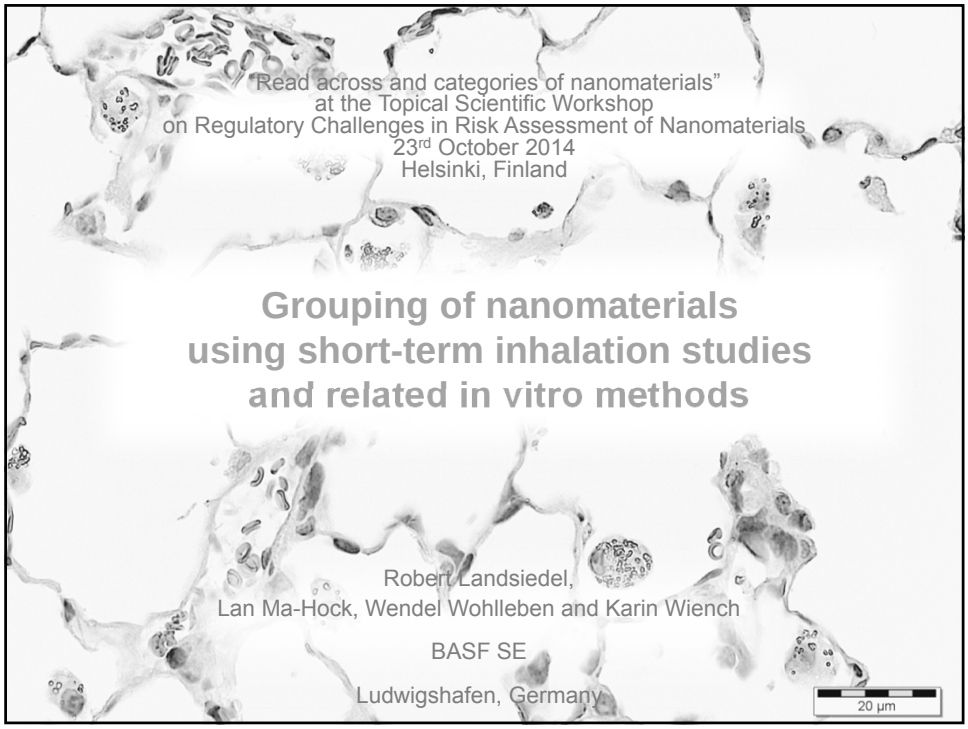

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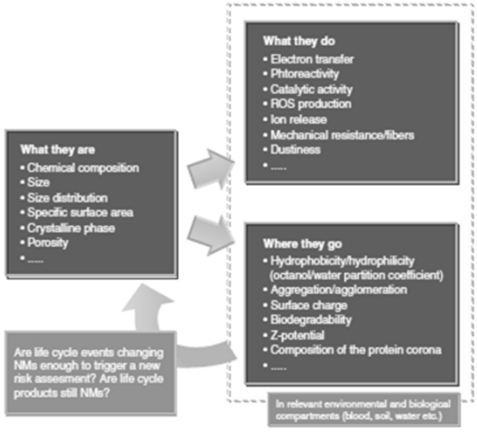
… since no single property groups all materials, need a multi-perspective grouping & testing strategy

Persistence Size Shape
Uptake Agglomeration
Surface-charge
Solubility
Toxicity
Zeta-Potential
Chemistry
Surface-Chemistry
Release Dispersibility Use

… since no single property groups all materials, need a multi-perspective grouping & testing strategy – **but how?**



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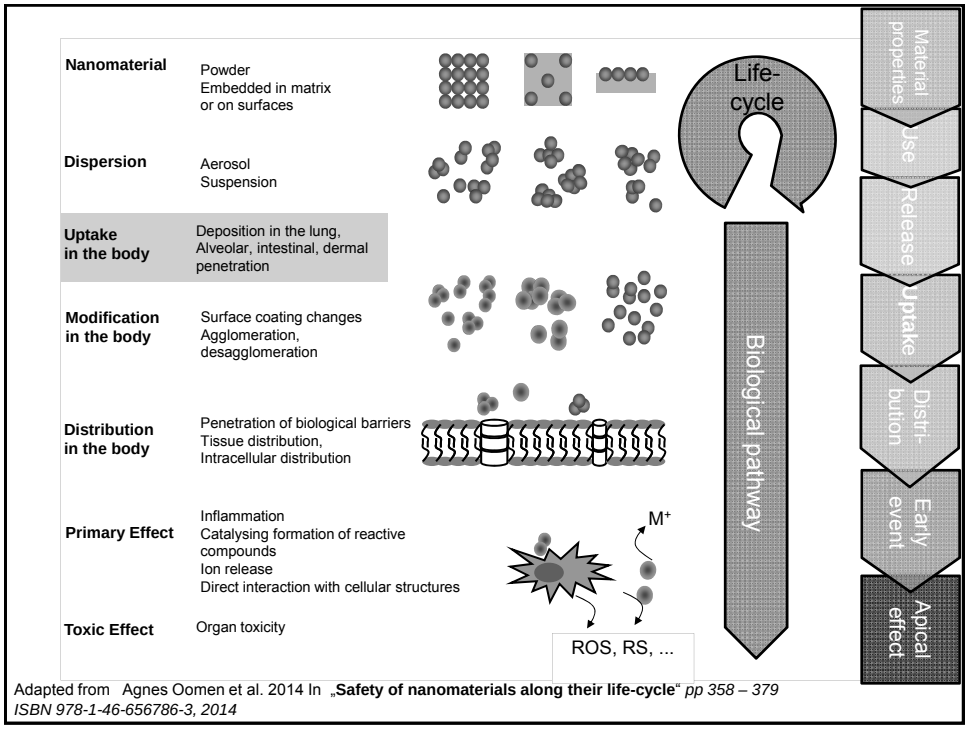
Multiperspective Grouping of nanomaterials



Adapted from Agnes Oomen et al. 2014 In „**Safety of nanomaterials along their life-cycle**“ *pp 358 – 379*
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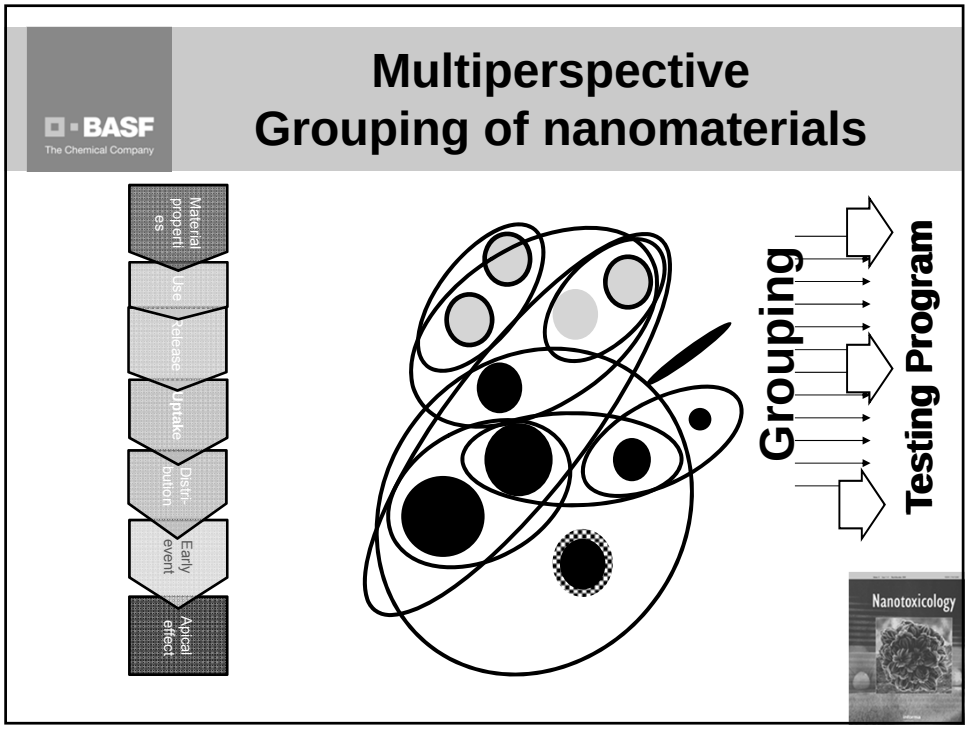

