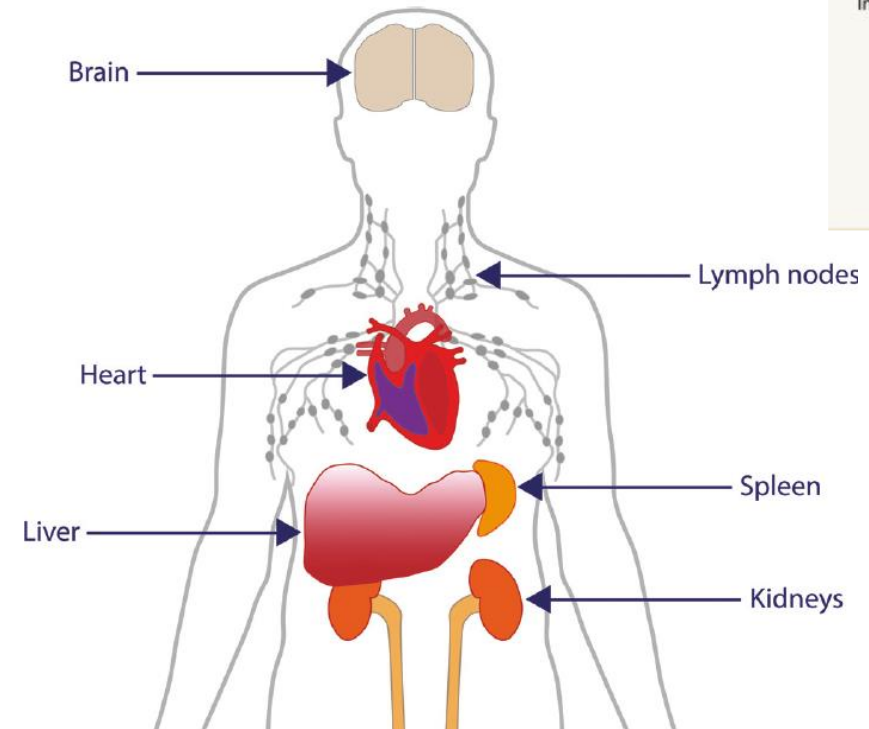
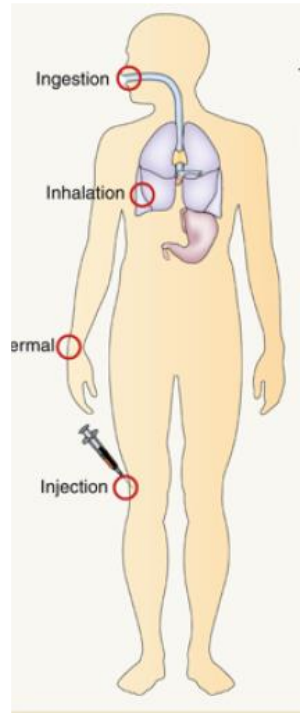
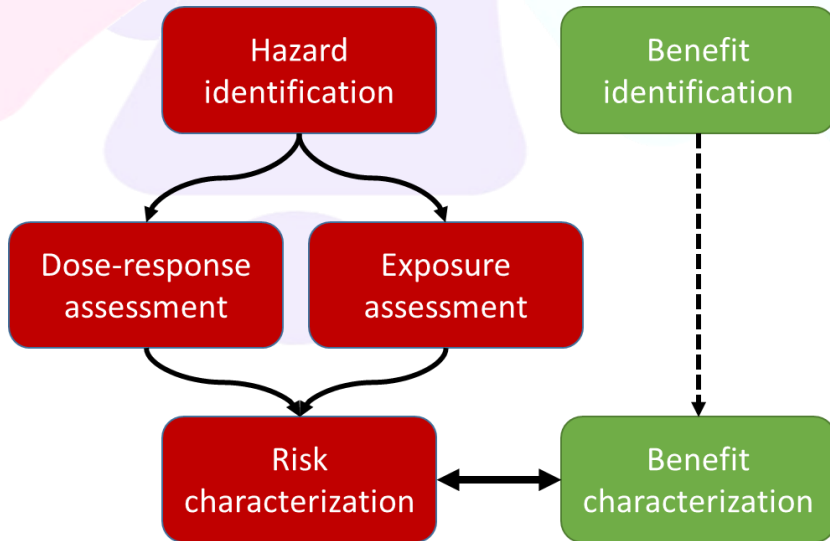
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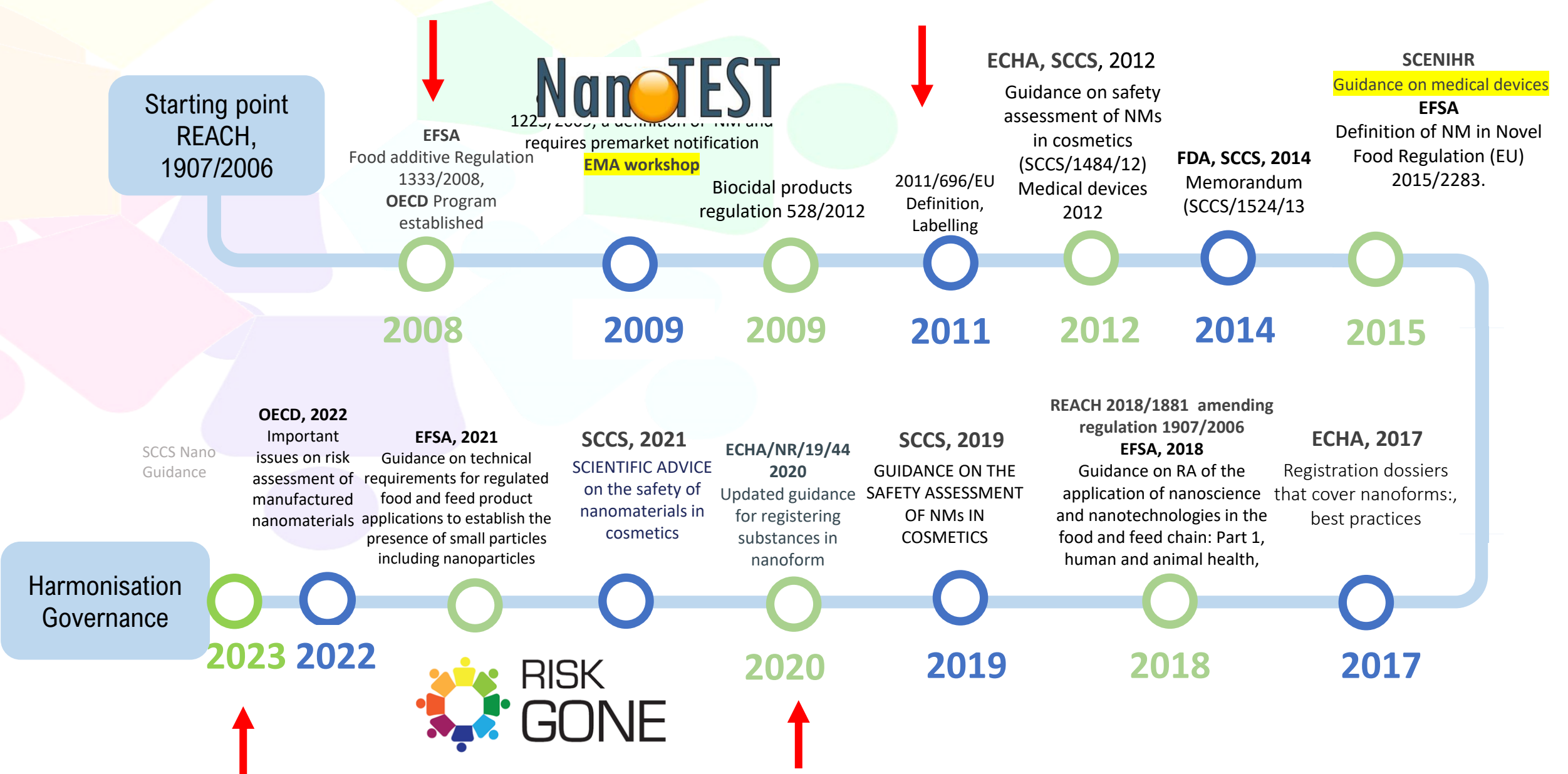
Risk Assessment

