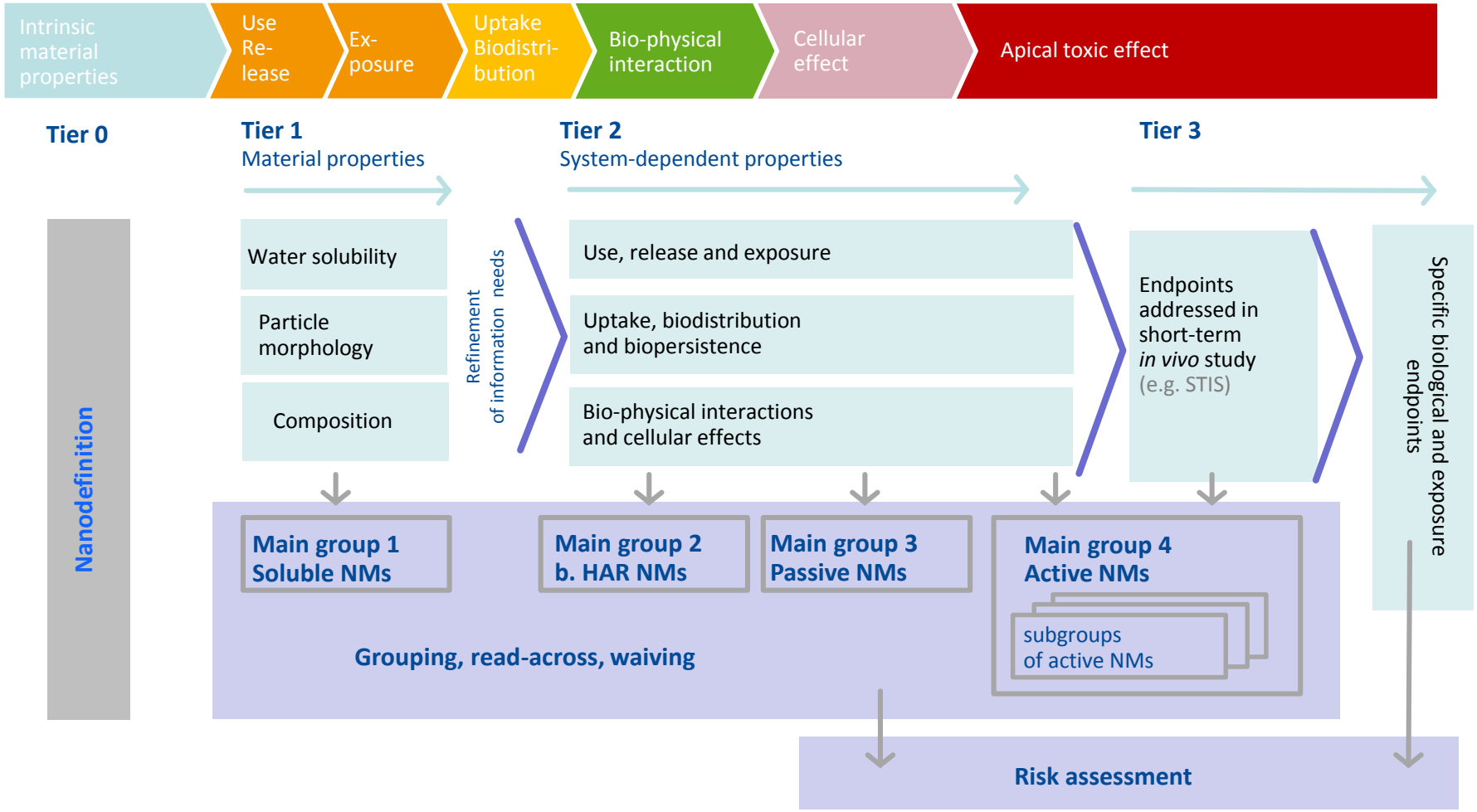
nanoGRAVUR - **Gr**uppierung hinsichtlich **A**rbeits-, **V**erbraucher- und **U**mweltschutz und **R**isikominimierung

SURFACE REACTIVITY AS CRITERION FOR GROUPING AND READ-ACROSS

Christian Riebeling[1], Martin Wiemann[2], Thomas A. J. Kuhlbusch[3], Bryan Hellack[4,5], Andreas Luch[1], Andrea Haase[1], Robert Landsiedel[6], Wendel Wohlleben[6],

(1) German Federal Institute for Risk Assessment (BfR), Berlin
(2) IBE, Institute for Lung Health, Münster
(3) Federal Institute for Occupational Safety and Health (BAuA), Dortmund
(4) Institute of Energy and Environmental Technology (IUTA), Duisburg
(5) Center for Nanointegration CENIDE, Duisburg
(6) BASF SE, Ludwigshafen.