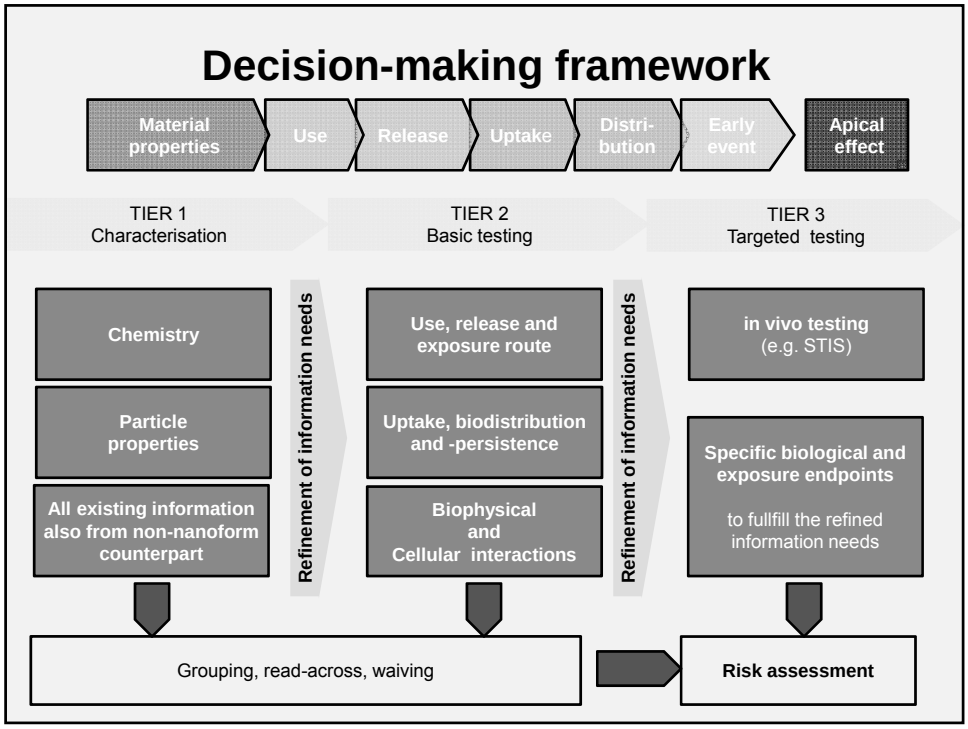

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pp 358 – 379 in ISBN 978-1-46-656786-3, 2014.

NanoSafety Cluster

MARINA
MANAGING RISKS OF NANOMATERIALS

ecetoc

Short-term inhalation studies (STIS)

9

Short-term inhalation studies (STIS)

Short Term Inhalation Study (STIS)

Study day	1	2	3	4	5	6	7	8	9-27	28
Study phase	x	x	x	x	x	R	R	R	R	R
Examinations					E			L		E+L

X: Head-nose exposure to aerosols for 6 hours per day on 5 consecutive days

R: Recovery period

L: Lavage

E: Examinations

- Organ burden (lung, mediastinal lymph nodes, liver, kidney, spleen and basal brain with olfactory bulb)
- Distribution and translocation
- Particle size distribution within the lung
- Histology of selected organs, cell proliferation / apoptosis
- Cytological and biochemical parameters in the broncho alveolar lavage fluid

10

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STIS
Biological parameters

Histopathology

Proliferation and apoptosis

Clinical chemistry (lavage)
Protein
Lactate dehydrogenase (LDH)
Alkaline phosphatase (ALP)
γ-Glutamyltransferase (GGT)
N-acetyl-β-Glucosaminidase (NAG)
Total cell count
Cell differential analysis
-Macrophage (MPH)
-Polymorph nuclear granulocytes (PMN)
-Lymphocyte (LYMPH)

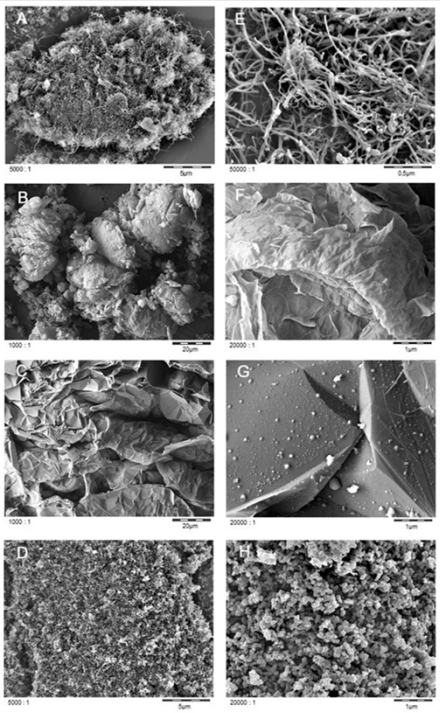
Troponin I

Parameters of oxidative stress
Carboxymethyllysin (CML)
Malondialdehyd (MDA)
8-OHdG

Cytokines *et al.*

1. Apolipoprotein A1
2. ß-2 Microglobulin
3. Calbindin
4. CD40
5. CD40L
6. Clusterin
7. C-Reactive Protein
8. Cystatin
9. EGF
10. Emdothelin-1
11. Eotaxin
12. Factor VII
13. FGF-basic
14. FGF-9
15. Fibrinogen
16. GCP-2
17. GM-CSF
18. Growth Hormone
19. GST-α
20. GST-1 Yb
21. Haptoglobin
22. IFN-γ
23. IgA
24. IL-1α
25. IL-1ß
26. IL-2
27. IL-3
28. IL-4
29. IL-5
30. IL-6
31. IL-7
32. IL-10
33. IL-11
34. IL-12p70
35. IL-17
36. Insulin
37. IP-10
38. KC/GROα
39. Leptin
40. LIF
41. Lipocalin-2
42. MCP-1
43. MCP-2
44. MCP-3
45. MCP-5
46. M-CSF
47. MDC
48. MIP-1α
49. MIP-1ß
50. MIP-1γ
51. MIP-2
52. MIP-3ß
53. MMP-9
54. Myoglobulin
55. OSM
56. Osteopontin
57. RANTES
58. SCF
59. Serum Amyloid P
60. SGOT
61. TIMP-1
62. Tissue Factor
63. TNF-α
64. TPO
65. VCAM-1
66. VEGF
67. von Willebrand Factor

11



Carbonaceous nanomaterials

Carbon black

MWCNT

Graphene

Graphite nanoplatelets

pft PARTICLE AND FIBRE TOXICOLOGY

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STIS
Carbonaceous nanomaterials