The traditional risk assessment methodology comprises the following stages:

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



Regulation of NM, risk assessment of NM



Regulatory landscape in nanomedicine for RA



European Medicines Agency - EMA

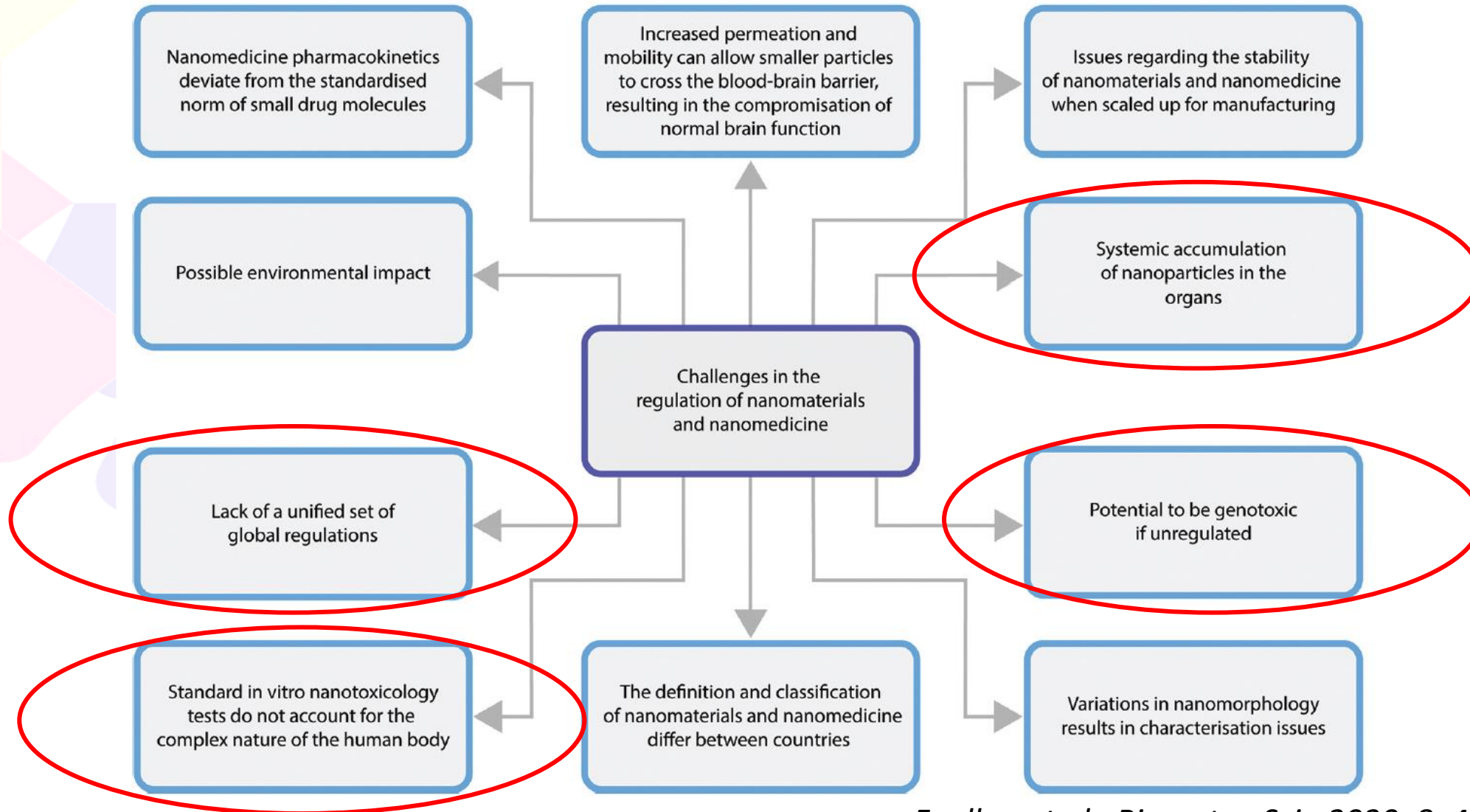
- General Medicinal Product legislation on regulating nanomedicines using current risk/benefit-analysis principles- no specific regulatory framework
- Regulated either as medicinal products or medical devices.
- Limited guidance on regulatory information needs
- Several guidance documents- a range of 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

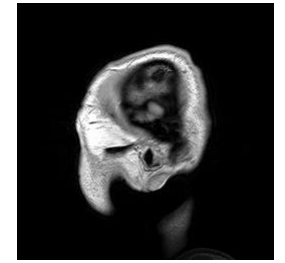
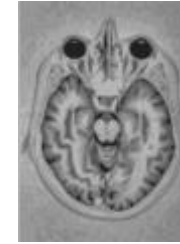


US Food and Drug Administration - FDA

- Produced a draft guidance on drug products, including nanomaterials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