Decision-making framework with quantitative cut-offs



Arts et al **2015a**, http://dx.doi.org/10.1016/j.yrtph.2015.03.007

Decision Criteria with Quantitative Cut-offs

TIER 1

Intrinsic material properties

- Water solubility
- Particle morphology
- Composition

TIER 2

System-dependent material properties

- Dissolution rate
- Surface reactivity (incomplete)
- Dispersibility

Use, release, exposure route

- Exposure route
- No exposure

Uptake, biodistribution, biopersistence

- Penetration of biological barriers
- Persistence in biological fluids

Effects *in vitro*

- Cellular effects: Macrophages

TIER 3

Short-term study *in vivo*

- Toxic effects and Potency
- Reversibility
- Organ burdens and clearance, Biopersistence
- Translocation

Secondary criteria

- Size
- Hydrophobicity
- Surface charge

Criteria commonly discussed, but not used as stand-alone criteria

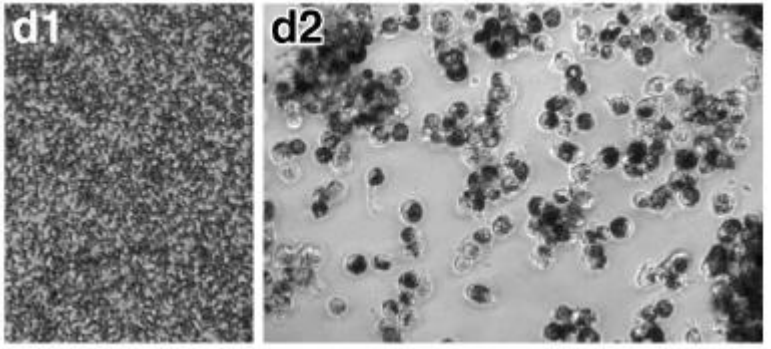
- *Crystallinity (addressed via 'composition', replaced by 'reactivity')*
- *Corona formation (replaced via 'surface charge' and 'hydrophobicity')*

Arts et al 2015a, http://dx.doi.org/10.1016/j.yrtph.2015.03.007

Reactivity: *in-vitro* by NR8383 alveolar macrophages achieve 95% accuracy vs. STIS: essential to prevent false negatives in tier 2

- Derived threshold of 100 pg/macrophage (converted to: 6,000 mm^2/mL) to determine the biological relevance of the lowest observed significant in vitro effects.
  - active if 2, 3 or 4 in vitro parameters significantly altered.
  - passive if 0 or 1 parameter was altered.

→Macrophage data reflected the STIS categorization with 95% accuracy



CeO_2 NM212 CeO_2 NM212 + NR8383

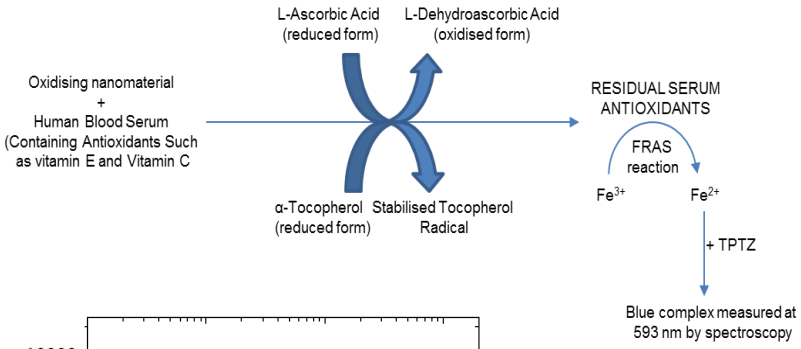
Table 4 Determination of the accuracy, sensitivity and specificity of the in vitro NR8383 alveolar macrophage assay