Material	Concentrations (mg/m^3)	NOAEC (mg/m^3)	Findings in BALF	Histopathological findings	Reversibility of effects
MWCNT	0.1; 0.5; 2.5	0.1	Severely increased PMN, (total cell count), lymphocyte, total protein, GGT, LDH, ALP and NAG, increased levels of CINC-1, IFNg, IL-1a, MCP-1, M-CSF, osteopontin in BALF and lung tissue	Lung: granulomatous inflammation	No
Graphene	0.5; 2.5; 10	0.5	Increased PMN, total cell count, lymphocyte, total protein, GGT, LDH, ALP. increased levels of CINC-1, MCP-1 and osteopontin in BALF and lung tissue	Lung: granulomatous inflammation	No
Graphite nanoplatelets	0.5; 2.5; 10	10	No adverse effect	10 mg/m^3: few intra-alveolar located multifocal aggregates of alveolar marcrophages	-
Carbon black	0.5; 2.5; 10	10	No adverse effect	No adverse effect	-

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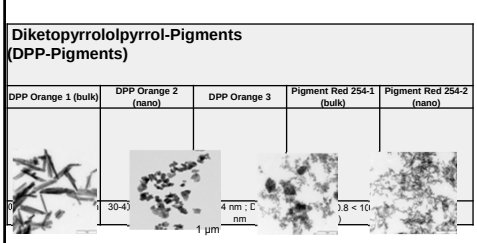
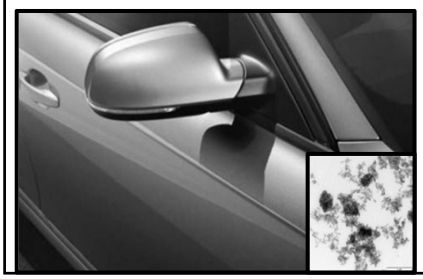
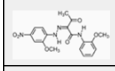
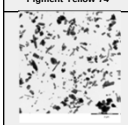
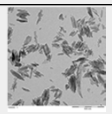
STIS
Pigments

Diketopyrrololpyrrol-Pigments (DPP-Pigments)





Arylide Yellow Pigment	Copper Phthalocyanine Pigment	Fe_2O_3	
			
Pigment Yellow 74	Pigment Blue 15	Pigment Red 101 (nano)	Pigment Red 101 (bulk)
			
38 % < 100 nm	10-108 nm ; D 50 30 nm (TEM)	3.8nm - 26.3 nm	100 - 300 nm

Material	Concentration (mg/m^3)	NOAEC (mg/m^3)	BALF findings	Histopathological findings	Reversibility of effects
DPP Orange 1 (bulk)	3; 10; 30	10	30 mg/m^3: increased PMN, marginally increased total cell count, increased MCP-1 and osteopontin level	30 mg/m^3: decreased absolute and relative thymus weight	Yes
DPP Orange 2 (nano)	1; 3; 10; 30	30	No adverse effect	No adverse effect	-
DPP Orange 3	30	-	Marginally increased PMNs	Lung: slight hypertrophy/hyperplasia: epithelial, in bronchioles, terminal bronchioles and alveolar ducts	Yes
P.R. 254 – 1 (bulk)	30	-	No adverse effect	Lung: minimal hypertrophy/hyperplasia: epithelial, in bronchioles, terminal bronchioles and alveolar ducts Adrenal glands: increase absolute and relative mean weights	Yes
P.R. 254 – 2 (nano)	30	-	No adverse effect	Lung: minimal hypertrophy/hyperplasia: epithelial, in bronchioles, terminal bronchioles and alveolar ducts	Yes
Pigment Yellow 74	1; 3; 10	10	No adverse effect	No adverse effect	
Pigment Blue 15	3; 10; 30	30	No adverse effect	Lung: 30 mg/m^3: minimal hypertrophy/hyperplasia: epithelial terminal bronchioles	
Pigment Red 101 (nano)	10, 30	30	No adverse effect	Ongoing	
Pigment Red 101	30	30	No adverse effect	Ongoing	

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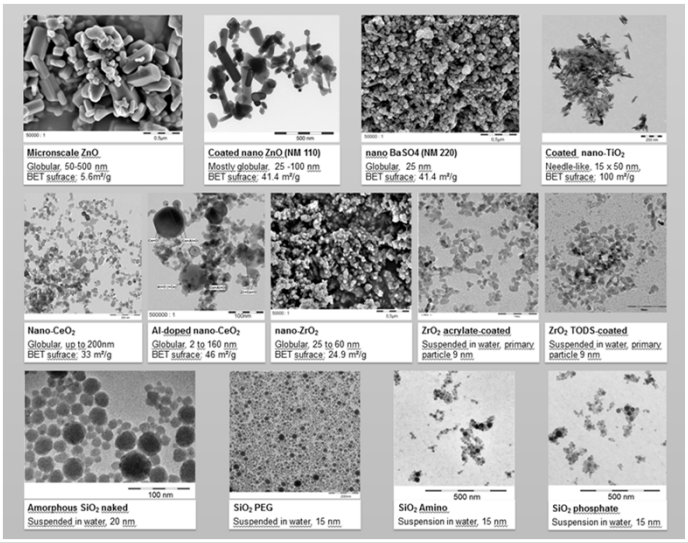
STIS
14 Metal- and metaloid-oxides



pft PARTICLE AND FIBRE TOXICOLOGY

STIS
No adverse effects observed at top concentration

Material	Measured top concentration [mg/m³]	NOAEC (mg/m³)	BALF findings	Histopathological findings	Reversibility of effects
SiO_2. acrylate	9.7	Local effect : ≥ 10 Systemic effect: 0.5	No adverse findings	**Respiratory tract**: no adverse effects; **Spleen**: increased weight (+ 25 %) without histological correlate; particles and high numbers of thrombocytes (TEM)	Reversible
SiO_2. PEG	54.1	≥ 50	No adverse findings	No adverse findings	n.a.
SiO_2. phosphate	51.5	≥ 50	No adverse findings	No adverse findings	n.a.
SiO_2. amino	50.4	≥ 50	No adverse findings	No adverse findings	n.a.
Nano-$BaSO_4$	53.4	≥ 50	No adverse findings	No adverse findings	n.a.
Nano-ZrO_2	9.6	≥ 10	No adverse findings	No adverse findings	n.a.
ZrO_2.TODS	52.2	≥ 50	No adverse findings	No adverse findings	n.a.
ZrO_2. acrylate	50.5	≥ 50	No adverse findings	No adverse findings	n.a.

STIS
Adverse effects observed

Material	Measured conc. [mg/m³]	NOAEC (mg/m³)	BALF (and lung tissue) findings	Histopathological findings	Rever-sibility of effects
Coated nano-TiO_2 (T-Lite SF)	0.6, 2.0, 10.7	0.6	Increased PMN, total cell count, lymphocytes, monocytes, total protein, enzymes (cytokines not measured)	**Lung**: pigment-laden macrophages	Not complete
Micron-scale ZnO	15.3	n.a.	Increased PMN, total cell count, lymphocyte, monocyte, total protein, enzymes, cytokines	**Nasal cavity**: moderate multi-focal necrosis of olfactory epithelia **Lung**: increased absolute & relative weight, broncho-alveolar hyperplasia, histiocytosis, granulocytic infiltration; **Mediastinal lymph nodes**: lympho-reticulo-cellular hyperplasia	Yes
Coated nano- ZnO (Z-Cote HP1)	0.6, 2.8, 13.8	< 0.6	Increased PMN, total cell count, lymphocyte, monocyte, total protein, enzymes, several cytokines	**Nasal cavity**: moderate multi-focal necrosis of olfactory epithelia (less severe than ZnO); **Lung:** histiocytosis, granulocytic infiltration; **Mediastinal lymph nodes**: lympho-reticulo-cellular hyperplasia	Yes
Amorphous silica (Levasil 200)	0.5; 2.4; 10.4; 52.6	2.5	Slightly increased PMN and lymphocytes	Slightly increased neutrophils in **blood** after the end of exposure **Respiratory tract**: Multifocal macrophage aggregates; exacerbation towards a slight multifocal inflammation after 3 weeks	No, slight progres-sion
Nano-CeO_2	0.8, 3.0, 11.6	0.8	Changes of all cytological and biochemical parameters in BALF; increased levels of specific cytokines in BALF and lung tissue	**Lung**: diffuse histiocytosis	Not complete
Al-doped nano-CeO_2	0.6, 2.1 9.2	< 0.6	Changes of all cytological and biochemical parameters in BALF, increased MCP-1 and CINC-1 in BALF, incr. IL1-α in lung tissue	**Lung**: single or aggregated particle-laden macrophages	Not complete