Challenges in regulation of nanomedicinal products

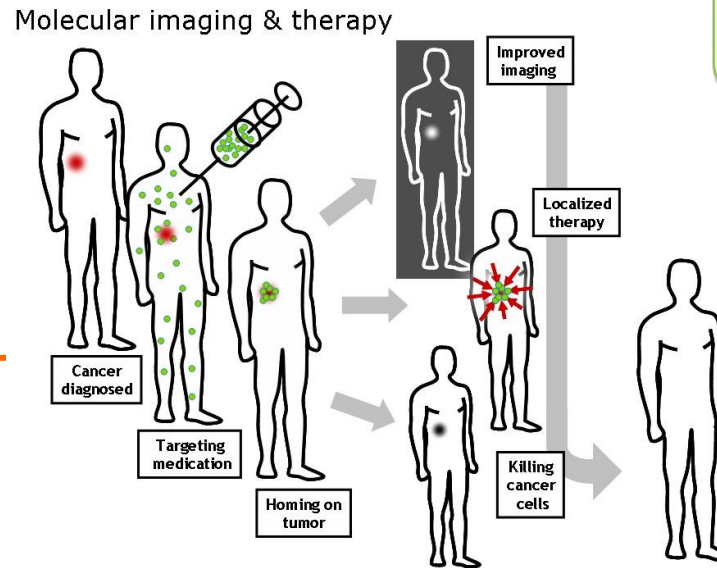




Work programme topic:
HEALTH-2007-1.3-4 "Alternative testing strategies for the assessment of the toxicological profile of nanoparticles used in medical diagnostics".

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



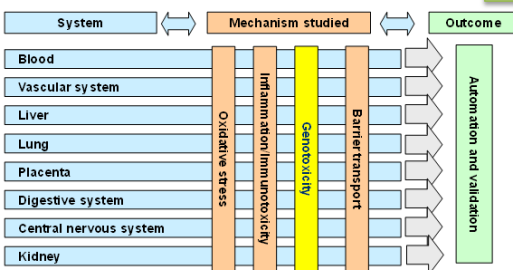
Blood: human blood cells-leucocytes, granulocytes, monocytes, etc. TK6

Vascular: HCEC, EC219, ECp23, HL1

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

Kidney: monkey kidney Cos-1 cells, human HEC

Central nervous system : HCEC, EC219, Murine N11 micro



Placenta: Placenta perfusion, BeWo cells

Digestive system :

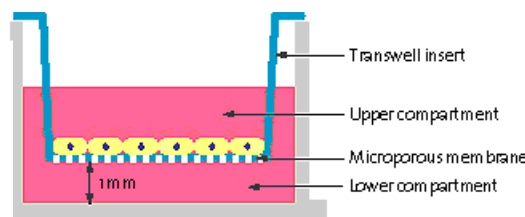
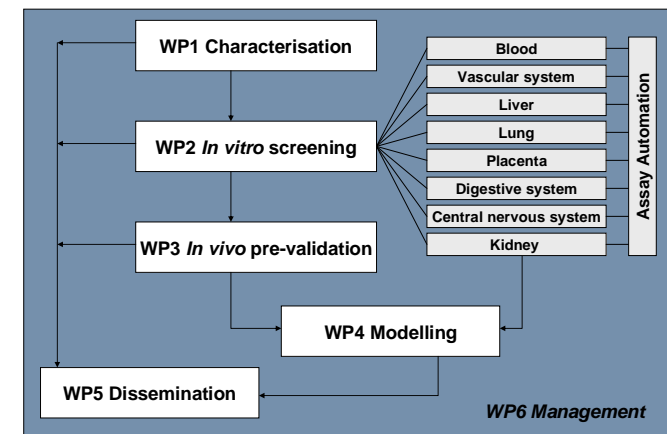
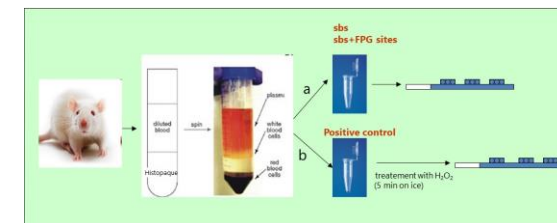
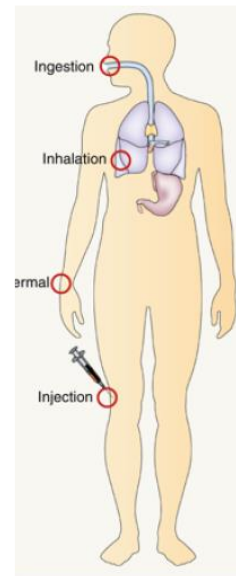
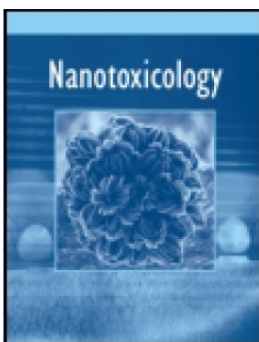
Liung: bronchial epithelial

Nanotoxicology

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

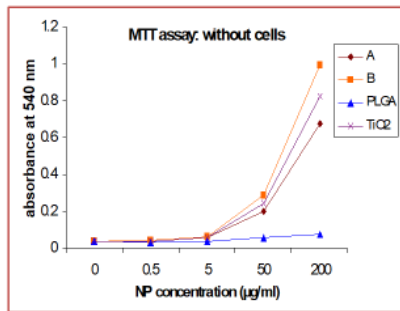
NanoTEST in a Nutshell

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



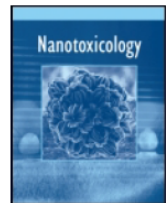
Interference

Nanomaterial interference with read out systems has been observed for colorimetric/fluorescence spectrophotometric assays

(A) Uncoated and (B) oleic acid coated Fe₃O₄ NPs, PLGA-PEO NPs and TiO₂ NPs.

Interference observed: WST-1, MTT, lactate dehydrogenase, neutral red, propidium iodide, ³H-Tymidine incorporation, automated cell counting, pro-inflammatory response evaluation (ELISA for GM-CSF, IL-6 and IL-8), and oxidative stress detection (monoBromoBimane, dichlorofluorescein, NO assays).

Interference control needs to be included for all tests methods



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^d, Zuzana Magdolenova^e, Dagmar Bilanicova^f, Margaret Saunders^g, Lucienne Juillerat-Jeanneret^h, Antonio Marcominiⁱ, Anna Huk^j, Maria Duszinska^k, Lise M Fjellsbo^l, Francelyne Marano^m & Sonja Bolandⁿ

^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 (RM CX), 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 Philosophy and Cultural Heritage, Dorsoduro 3484/D, 30123 Venice, Italy

Published online: 06 May 2015.