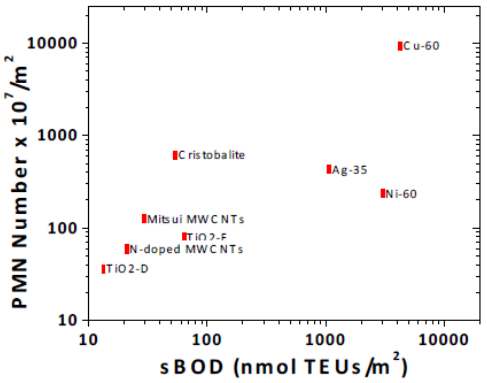
	Test material activity, STIS	Test material passivity, STIS	SUM	
Test material activity, in vitro	9	1	10	90 % positive prediction
Test material passivity, in vitro	0	10	10	100 % negative prediction
SUM	9	11	20	
	100 % sensitivity	91 % specificity		
	Accuracy 95 %			

Wiemann *et al. J Nanobiotechnol (2016) 14:16* DOI 10.1186/s12951-016-0164-2

Reactivity: Abiotic assays do not require settling – easier dosimetry ?

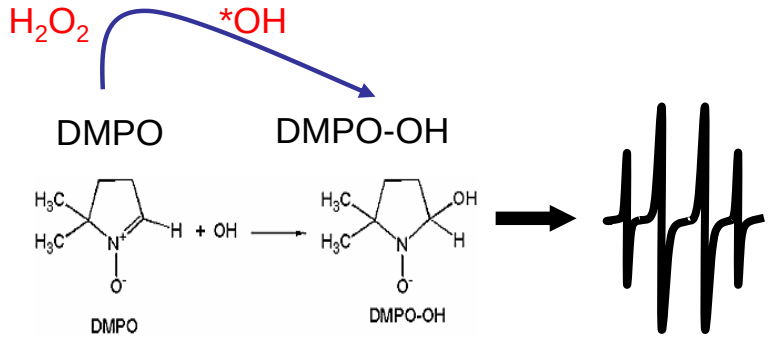
Ferric Reduction Ability of Serum (FRAS)



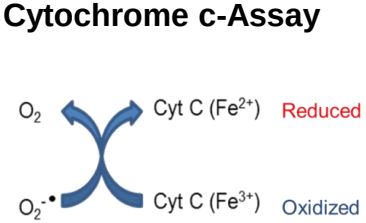


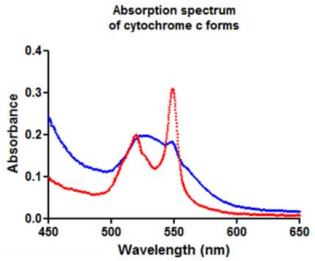
D. Bello et al., Small 2013
Thesis A . Pal 2014

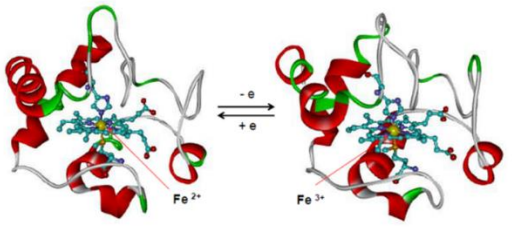
Electron Spin Resonance (ESR) to quantify spin traps



Cytochrome c-Assay







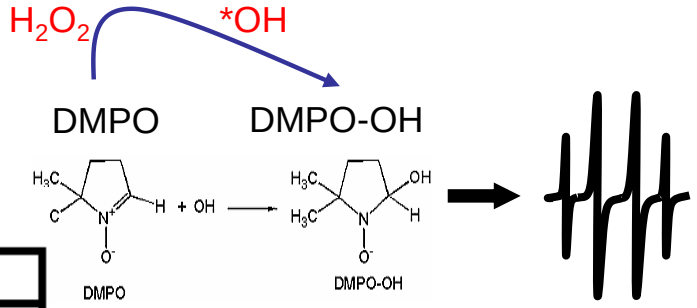
T. Xia ACS nano 2012 → M. Delaval Arch Toxicol **2016**

Reactivity: Abiotic assays
ESR, FRAS good enough for tier 2 ?

