PATROLS

Advanced Tools for NanoSafety Testing

Methodologies for nanomaterial characterisation in complex *in vitro* and human systems - Solubility, Dissolution and Reactivity Testing

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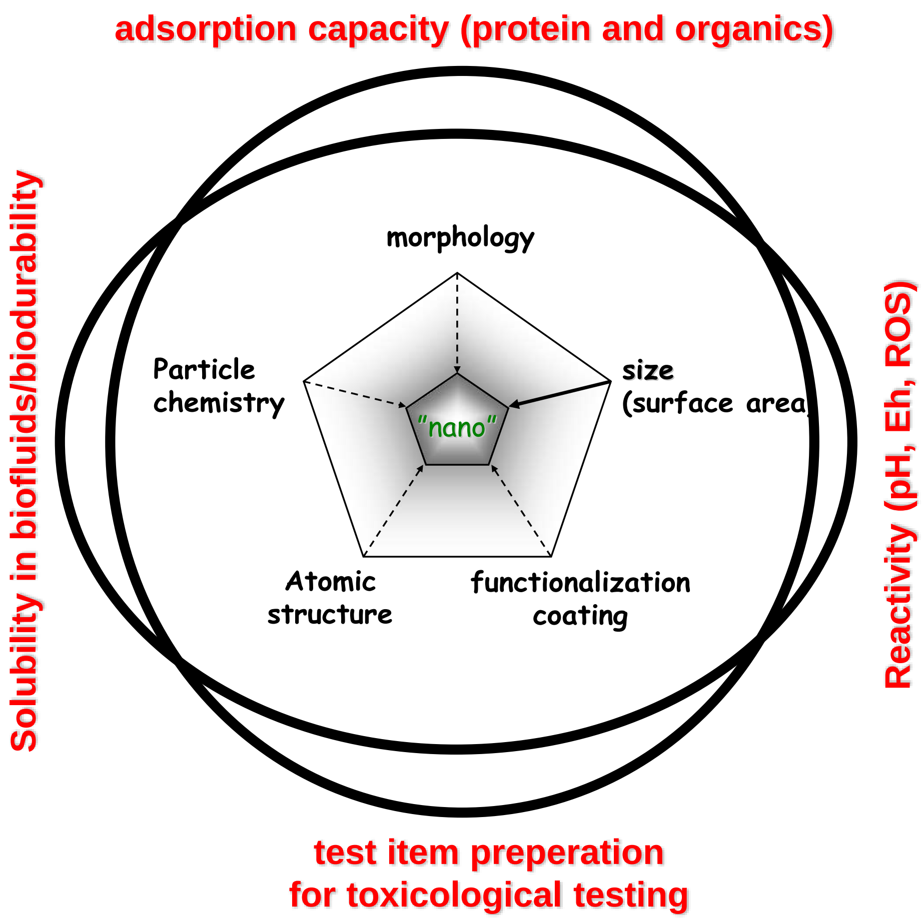
Data requirements for understanding toxicology, grouping, read-across and risk assessment.