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, ³H-T, mBBr, DCF, Griess reagent...

Cell proliferation, PI uptake...

MTT, ³H-T...

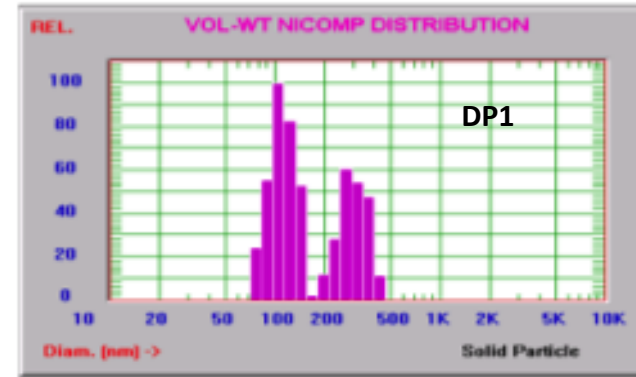
ELISA, LDH...

MTT, LDH...

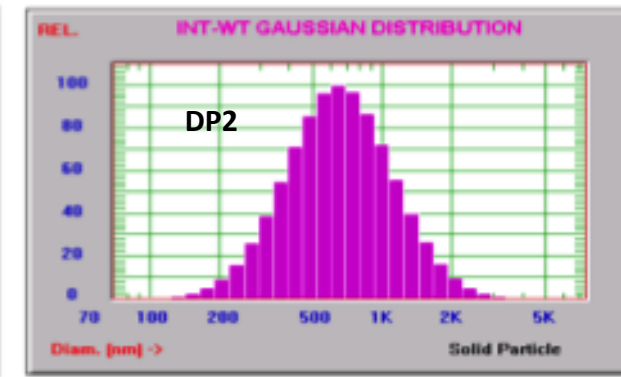


Dispersion of NMs is important

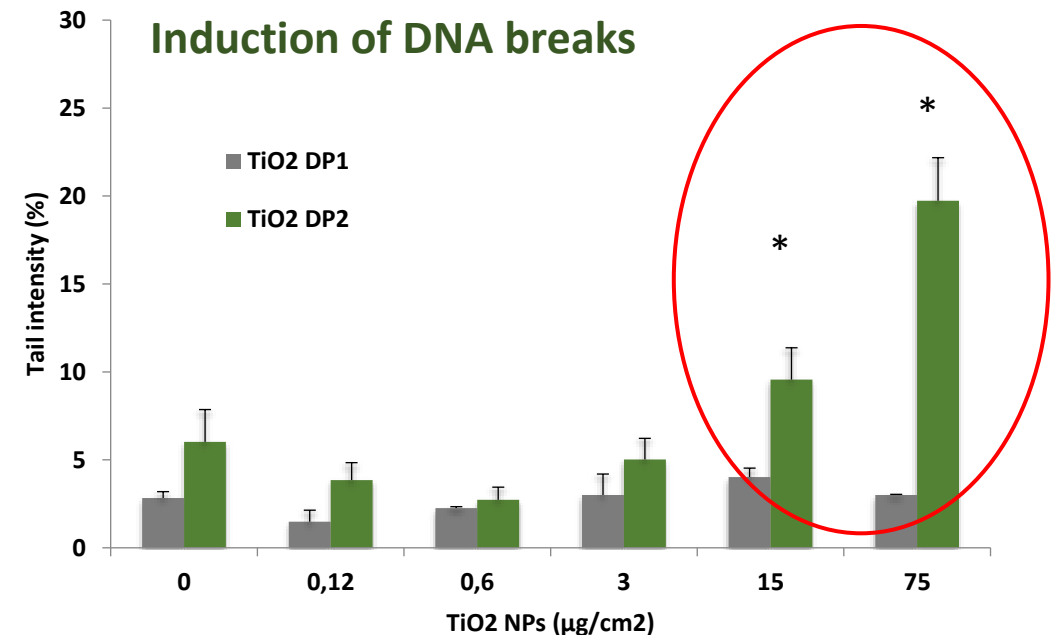
- The stability of a dispersion; two dispersion protocols
- Same TiO₂ - protein corona formation
- Different results
- Concentration can be affected by agglomeration, sedimentation, binding with other moieties in the medium, or adhesion to glass/plastic



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.



ENM genotoxicity (AOP and MoA)

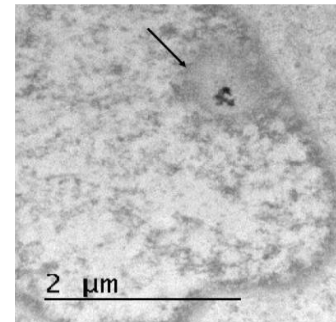
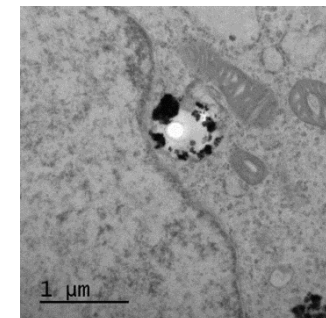
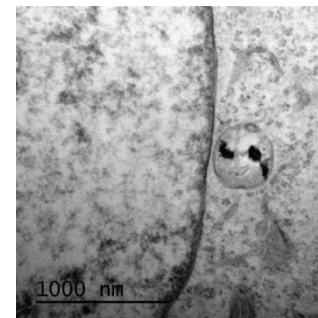
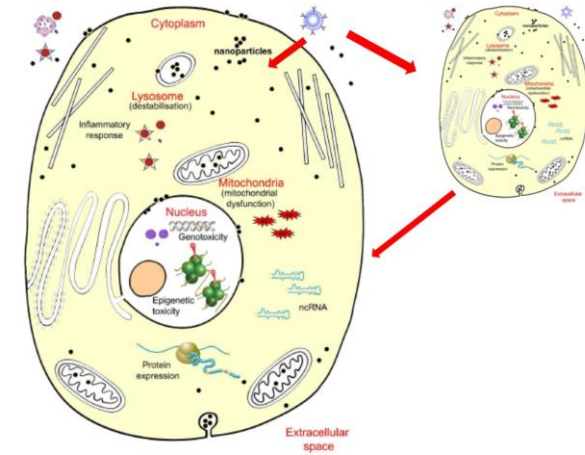
Primary genotoxicity:

- **Direct interaction with DNA**
- **Indirectly:** via oxidative stress, cell membrane damage, lipid peroxidation, mitochondrial dysfunction, 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