STIS
effects and biodistribution

Material	Conc. [mg/m³]	NOAEC [mg/m³]	BALF (clin. Path.)	Histo-pathology	Reversi-bility	Translocation
TiO_2	2; 10; 50	2	Inflammation	Histocytosis	Not complete	No indication
ZnO	0.5; 2.5; 12.5	< 0.5	Inflammation	Lung: inflammation / cell death, nasal necrosis	Yes	Yes (Zn ions)
SiO_2	0.5; 2.5; 10	10	No effects	No effects	-	No indication
SiO_2 coated	0.5; 2.5; 10	10	No effects	No effects	-	Spleen
CeO_2	0.5; 2.5; 10	< 0.5	Inflammation	Histocytosis, mild inflammation	Not complete	n.d.
MWCNT (NM-402)	0.1; 2.5; 2.5	≤ 0.1	Inflammation	Inflammation	No	No indication
$BaSO_4$	2; 10; 50	50	-	-	-	No indication

STIS
Toxic potency

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; however systemic NOEC at 0.5 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**

Adverse effects observed at 10 mg/m³

SiO_2.naked, graphene , Pigment Orange (bulk)
nanostructured calcium silicate hydrate seeds

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

NOAEC levels < 0.5 mg/m³ and effects progressive

MWCNT , *quartz*

Case study: CeO_2 , $BaSO_4$
STIS, subacute and subchronic study

Female rats, GLP, according to OECD TG
Test concentrations in STIS and 28 d study: 0.5 / 5 / 25 mg/m³ CeO_2; 50 mg/m³ $BaSO_4$
Test concentrations in 90 d: 0.1 / 0.3 / 1 / 3 mg/m³ CeO_2; 50 mg/m³ $BaSO_4$

5 days of exposure

Study day	1	2	3	4	5	6-7	8	9-25	26	27-28	29
Study Phase	X	X	X	X	X	R	R	R	R	R	R
Examination					OB H		L		OB H		L

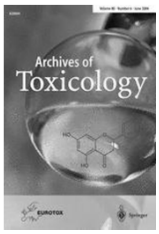
4 weeks of exposure (20 exposures)

Study day	1-28	29	30	31	32-36	37	38-61	62	63	64-92	93	94-156	157
Study Phase	X	R	R	R	R	R	R	R	R	R	R	R	R
Examination		OB L H	OB	OB		OB		H OB L			OB		OB

13 weeks of exposure (67 exposures)

Study day	1-93	94
Study phase	X	R
Examination		OB L

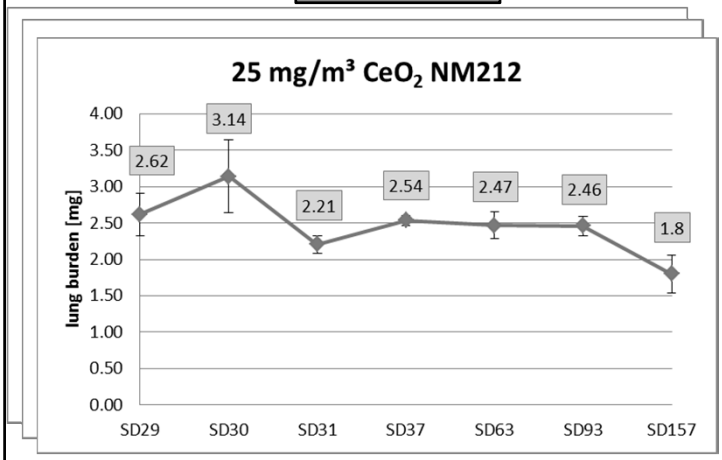
X: Whole body exposure to aerosol for 6 hours per day on 5 days or 5 days per weeks
R: Post-exposure period (24 days or 129 days)
OB: Organ burden
L: Examination broncho-alveolar lavage fluid
H: Histology of selected organs



pft PARTICLE AND FIBRE TOXICOLOGY

Lung burden
CeO_2 NM-212

4 weeks



5 days

0.5 mg/m³ CeO_2 NM-212	SD 5	SD 26
Lung burden [mg]	**0.011** ± 0.001	**0.006** ± 0.001

5 mg/m³ CeO_2 NM-212	SD 5	SD 26
Lung burden [mg]	**0.1** ±0.009	**0.088** ±0.009

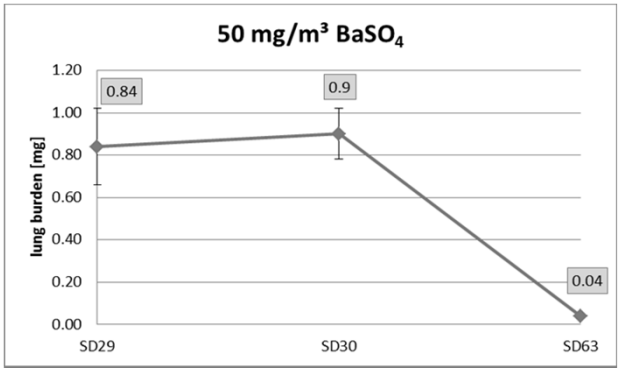
25 mg/m³ CeO_2 NM-212	SD 5	SD 26
Lung burden [mg]	**0.53** ± 0.12	**0.4** ± 0.07

13 weeks

CeO_2 NM-212	0.1 mg/m³	3 mg/m³
	SD 94	SD 94
Lung burden [mg]	**0.012** ± 0.0028	**1.39** ± 0.16

Lung burden $BaSO_4$ NM-220

4 weeks



5 days

50 mg/m³ $BaSO_4$	
Lung burden [mg]	1.056
Lung burden [mg] (3 week post exposure)	0.24