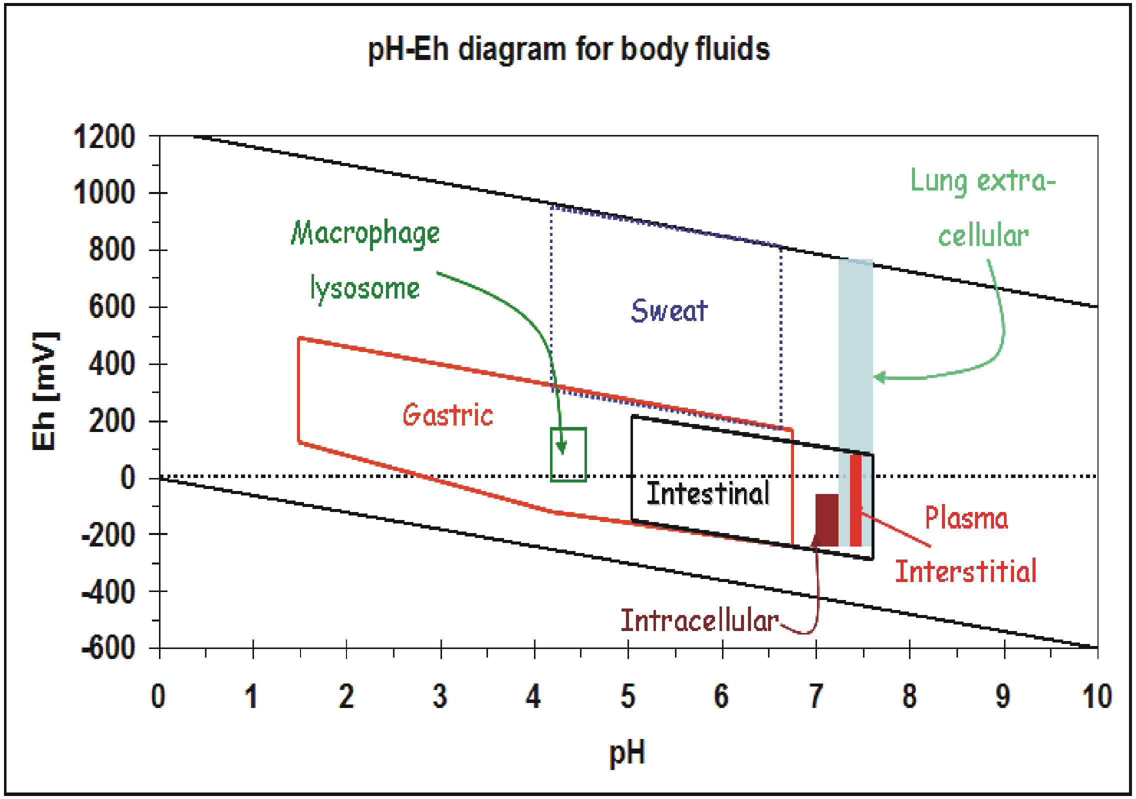


OECD WPMNM list of end-points

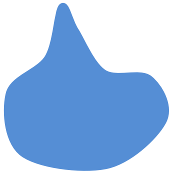
- Molecular structure/crystalline phase
- Composition/purity
- Surface chemistry (coating/functionalization)
- Size (primary/aggregate/agglomerate)
- Crystallite size
- Morphology (nano-object)
- Specific surface area (and relative density)
- Porosity
- Zeta-potential
- (Photo-)catalytic activity
- Redox potential
- Radical formation capacity
- **Water-solubility/dispersability**
- Octanol-water coefficient
- Pour density
- Dustiness
- Other when relevant

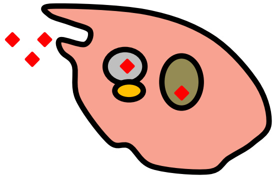
OECD ENV/JM/MONO(2010)46

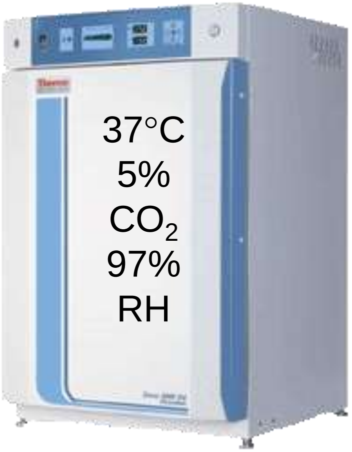
Reliable Data **requires** ***Analysis*** **under** ***Controlled*** **and** ***Relevant*** **conditions!**



Modified after Plumlee & Ziegler (2004)