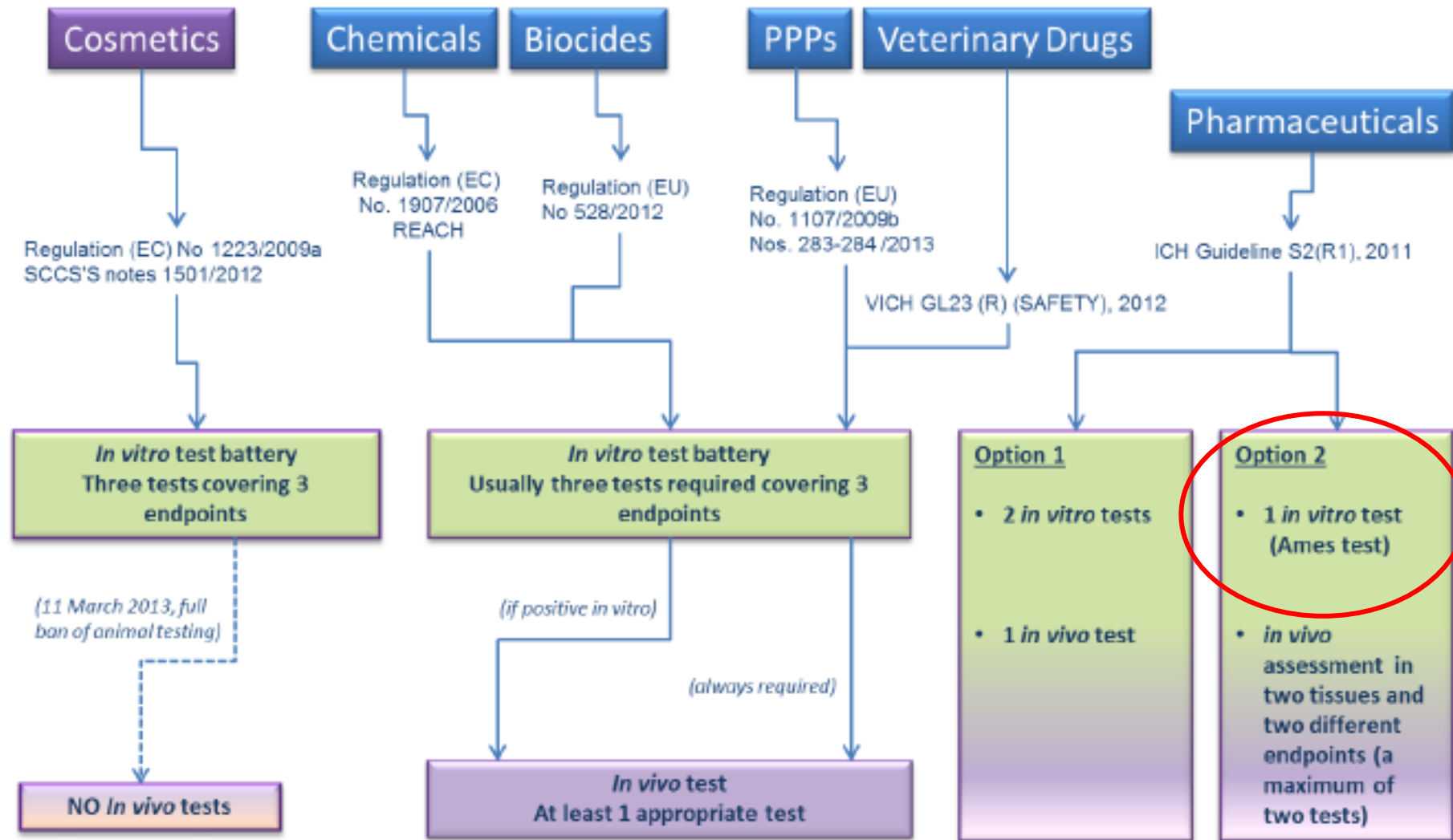
Secondary genotoxicity:

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



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 - micronucleus (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)

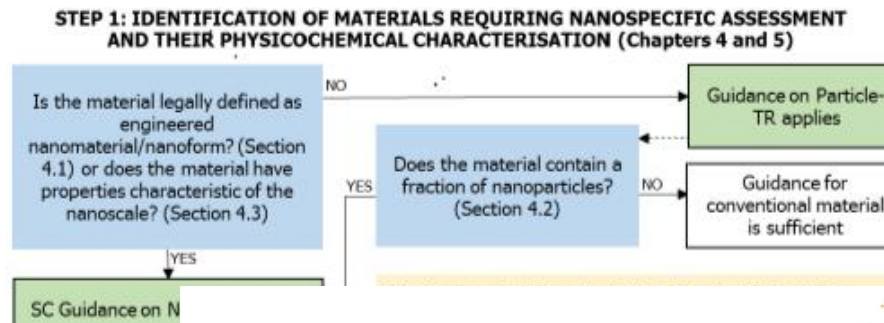


Towards an alternative testing strategy for nanomaterials used in nanomedicine: Lessons from NanoTEST

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. Kovackikova, 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



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



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

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

Step 3 Specific studies

Blue questions to address;
Green: nano risk assessment
Yellow: testing (nanospecific)

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

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.

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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.

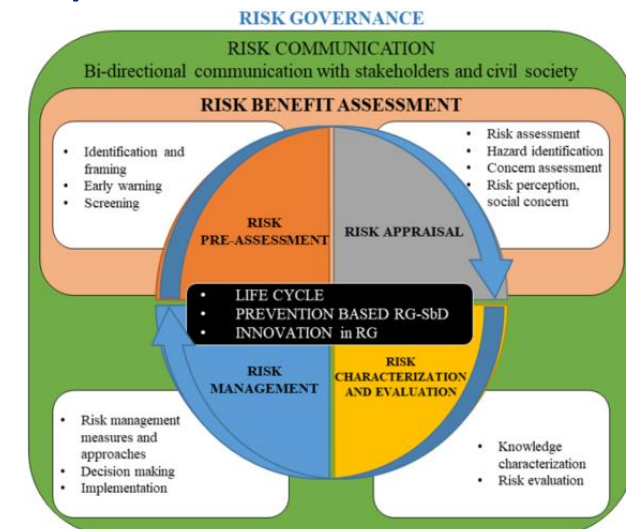
Expert judgement should be used to select and justify one or more of the available *in vivo* tests e.g.

- *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)).