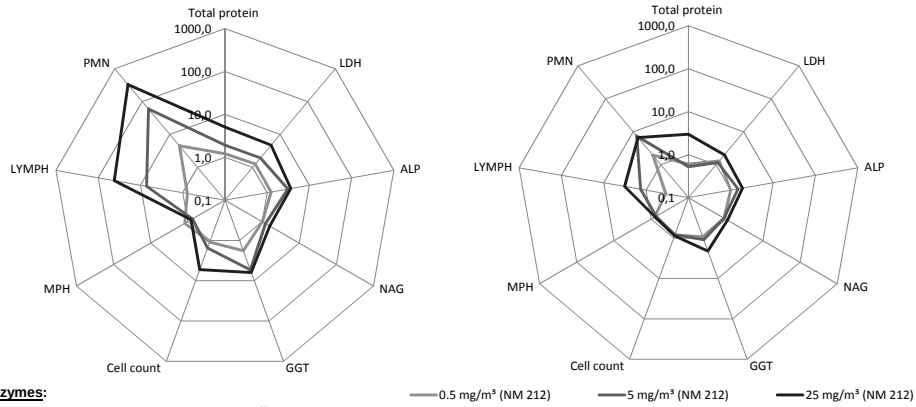
13 weeks

50 mg/m³ $BaSO_4$	SD 94
Lung burden [mg]	**1.73** ± 1.88

BALF after 5 day inhalation CeO_2 NM-212

Main group
SD 8

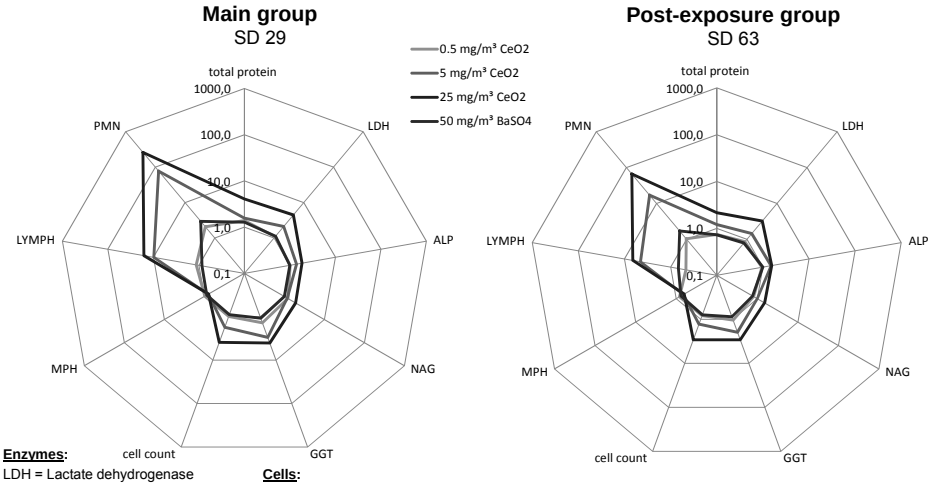
Post-exposure group
SD 29



Enzymes:
LDH = Lactate dehydrogenase
ALP = Alkaline phosphatase
NAG = N-Acetylaminglucosaminidase
GGT = γ-Glutamyltransferase

Cells:
MPH = Macrophage
PMN = Polymorphonuclear neutrophilic granulocyte
LYMPH = lymphocyte

BALF after 28 day inhalation CeO_2 NM-212 and $BaSO_4$ NM-220



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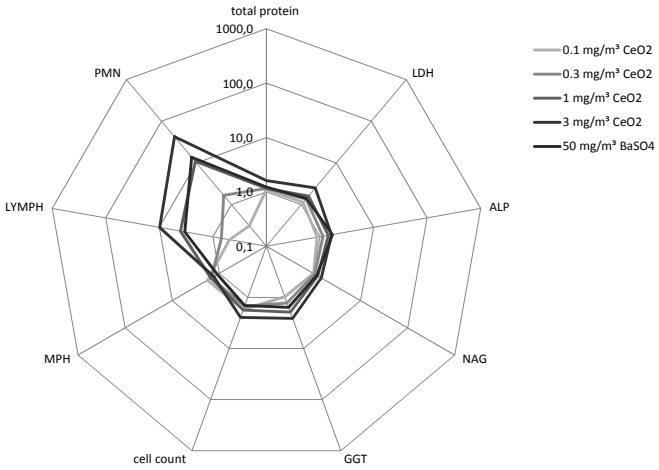
LYMPH = lymphocyte

BALF after 13 weeks inhalation CeO_2 NM-212 and $BaSO_4$ NM-220



Enzymes:
LDH = Lactate dehydrogenase
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GGT = γ-Glutamyltransferase

Cells:
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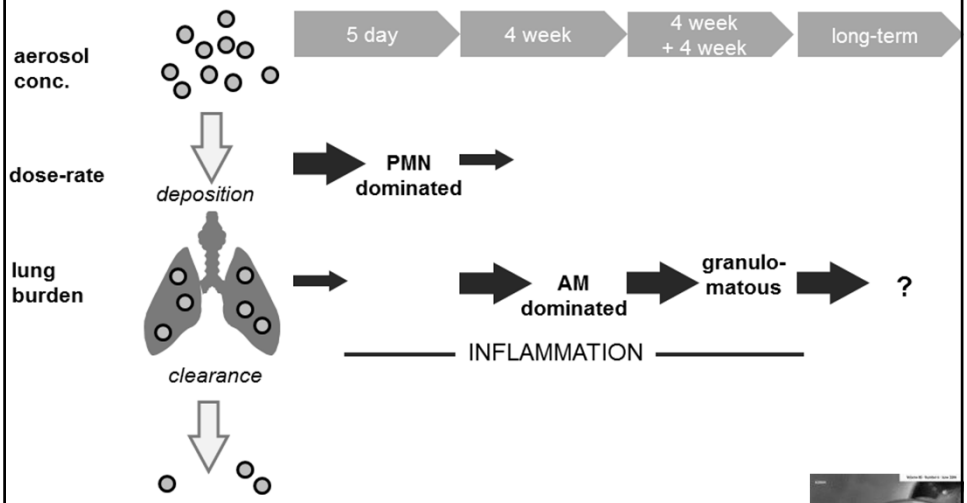
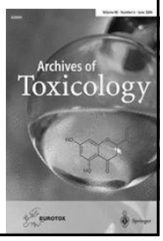
PMN = Polymorphonuclear neutrophilic granulocyte
LYMPH = lymphocyte



Time-course of lung retention and effects



Comparison of toxic potency in STIS and longer-term studies

STIS

- $BaSO_4$
- precipitated SiO_2, Carbon black (low surface), ZrO_2
- TiO_2, pyrogenic SiO_2
- MWCNT

NOAEC mg/m³: 50 > 10 > 1 > 0.1

Long-term Studies

- $BaSO_4$
- precipitated SiO_2, Carbon black (low surface), ZrO_2
- TiO_2, pyrogenic SiO_2
- MWCNT

NOAEC mg/m³: 50 > 10 > 1 > 0.1

28

Conclusion
Short-term **i**nhalation **s**tudy (STIS)

examines more …

- Effects in the lung
- Persistence, progression or regression of the effects
- Effects outside the lung
- Lung burden and potential translocation to other tissues

… with less animals and resources

Ma-Hock, Lan, et al.
Development of a short-term inhalation test in the rat using nano-titanium dioxide as a model substance.
Inhalation toxicology 21.2 (2009): 102-118.

Landsiedel, Robert, et al.
Testing Metal-Oxide Nanomaterials for Human Safety.
Advanced Materials 22.24 (2010): 2601-2627.

Klein, Christoph L., et al.
Hazard identification of inhaled nanomaterials: making use of short-term inhalation studies.
Archives of toxicology 86.7 (2012): 1137-1151.

Ma-Hock, Lan, et al.
Comparative inhalation toxicity of multi-wall carbon nanotubes, graphene, graphite nanoplatelets and low surface carbon black."
Particle and fibre toxicology 10.1 (2013): 23.

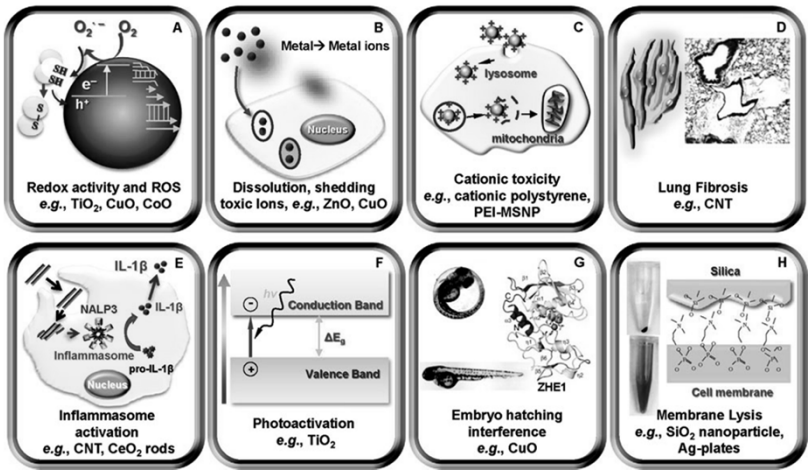
Landsiedel, Robert, et al.
Application of short-term inhalation studies to assess the inhalation toxicity of nanomaterials.
Particle and fibre toxicology 11.1 (2014): 16.