Overall excellent match
ESR vs. in vitro carbonylation

Cases of disagreement
ESR vs. in vitro carbonylation

Electron Spin Resonance (ESR) to quantify spin traps



Figure

A Acellular assessment of oxidative stress potential by ESR

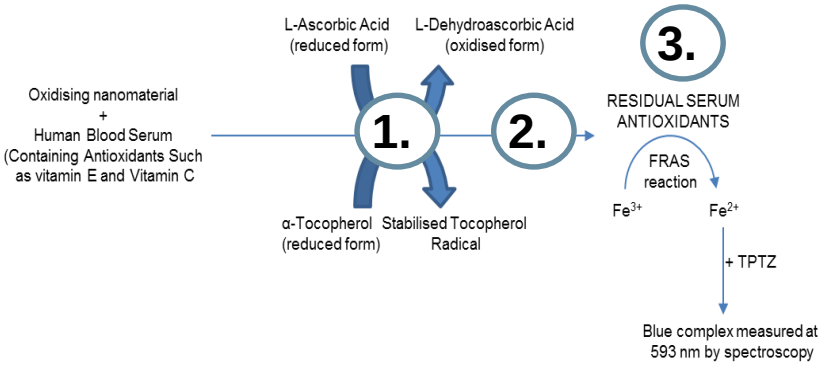
Positive			Negative		
SiO2 Amino	TiO2 NM-103	ZnO NM-110	ZrO2 PEG	SiO2 PEG	AlOOH
SiO2 Phosphate	TiO2 NM-105	ZnO NM-111	ZrO2 Amino	TiO2 NM-104	BaSO4 NM-220
SiO2 NM-200	Ag 200 PVP		ZrO2 Acryl	Ag NM-300K	
SiO2 NM-203	Ag 50 PVP		ZrO2 TODS	MWCNT NM-400	
SiO2 naked	MWCNT NM-402			MWCNT NM-401	

B *In vitro* screening for protein carbonylation

Weakly Positive		Negative		
SiO2 Amino	TiO2 NM-105	ZrO2 PEG	SiO2 PEG	AlOOH
SiO2 Phosphate		ZrO2 Amino	TiO2 NM-103	BaSO4 NM-220
SiO2 NM-200	Ag 50 Citrat	ZrO2 Acryl	TiO2 NM-104	
SiO2 NM-203		ZrO2 TODS	Ag 200 PVP	
Positive			MWCNT NM-400	
	Ag NM-300K ZnO NM-110		MWCNT NM-401	
SiO2 naked	Ag 50 PVP ZnO NM-111		MWCNT NM-402	

Riebeling et al. (**2016**) A redox proteomics approach to investigate the mode of action of nanomaterials. Toxicology and Applied Pharmacology,