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



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.**

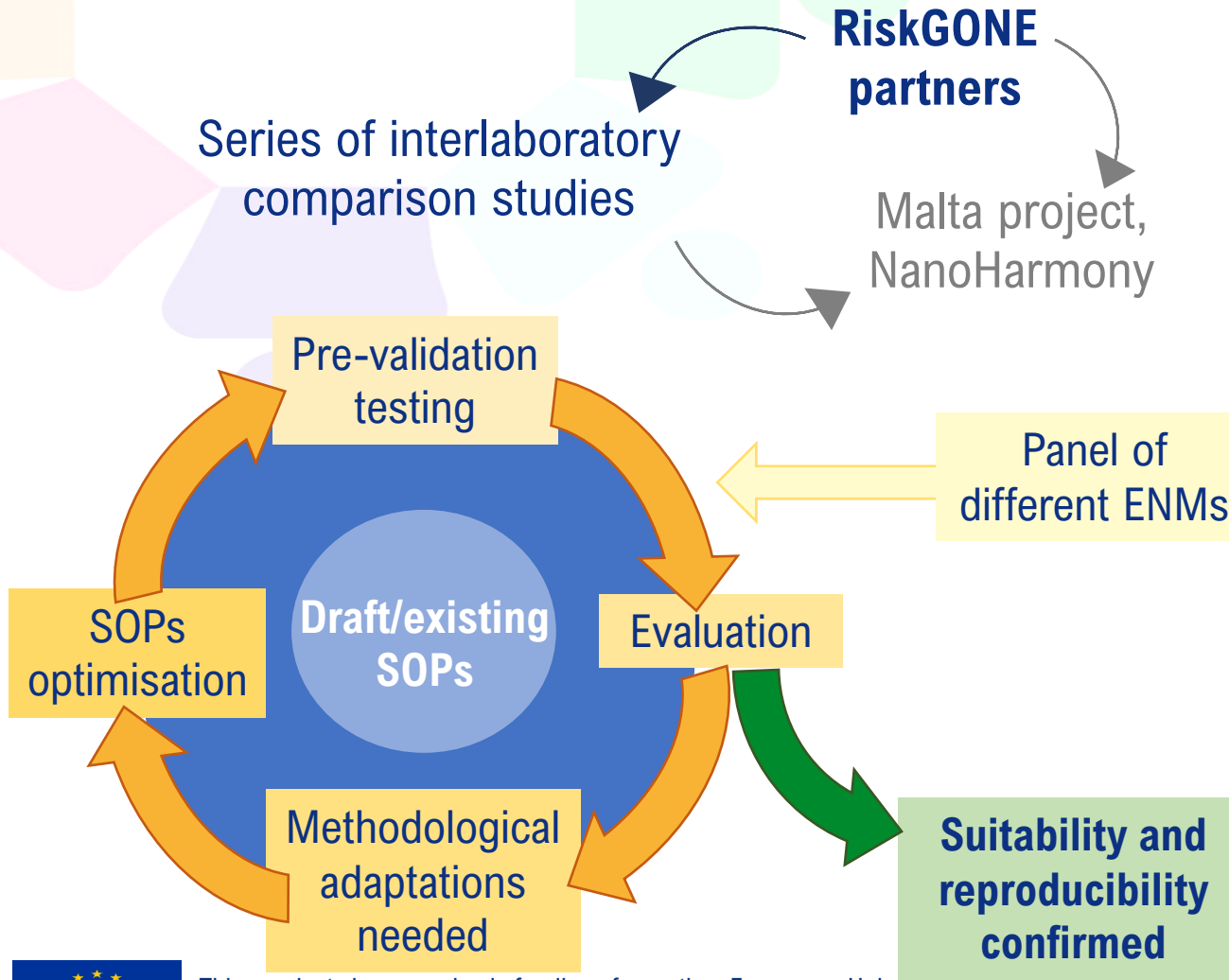
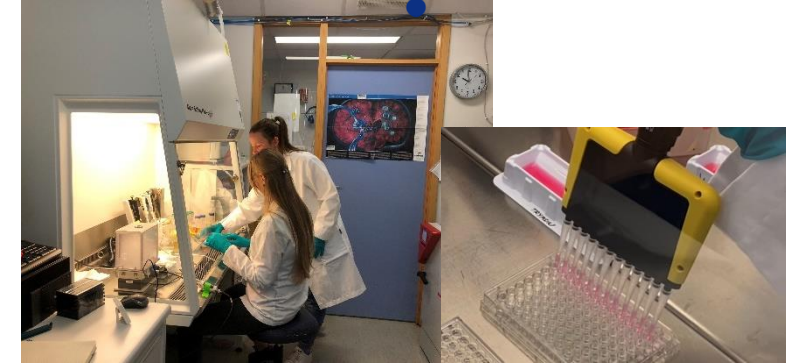
Selected ENMs		ENM Dispersion methods		ENM Characterization/endpoints	
POWDER	CTRL	Selected ENMs		Adaptation of existing methods for chemicals	
		AgNPs TiO ₂ (ERM00000064)	OECD TG318	1) Dispersion Stability of ENM in Simulated Environmental Media	
w-DISPERSIONS	POWDER	Selected ENMs		ENM Characterization/endpoints	
		ZnO (E TiO ₂ (E PL-Cu Wo/Cu	*VCM		
w-DISPERSIONS	POWDER	Selected ENMs		ENM Characterization/endpoints	
		ZnO (E PLGA- PLGA- AuNPs AuNPs MWCI	*VCM		
		Endpoint	OECD TG	Description	
		Reprod			
		Genotoxicity	TG487 TG476	Micronucleus assay Mammalian cell gene mutation test New <i>in vitro</i> guideline for comet assay to detect strand breaks and specific deoxyribonucleic acid (DNA) lesions	
		Multi-g			
		Chronic (herbici	Guidance documents 214 & 231	Cell transformation assays	
		Genotox	TG487 TG432	Relative population doubling (TG487) Colony forming efficiency (CFE) <i>In vitro</i> 3T3 NRU Phototoxicity Test	

Scientific acceptance
Regulatory acceptance



RiskGONE approach

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



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	

Scientific documents to support development of TG/GD



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

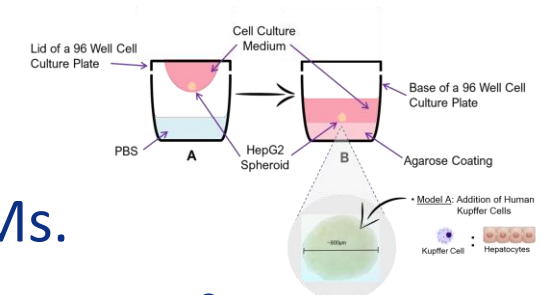
Human hazard assessment



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**



This project has received funding from the European Union's Horizon programme: grant agreement No 101019720

Check for updates

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

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M. Dusinska¹

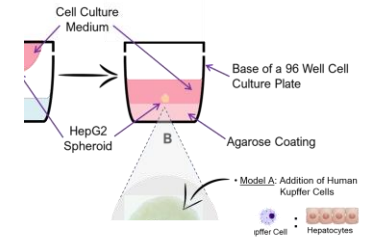
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The colony forming efficiency assay for toxicity testing of nanomaterials—Modifications for higher-throughput

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safety ng.