Keller, Jana, et al.
Time course of lung retention and toxicity of inhaled particles: short-term exposure to nano-Ceria.
Archives of toxicology (2014): 1-27.

Konduru, Nagarjun; Keller, Jana et al.
Biokinetics and Effects of Barium 1 **Sulfate Nanoparticles**
Particle and fibre toxicology in press

REVIEW
Testing nanomaterials *in vitro*

Nanomaterials' primary biological effects



Nel, A. *et al.,* 2013. Acc Chem Res 46, 607-621. Reproduced with permission of the author

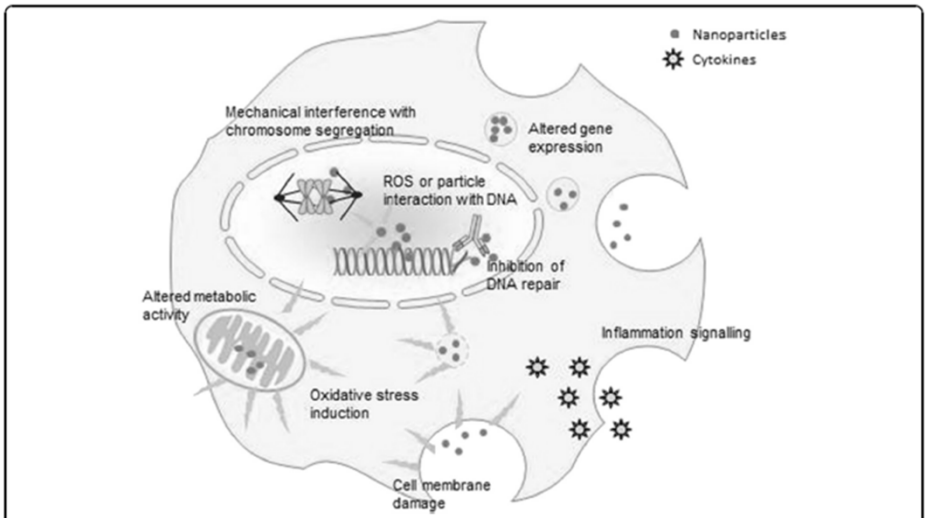


Figure 2 Suggested mechanisms underlying nanoparticle-induced responses at the cellular level. At sufficiently high or persistent levels nanoparticle-induced responses potentially lead to altered tissue function and damage. Uptake of nanoparticles by alveolar macrophages can result in the release of mediators and oxidative stress, which may lead to mitochondrial damage, damage to lipids and DNA, and inflammation [104].

Colognato R, Park MVDZ, Wick P, De Jong WH: Interactions with the Human Body. In Adverse Effects of Engineered Nanomaterials: Exposure, Toxicology and Impact on Human Health. Edited by Fadeel B, Pietroiusti A, Shvedova AA. Amsterdam: Elsevier; 2012.

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REVIEW
Testing nanomaterials *in vitro*

Poorly soluble NM	TiO_2 , CeO_2
Partly soluble NM	Amorphous SiO_2
Soluble NM (shedding toxic ions)	Ag, ZnO
Poorly soluble higher aspect ratio NM	MWCNTs

nanomedicine
Nanotechnology, Biology, and Medicine

REVIEW
Summary

- *In vitro* studies: multitude of cell culture conditions, exposure durations, endpoint detection methods
- *In vitro* studies*:* Inflammation and/or cytotoxicity:
  - Ag ~ ZnO >> TiO_2 > CeO_2 ~ SiO_2
  - MWCNT: inconsistent results
- *In vivo*, STIS:
  - Different degrees of pulmonary inflammation
  - Not fully reversible for some CeO_2 and SiO_2 NMs and MWCNTs within 3 weeks post-exposure
  - ZnO: necrosis of olfactory epithelium

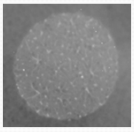
REVIEW
Limitations and hurdles of *in vitro* studies: Selected dosages

- *In vitro* NM doses
  - few µg/mL - several mg/mL
  - rarely correlated to aerosol concentrations for inhalation or *in vivo* lung burdens
  - Unknown effective dose (proportion reaching the cells)
- *In vitro* effects only at much higher doses than can be expected from *in vivo* exposure?
- *Caveat* NM interferences with, *e.g.,* assays dyes

Precision Cut Lung Slices (PCLuS)

Precision Cut Lung Slices (PCLuS)

PCLuS test system



Total protein: PCLuS **Destruction**

WST-1: **Cytotoxicity**

caspase-3/-7: **Apoptosis**

Histopathology

IL-1a, TNF-a, IL-8, MCP-1, M-CSF, OPN: **Cytokine induction**

GSH reduction / increase: **Oxidative stress**

Test substance conc. 10, 50, 100, 500, 1000 µg/mL

24h NM exposure

In PCLuS: WST-1
PCLuS lysate: caspase-3/7, GSH, IL-1a
PCLuS supernatant: other cytokines

		Tissue Destr.	Cyto-toxity	Apo-ptos.	Oxidat. Stress	Inflam-mation	No Effect
TiO_2, an.	NM-100						
anatase	NM-101						
anatase	NM-102						
rutil	NM-103						
rutil	NM-104						
rut.-an.	NM-105						
ZnO	NM-110						
	NM-111						
SiO_2	NM-200						
	NM-203						
CeO_2	NM-211						
	NM-212						
Ag	NM-300K						
MWCNT	NM-400						
	NM-401						
	NM-402						

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PCLuS Conclusions

PCLuS can detect different NM early effects

But

- Comparison *in vitro - in vivo* effects?
- *in vitro - in vivo* dosage?
- Potency? Ranking?

- Variability of test substance measurements
- No adequate negative and positive controls

Nel, Andre E., et al.

A multi-stakeholder perspective on the use of alternative test strategies for nanomaterial safety assessment.

ACS nano 7.8 (2013): 6422-6433.

Sauer, Ursula G., et al.

Applicability of rat precision-cut lung slices in evaluating nanomaterial cytotoxicity, apoptosis, oxidative stress, and inflammation.

Toxicology and applied pharmacology 276.1 (2014): 1-20.

Sauer, Ursula G., et al.

Influence of dispersive agent on nanomaterial agglomeration and implications for biological effects *in vivo* or *in vitro*
Toxicology in Vitro in press

Landsiedel, Robert, et al.