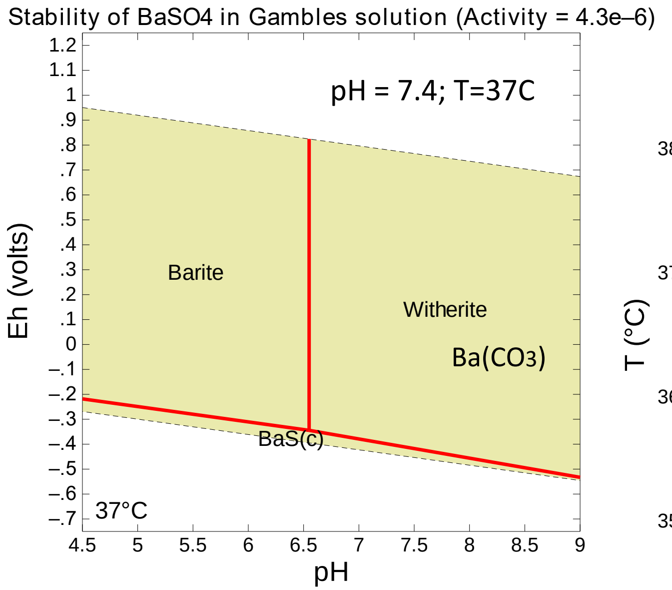


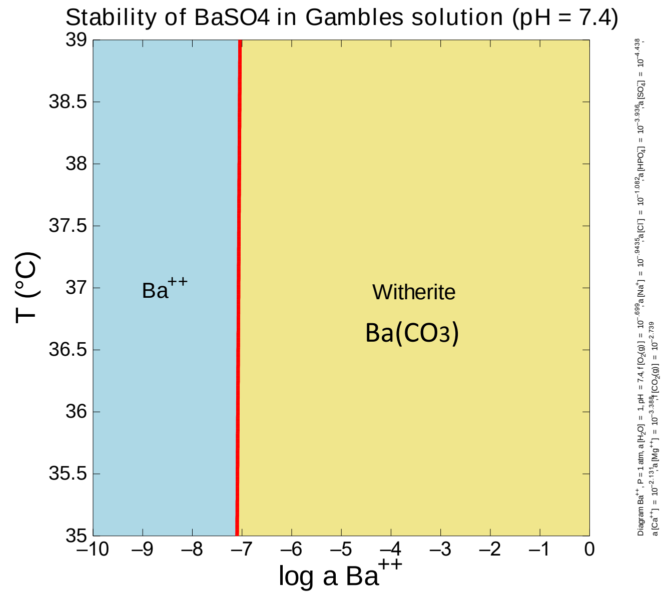


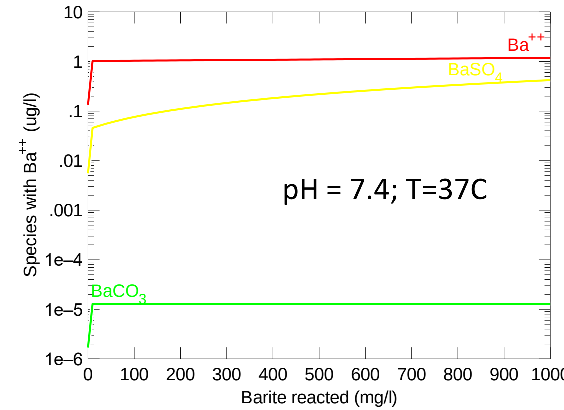
Outline

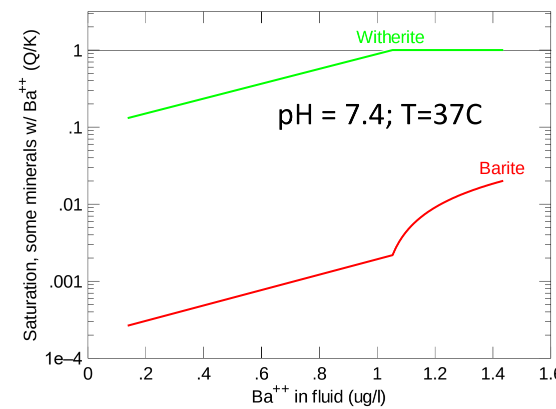
1) Chemical Reaction Modelling
2) Batch reactor test system
3) Continuous flow test system
4) Experimental simulation of test method
5) Applicability for interpretations and risk assessment

1) Modelling: Pre- and Post-test assessment of dissolution, transformation and reactivity
(Example: $BaSO_4$ in Gambles solution)