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Check for updates

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

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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

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Human hazard assessment



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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



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Common Considerations for Genotoxicity Assessment of Nanomaterials

Rosalie K. Elespuru^{1*}, Shareen H. Doak², Andrew R. Collins³, Maria Dusinska⁴, Stefan Pfuhler⁵, Mugimane Manjanatha⁶, Renato Cardoso⁷ and Connie L. Chen⁸

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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.

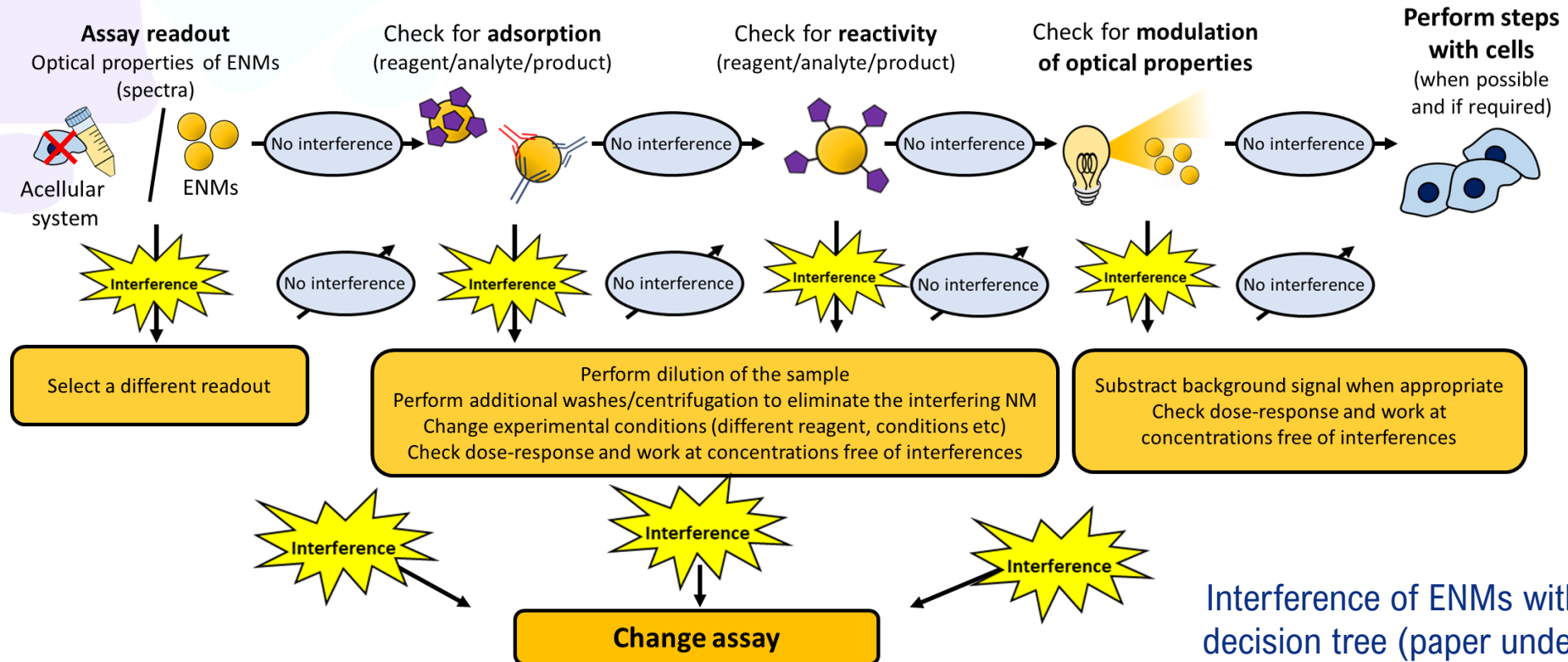
Keywords: nanomaterials, genotoxicity, methods, mutagenicity, clastogenicity, biocompatibility

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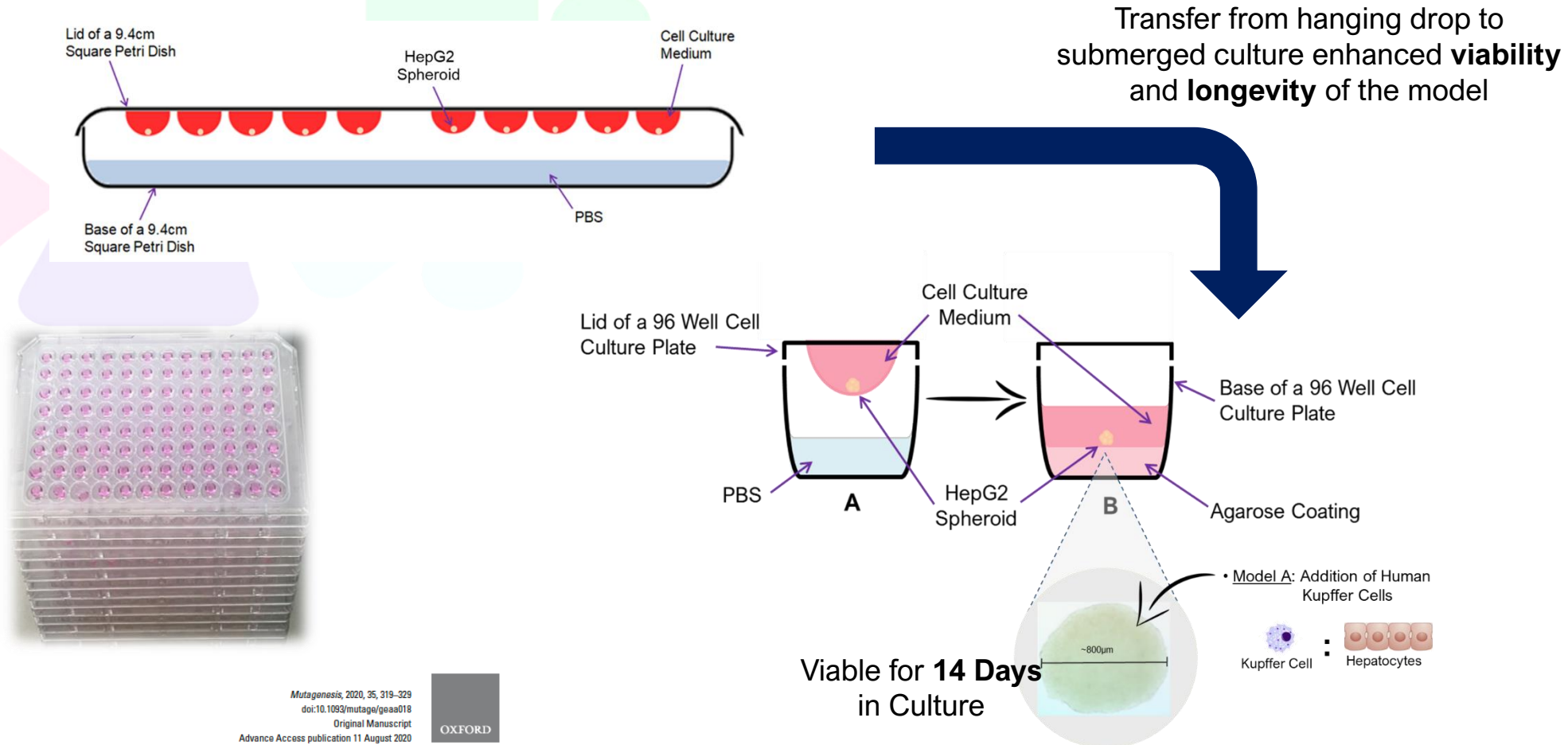
Guidance on controlling interference