Pulmonary toxicity of nanomaterials: a critical comparison of published *in vitro* assays and *in vivo* inhalation or instillation studies

Nanomedicine in press

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GEFÖRDERT VOM
Bundesministerium für Bildung und Forschung

nano GEM

Macrophage assay in vitro

(vector model)

Macrophage assay in vitro

(vector model)

	Toxicity classification in vitro							Animal		Human epide-miology
µg/10^6 cells	3.75	7.5	15	30	60	120	Over-load >120	mg/ Lunge bei Effekt	Tox.-stufe	
Korund							ROS	> 4.8	0	–
Nano TaC[1,2]							n.d.	6	0	–
Nano TiC[1,2]							n.d.	6	0	–
TiO_2 Ultrafein[3]							n.d.	4.8	0	±[4]
Printex 90[5,6]						ROS	n.d.	2.4	I	±[4]
Neuburger silica earth[7] (85% CS)						Gluc	n.d.	4.8	(I)	-/+ (vorl. Daten)
Quartz 5/1C (99% CS)					TNF		n.d.	2.4	II	+ ...+++ [8]
Quartz 11/1C (99% CS)					TNF		n.d.	2.4	I	+ ... +++[8]
Quartz 3/1C (99% CS)			TNF				n.d.	0.6	III	+ ... +++[8]
Quartz 2/1C (99% CS)			TNF				n.d.	0.6	IV	+ ... +++[8]
Quartz DQ12 (97% CS)			TNF				n.d.	0.6	IV	+++[8]
Chinese TM; Limu (16% CS) [9,10]		TNF					n.d.	0.3	V	++++[11-13]
Chinese TM; Tongken (12% CS) [9,10]		ROS	TNF				n.d.	0.3	V	++++[11-13]

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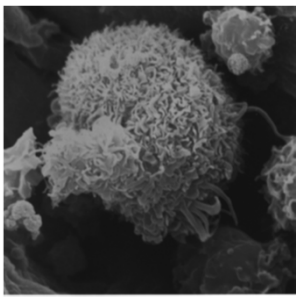
Slide by courtesy of Prof. Wiemann

Macrophage assay *in vitro*
The vector model with NR8383 cells

Serum-free testing of (nano)particles possible

Evaluation of tests for several endpoints gave results similar to those obtained with alveolar macrophages:

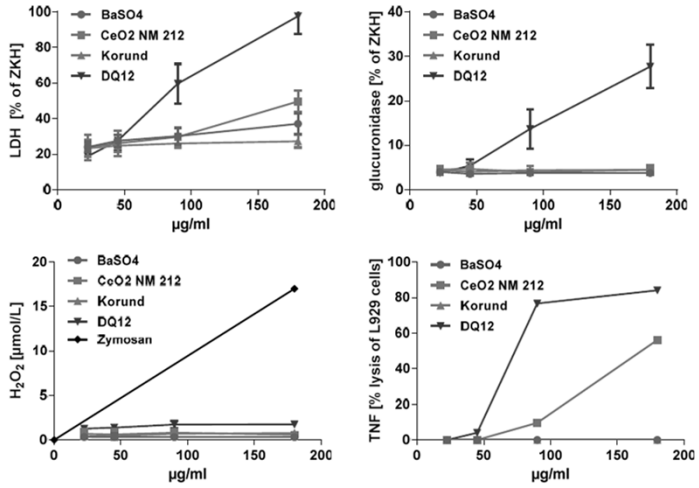


- Cytotoxicity and tissue damage (Release of LDH, WST-1)
- Oxidative Stress (Release of H_2O_2)
- Macrophage activation (Release of lytic enzyme)
- Inflammation/immunmodulation (Release of biologically active TNFα)

IBE R&D Institute for Lung Health

Slide by courtesy of Prof. Wiemann

Macrophage assay (NR8383 cells)
$BaSO_4$ NM-220 and CeO_2 NM-212



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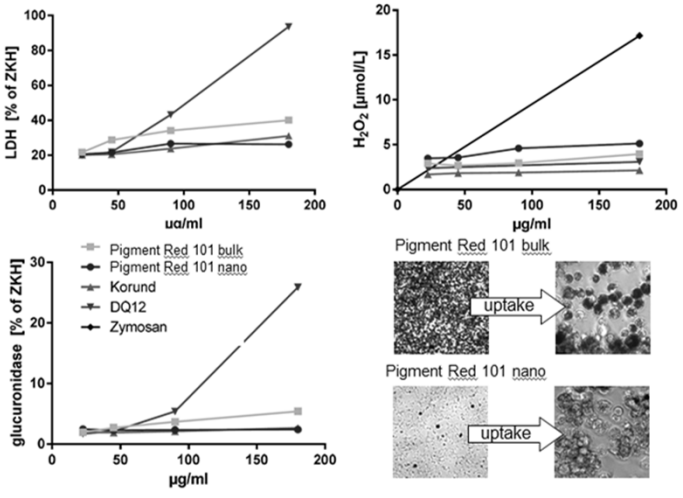
Macrophage assay (NR8383 cells)
Pigment Red 101 bulk and nano



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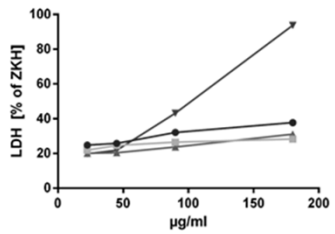
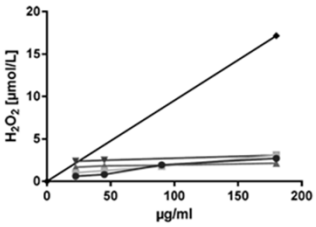
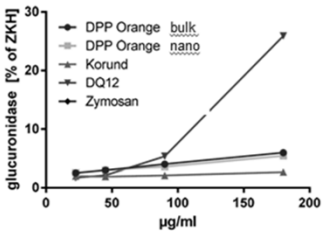
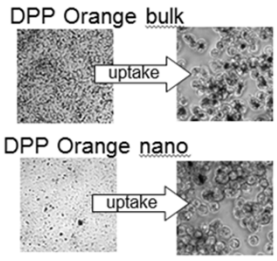
Macrophage assay (NR8383 cells)
Pigment Orange bulk and nano