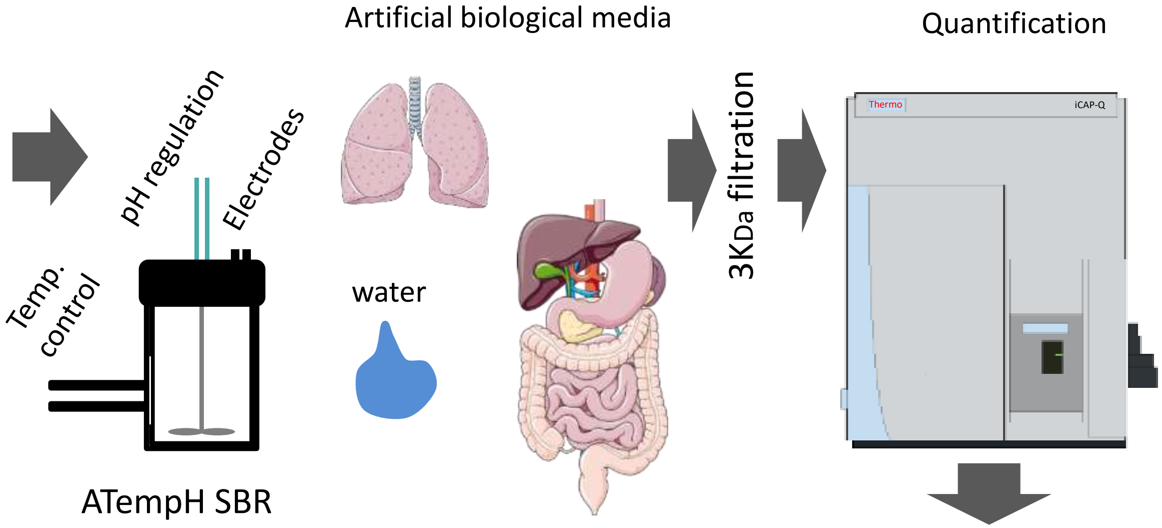
Thermodynamic equilibrium modelling using Geochemist Workbench®

2) Atmosphere-Temperature-pH-controlled Stirred Batch Reactor System (Screening and short-term)

Calibration by
P(ac) & NM-200 performance

	Z_{ave}	σ	PDI	σ
Average	252.7	13.7	0.384	0.038
σ	24.9		0.079	
	Data from 24 partners			

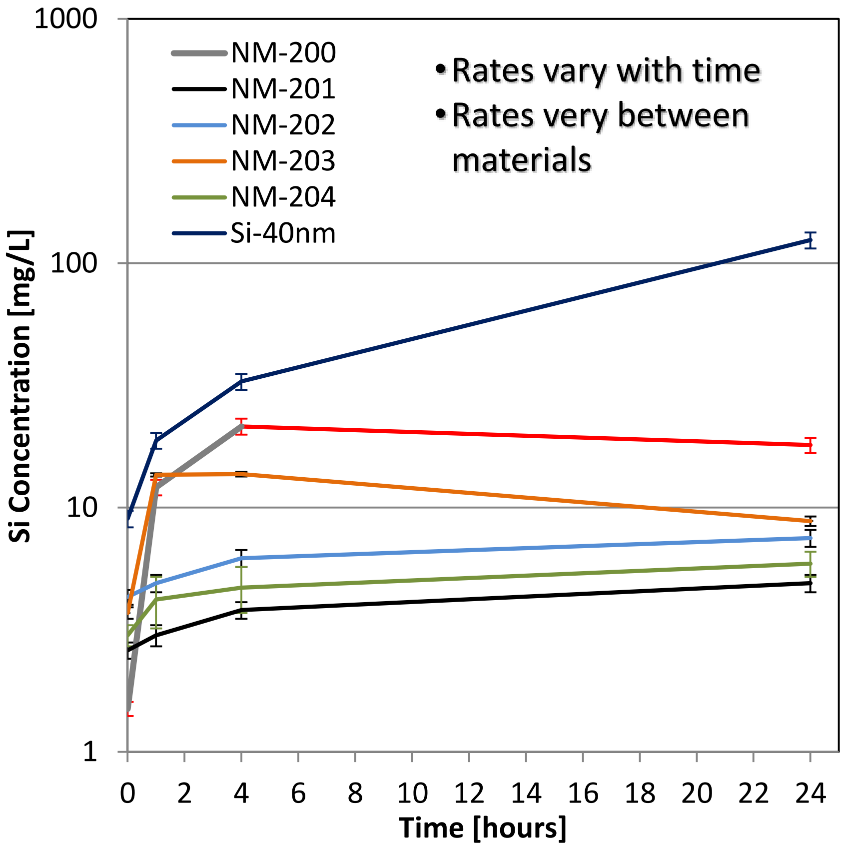
NANOGENOTOX SOP
(0.05% w/v BSA water EtOH pre-wetting)