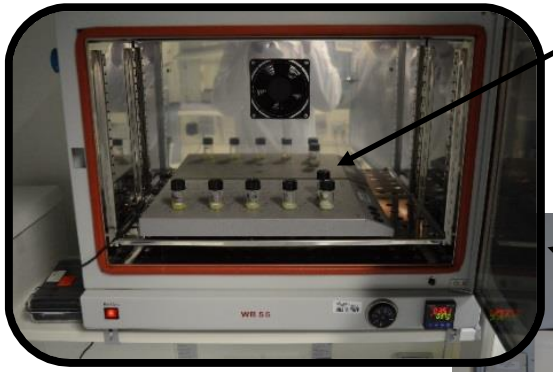
First detailed FRAS SOP



1. INCUBATION

Multiplate stirrer

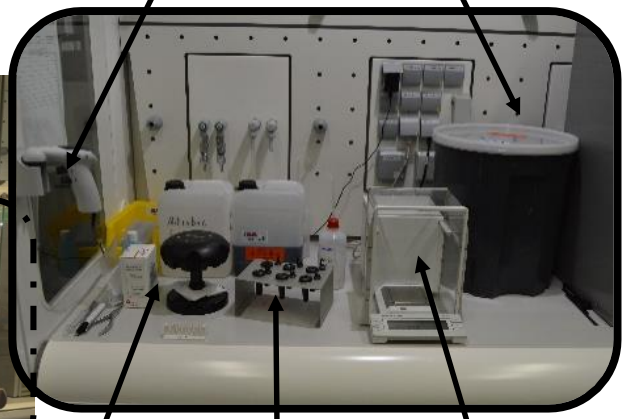
heating chamber



2. SEPARATION

pipetting aid

waste disposal



centrifuge tubes

swing-out rotor

balance

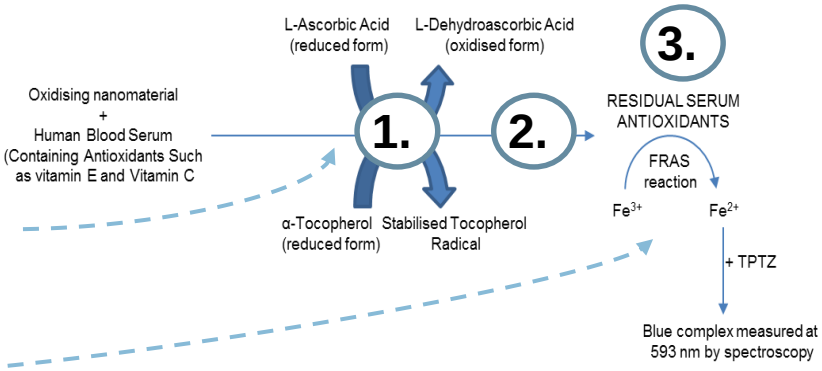
3. ANALYSIS

UV/Vis spectrometer

First detailed FRAS SOP

- Traditional SOP: single dose, linear slope

- **Protocol optimized for high sensitivity & increased significance**
  1. Optimized ENM@serum incubation (3h)
  2. Optimized centrifugal extraction to retain antioxidants, remove ENM.
  3. serum@FRAP reaction time (1h)

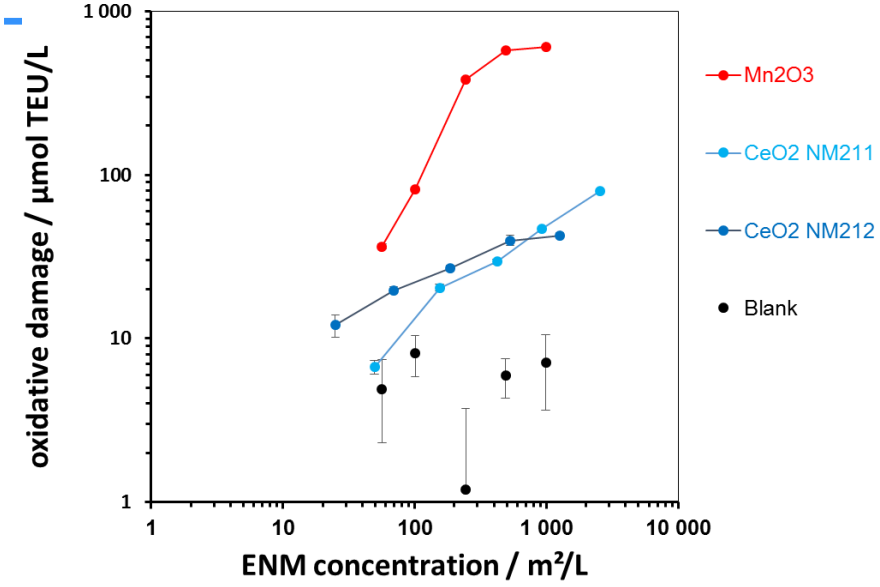


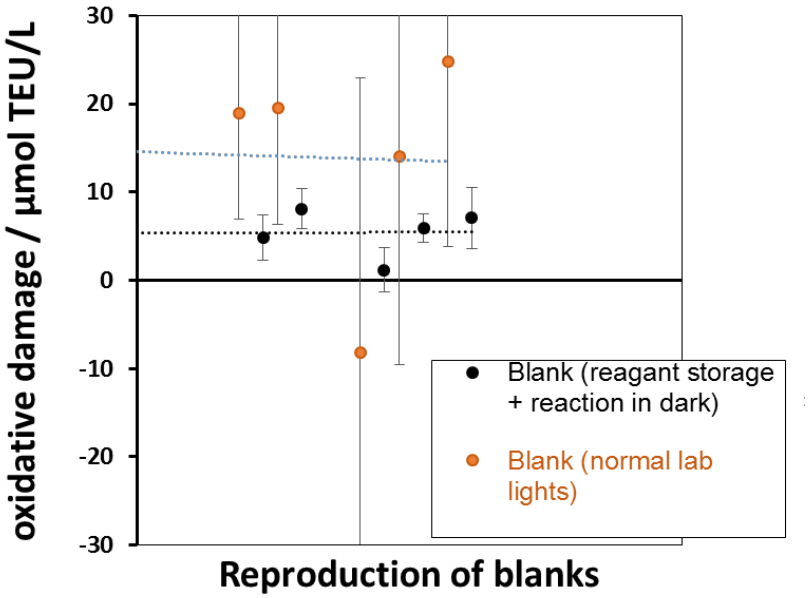
- 5 fixed *mass* doses, sonication to make ENM surface accessible
- Handling of the FRAS reagant in the dark is essential
- Triplicate testing of dose response
- Log slope fit in *surface* metrics for each dose response,