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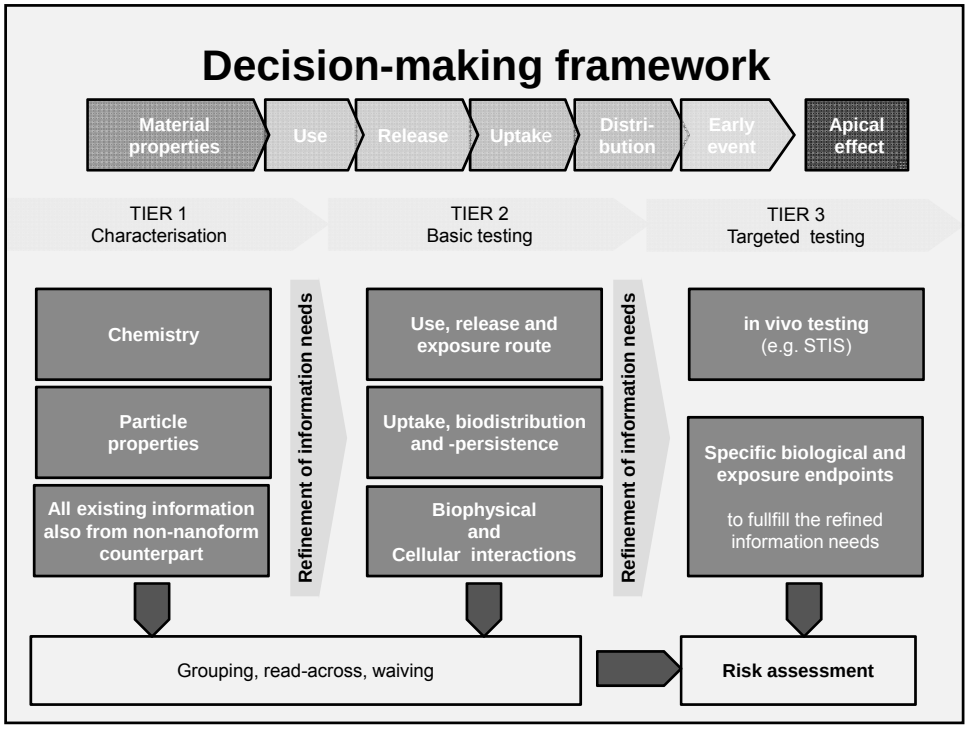
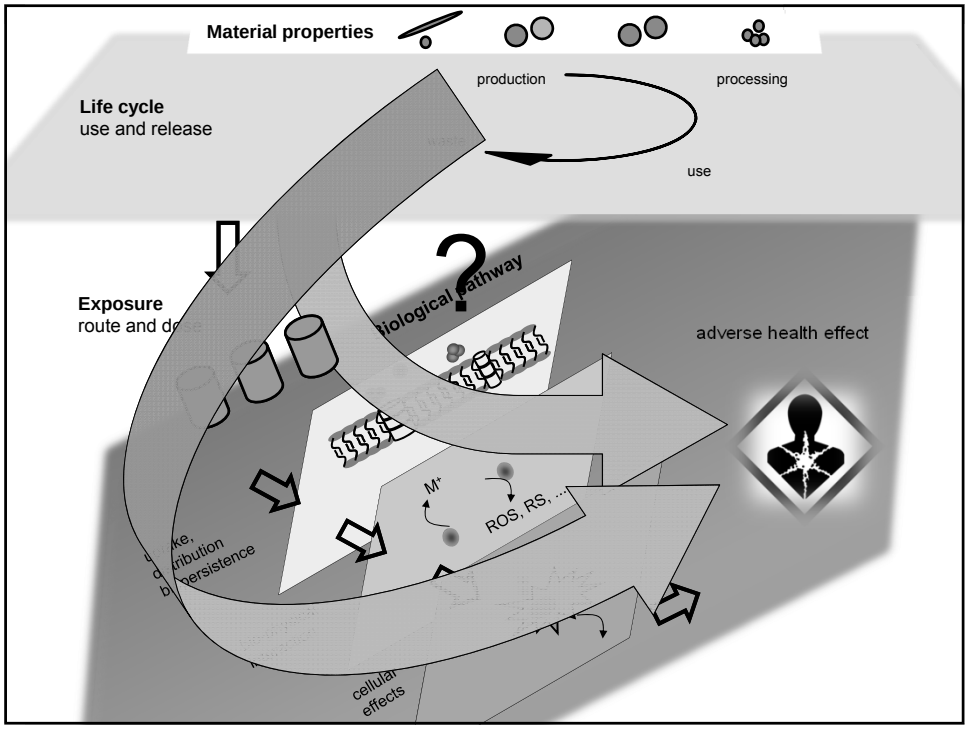
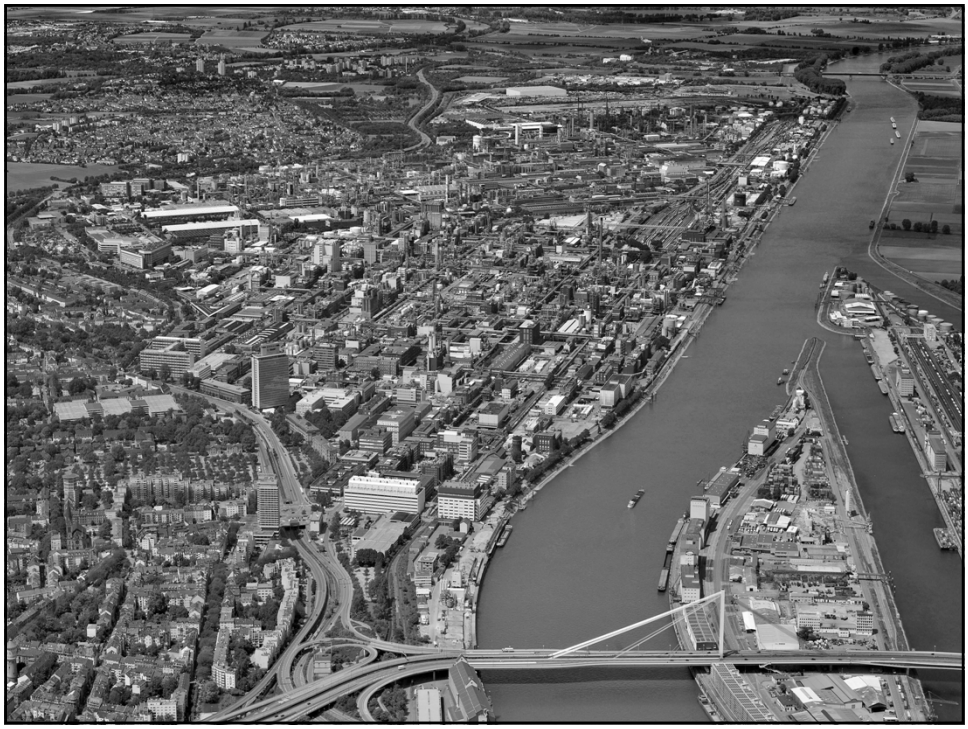
Macrophage assay *in vitro*
Conclusions

- Cultured NR8383 cells are able to differentiate between particles' toxicity
- A ranking based on sum indices or single parameters correlated with *in vivo* data from both intratracheal instillation and short term inhalation studies
- The model is applicable for subsets of similar particles (SiO_2) but also for the range of particles (poorly soluble, partly soluble and soluble ion shedding particles)
- Particle coating in culture media and unusual lung deposition may result in discrepant results

- The macrophage *in vitro* model is helpful to study effects of nanoparticles according to their biologic activity, which is necessary for grouping and ranking


Decision-making framework
Material properties
Use
Release
Uptake
Distri-bution
Early event
Apical effect
TIER 1
Characterisation
TIER 2
Basic testing
TIER 3
Targeted testing
Chemistry
Particle properties
All existing information also from non-nanoform counterpart
Refinement of information needs
Use, release and exposure route
Uptake, biodistribution and -persistence
Biophysical and Cellular interactions
Refinement of information needs
in vivo testing
(e.g. STIS)
Specific biological and exposure endpoints
to fullfill the refined information needs
Grouping, read-across, waiving
Risk assessment


Material properties
Life cycle
use and release
production
processing
use
Exposure
route and d
?
biological pathway
adverse health effect
M+
ROS, RS, ...
cellular effects



Characterization of reference nanomaterials

OECD No. / Test substances		1ary NM size [nm]	Dispersed [nm] *
NM-100	TiO_2, anatase	42-90 (TEM)	262
NM-101		8	428
NM-102		22	495
NM-103	TiO_2, rutile	20	118
NM-104		20	105
NM-105	TiO_2, rutile-anatase	21	79
NM-110	ZnO, uncoated	70-200	176
NM-111	ZnO,coated	33 (XRD)	310
NM-200	SiO_2, amorphous	20 (TEM)	65
NM-203		20 (TEM)	58

OECD No. Test substances		1ary NM size [nm]	Dispersed [nm]*
NM-211	CeO_2, uncoated	10.3	146 & 309*
NM-212	CeO_2, uncoated	33	107
NM 300K	Ag <20 nm; colloidal, 10% w/w	15 (TEM)	11
NM-300K DIS	Ag dispersant	-	-
NM-400	MWCNT	9.5 nm x 1.5 µm	30
NM-401	MWCNT	10-30nm x 5-15 µm	219
NM-402	MWCNT	5-15nm x 0.1-10µm	36

* all NM: dispersed in DMEM/F-12 containing 5% Bovine Serum Albumin to reduce agglomeration; characterisation of dispersed NM: Analytical Ultracentrifugation (AUC)