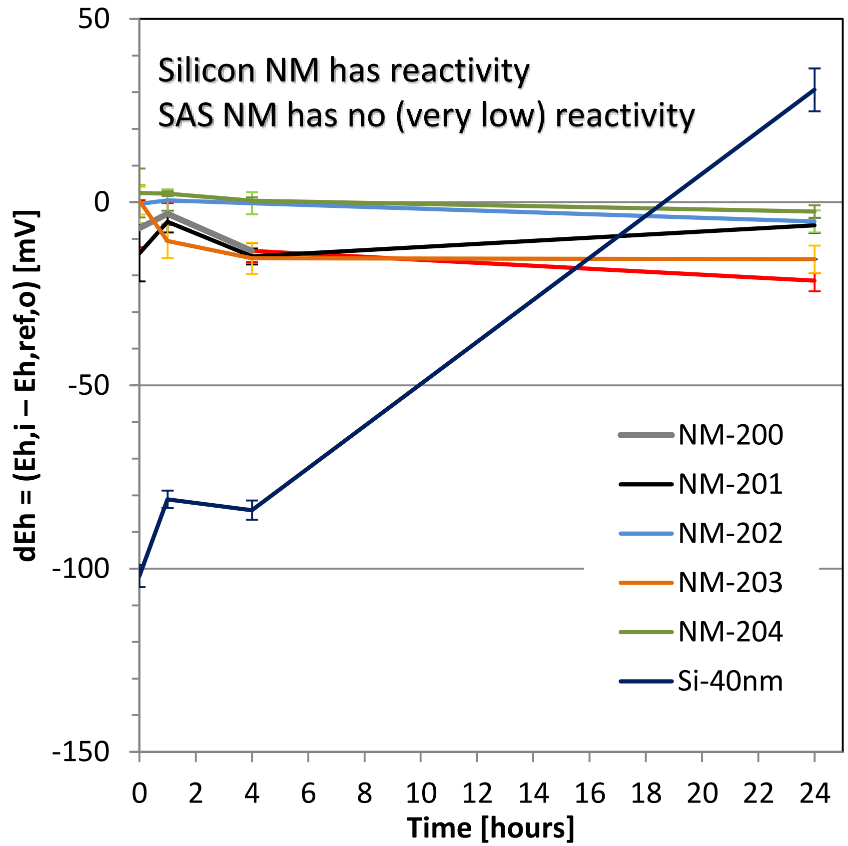


1. Temporal dissolved fraction
2. Initial dissolution rates
3. Transformation by offline analysis

Data on metals, oxides, silicates, carbon based materials.
Improvement on existing methods (e.g., TG-105 and GD29)
Intralaboratory method validation for NM on-going in OECD WNT Project 1.5 (supported by PATROLS & GO4NANO)