- Positive control Mn_2O_3 induces maximum antioxidant damage already at low dose
- Negative control error bars indicate LoD = 1% of Mn_2O_3 reactivity.

→ New SOP significantly reduces standard deviation, increased significance & resolution

First detailed FRAS SOP





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„active ENM if FRAS > 10% of Mn_2O_3“ Verification by in vivo ranking (STIS)

nanoGRAVUR

Inhalation Toxicology, **2016**, 10.1080/08958378.2016.1200698:1-17
Advanced Materials, **2010**, 22:2601
Arch. Toxicol **2012**, 86:1077
Particle & Fibre Toxicology, **2014**, 11:16

No adverse effects observed up to highest concentration, i.e. 10-50 mg/m³

$BaSO_4$, SiO_2.PEG, SiO_2.phosphate , SiO_2.amino, nano.ZrO_2, ZrO_2.TODA, ZrO_2.acrylate, SiO_2.acrylate (no lung effects up to 10 mg/m³)**, graphite nanoplatelets , low surface area carbon black , Pigment Orange (nano), Pigment Red 254 nano and bulk, Pigment Yellow 74, Pigment Blue 15, Pigment Red 101 nano and bulk**

Most ENM correctly recognized as passive. Three ENM false positives by surface reactivity

Adverse effects observed at 10 mg/m³

SiO_2.naked, graphene , Pigment Orange (bulk), SiO_2.acrylate (systemic NOEC 0.5 mg/m³)**,** *nanostructured calcium silicate hydrate seeds*

Recognized as non-passive by Tier 1 aspect ratio, by Tier 2 surface reactivity, macrophage assay, dispersability

Adverse effects observed at approx. 0.5 mg/m³

nano-CeO_2, Al doped nano-CeO_2, coated nano-ZnO, coated nano-TiO_2
uncoated nano-TiO_2

Recognized as non-passive by Tier 2 macrophage assay, biopersistence

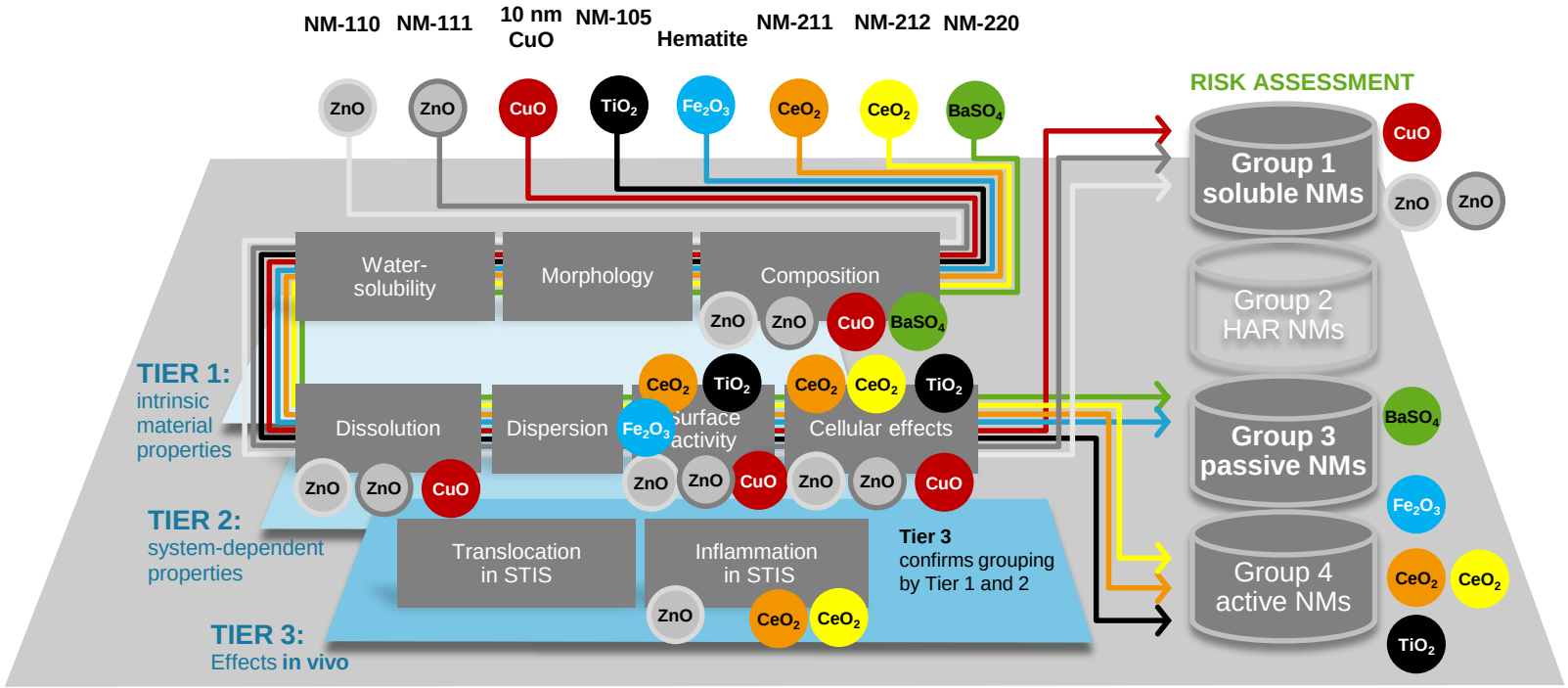
NOAEC levels < 0.5 mg/m³ and effects progressive