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



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



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Original Manuscript
Advance Access publication 11 August 2020



Original Manuscript

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

Gillian E. Conway^{1,2}, Ume-Kulsoom Shah¹, Samantha Llewellyn¹, Tereza Cervena^{1,2}, Stephen J. Evans¹, Abdullah S. Al Ali¹, Gareth J. Jenkins¹, Martin J. D. Clift¹ and Shareen H. Doak^{1*}

jove Journal of Visualized Experiments

Video Article

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

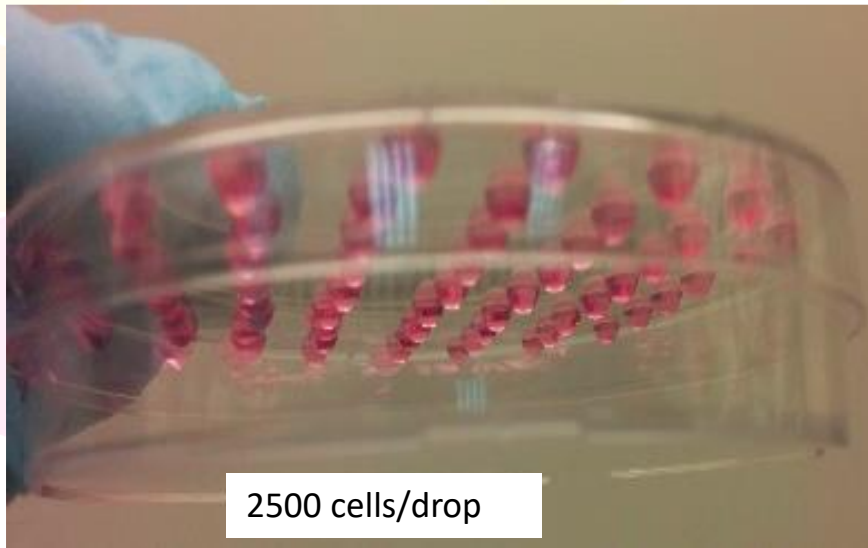
Samantha V. Llewellyn¹, Gillian E. Conway¹, Ume-Kulsoom Shah¹, Stephen J. Evans¹, Gareth J.S. Jenkins¹, Martin J.D. Clift¹, Shareen H. I.

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

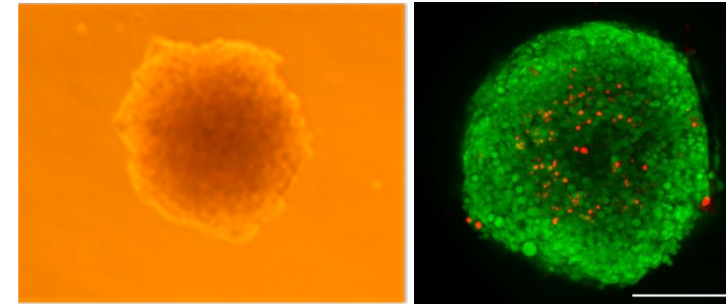
Samantha V. Llewellyn¹, Ali Keramanizadeh², Victor Ude³, Nicklas Raun Jacobsen⁴, Gillian E Conway¹, Ume-Kulsoom Shah¹, Marije Niemeijer⁵, Martijn J. Moné⁵, Bob van de Water⁵, Shambhu Roy⁶, Wolfgang Moritz⁷, Vicki Stone³, Gareth J.S. Jenkins¹ and Shareen H. Doak^{1*}.

3D liver model – HepG2 Spheroids



2500 cells/drop

Seeded as hanging drops



HepG2 spheroids

Cytotoxicity
by AlamarBlue

after 4 days

Cultured
for 1 week

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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,*}

ARTICLE INFO

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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



Article

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

Elisabeth Elje^{1,2}, Espen Mariussen¹, Oscar H. Moriones^{3,4}, Neus G. Bastús³, Victor Puentes^{3,5,6}, Yvonne Kohl⁷, Maria Dusinska¹ and Elise Rundén-Pran^{1,*}

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Need for harmonisation of hazard and risk assessment of nanomaterials across legislations.

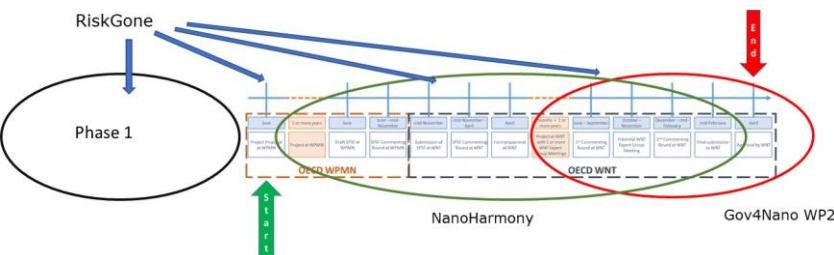
OECD TGs and standards needed on NAMs

NanoTEST

RISK GONE

- 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)



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THANK YOU!

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