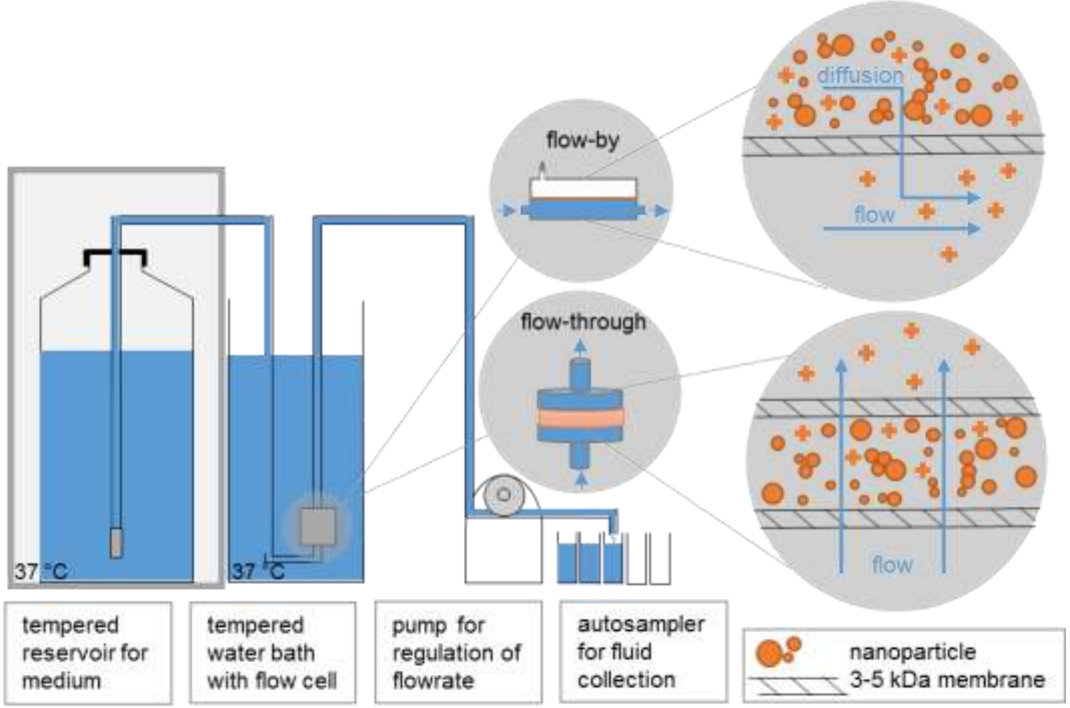
Example: Dissolution and reactivity of silica and silicon NMs in Gambles solution (lung-lining)





3) Temperature-controlled Continuous Flow-By or Through Systems (long-term rate determination)

- Originally developed to understand & predict mineral fiber biodissolution (1995)
- Extensive documentation in scoentific literature
- Described for nanomaterials in ISO TR 19057 (2017)



1. Long-term dissolution rates from ion concentrations (ICP-MS)
2. Assess transformation on remaining solids by offline analysis

Checked on GIT, lysosomal,

Lung lining, freshwater.

Matches in vivo kinetics on benchmark materials

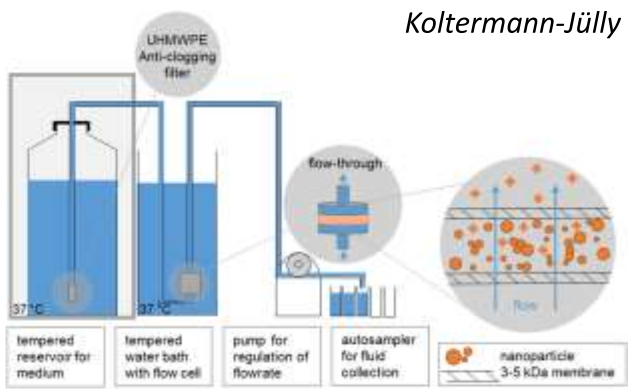
Koltermann-Jülly et al., Nanoimpact (2018)

Considered for ongoing OECD WNT Project 1.5.

Example: Dissolution rates and transformation of 5 different NMs in phagolysosomal fluids (pH4.5)

	Rate [ng/cm²/h]	Half-time [days]
CeO_2 NM-212	0.059	2,880
TiO_2 NM-105	0.056	1,440
SiO_2 NM-200	0.58	41
$BaSO_4$ NM-220	10	5.8
ZnO NM-111	177	0.7