MWCNT , *quartz*

Correctly grouped by Tier 1 shape, composition

Arts et al 2015 doi:/10.1016/j.yrtph.2015.11.020 0273-2300
Case studies putting the decision-making framework for the grouping and testing of nanomaterials (DF4nanoGrouping) into practice

Case study: Metal oxides and Metal sulfate

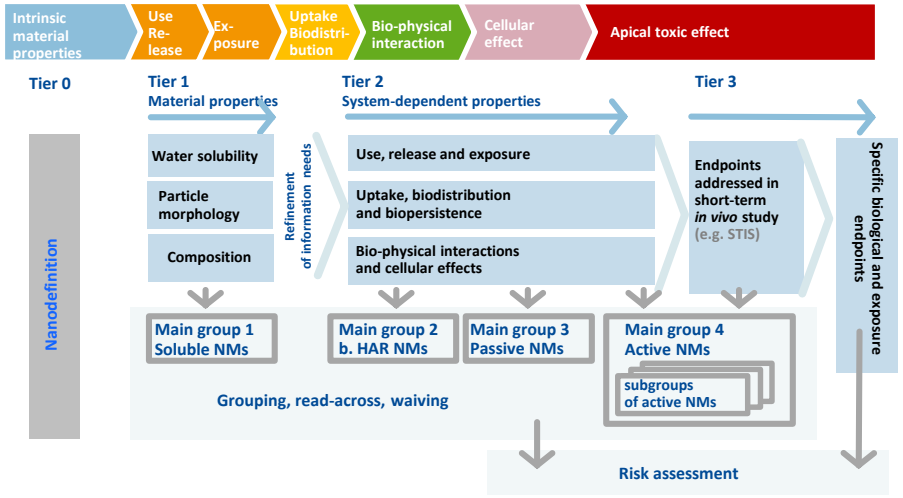


CONCLUSION

- FRAS assay can differentiate between nanoforms with plausible ranking.

- nanoGRAVUR elaborates ESR, FRAS, NR8383 as elements of grouping / read-across
  - Validation against in vivo STIS ongoing.

- The ECETOC scheme is efficient in sorting out nanomaterials that could undergo *human* hazard assessment without further testing:
  - soluble nanomaterials (MG1)
  - high aspect-ratio nanomaterials (MG2)
  - passive nanomaterials (MG3)



- nanoGRAVUR currently elaborates how Tier 2 is guided by lifecycle (use, release, exposure) considerations and lifecycle testing.

- nanoGRAVUR currently transfers the concept to an overarching scheme including *environmental* hazard assessment, and to identify sub-groups of active nanomaterials (MG4) by specific concern.