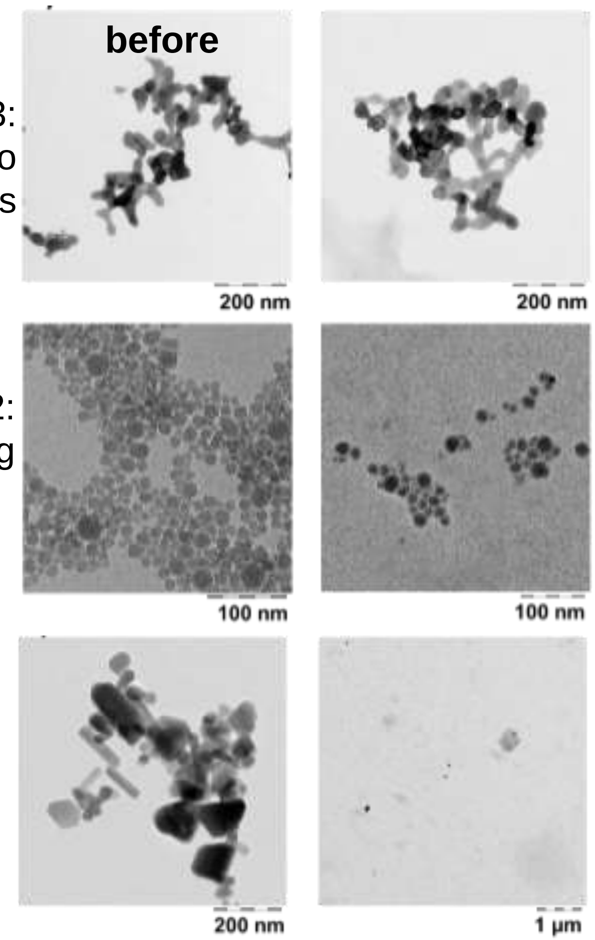
Koltermann-Jülly et al., Nanoimpact (2018)



Fe2O3: no changes

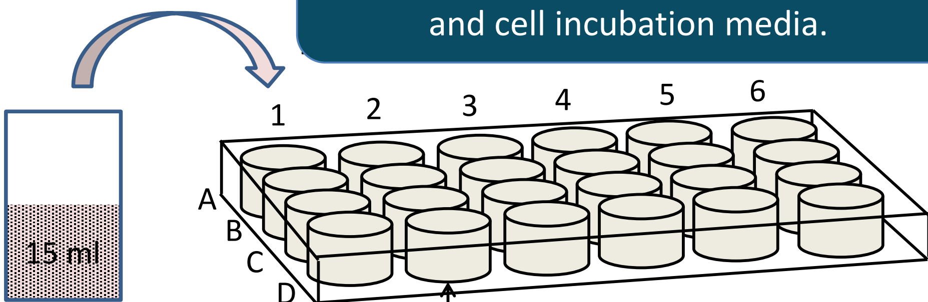
SiO2: gelling

ZnO NM111 dissolved

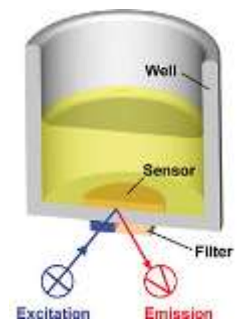


5) Sensor-Dish Reader) pH- and O2 Reactivity and Dissolution Testing (simukating *in vitro* systems)

Tested in numerous media ranging from e.g., serum-water, BSA-water, PBS, and cell incubation media.



A: 1.750 ml medie + 250 µl batch
B: 1.875 ml medie + 125 µl batch
C: 1.975 ml medie + 62.5 µl batch
D: 2.000 ml medie

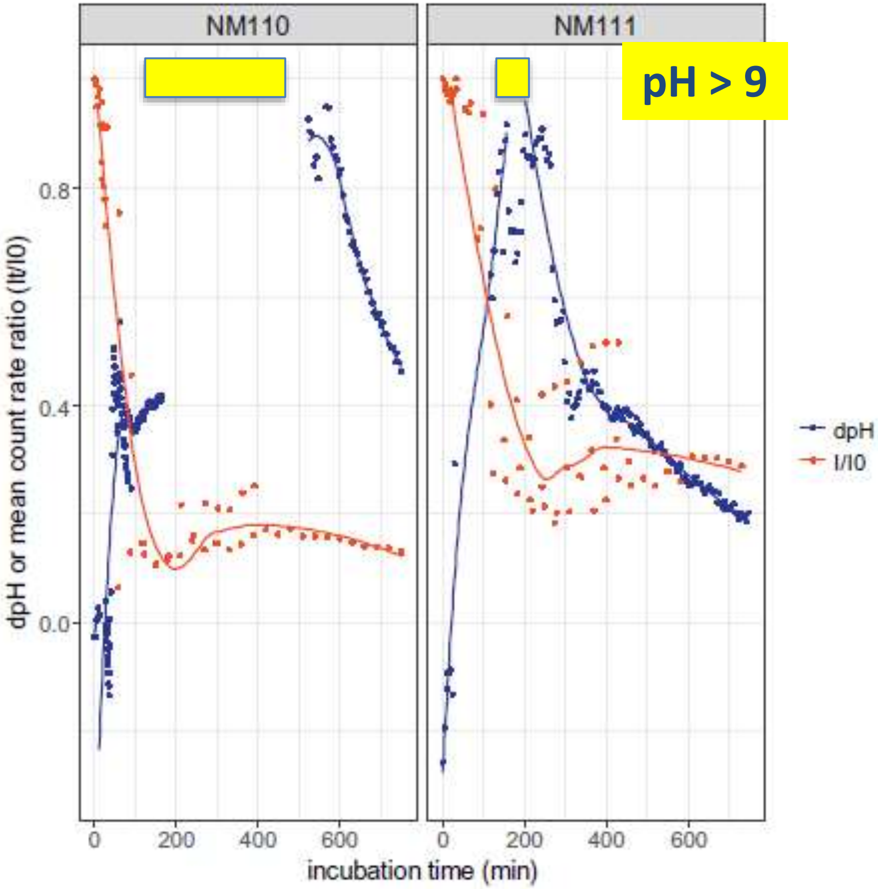


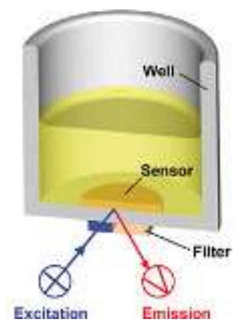
"New" Method!
Intralaboratory validation demonstration completed
Jørgensen et al. (in prep.)
Ongoing: expanding data and knowledge

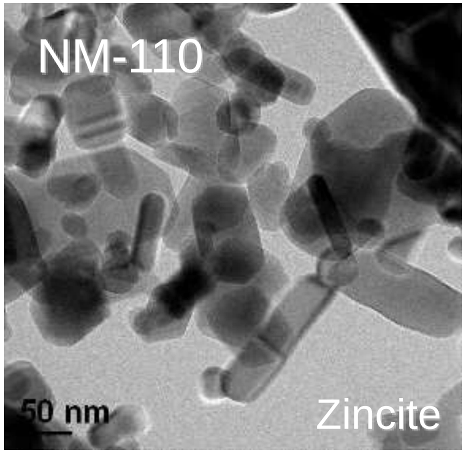
Monitor
pH = sensor (calculate dpH)
O_2 = sensor (calculate dO_2)
Liquid sampling for solubility testing
3kDa filtration in centrifuge

Solubility and dissolution by chemical analyses of dissolved ion concentrations
Assess transformation on remaining solids by offline analysis

Example: Dissolution and reactivity of two ZnO NMs in cHAMs F12 (in vitro)







TEM: P.J. De Temmerman & J. Mast

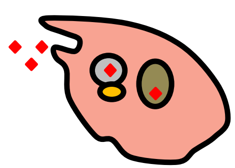
Sampling time (h)	Zn (total, µg/L)			
	NM-110	σ	NM-111	σ
0[a]	872.6	22.6	864.3	19.0
0.25	5802.3	67.5	5177.4	148.9
1	5978.4	162.2	5595.2	22.0
2	5956.5	101.3	5827.5	139.4
4	6154.7	131.4	6056.2	242.0
24	7431.1	373.5	8265.2	178.0
cHam's F12	49.4	14.6	41.1	7.5

[a] Estimated concentration at $t = 0$ h.

Da Silva et al. Tox In Vitro (2019)

Applicability for toxicological interpretations and risk assessment?

- **Analysis of dissolution and reactivity of test materials under well-controlled relevant conditions can provide:**
  - Important results for prediction of test material behaviour in biological test systems
  - Important information for understanding and interpretation of toxicological test results
  - Important information for grouping, read-across and QSAR
- **Proposed test methods should be possible to implement in most laboratories as they are further refinements and developments of previously established methods**
- **Intralaboratory (ongoing) - and interlaboratory (forthcoming) validation on NMs remain, but are planned allow establishment of a GD as part of OECD project 1.5 (currently no other similar use of the Sensor Dish Reader® System).**
- **Further demonstration of application of data in case studies and authentic use scenarios remains.**

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