Safe design of Engineered NanoParticles (ENP)

- work in progress -

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Introduction

- Institute for applied science - Netherlands
- Multidisciplinary (material science, toxicology,, computational chemistry, exposure/risk assessment, LCA)

- Project leader/researcher
- Occupational exposure and risk assessment
- Nanomaterials

SD

- Work on safe design of ENP:
  1. Development of general framework for safe design
  2. Case study on quantum dots


1. General framework for safe design of NP

Identify and describe relations between phys/chem properties of NP and:

a) Functionality

b) Toxicity

c) Exposure/release

With the purpose to design optimal NP for specific applications: minimal toxicity and exposure/release and maximal functionality


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a) Relation phys/chem properties of NP and *functionality*

- Goal: Identify phys/chem properties required for NP to be functional
- Method: literature and expert interviews
- Complicating factor:
  - The product matrix in which the NP is applied may require additional properties → not taken into account due to diversity of product matrix. To be further developed in relation to release.


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TNO innovation for life

Phys/chem properties required for ENP to be functional

Consequence of NP choice | Freedom to modify

Functionality	Chemistry	Prim. size	Shape	Crystallinity	Steric stabilisation	Polarity	Surface charge	Solubility in water
Fluorescence (QD)	Semi-conductors	<10	Sphere	Crystalline	Yes, covalent	Hydrophilic/ hydrofobic	?	No
Antimicrobial	Ag, Cu	10-50	Sphere	Metallic	Coordination	-	Neutral	Yes
UV blocking	ZnO	10-50	Sphere	Crystalline	No	-		No
	TiO_2 rutile	10-50	Sphere	Crystalline	No	-	Negative	No
	CeO_2	10-50	Sphere	Crystalline	Yes, ionic	Hydrophilic	Negative	No
Reinforcement	Clay	<10	Platelet	Crystalline	Yes, ionic	Hydrophobic	Negative	No
	SiO_2	<200	Sphere	Amorphous	Yes, covalent	Hydrophobic/ hydrophilic	Negative	No
	CNT	<10	Tube	Crystalline	Yes, covalent	Hydrophobic	Neutral	No
Scratch resistance	SiO_2	10-50	Sphere	Amorphous	Yes covalent	-	Negative	No
Conductivity	CNT	<10	Tube	Crystalline	No	Hydrophobic	Neutral	No
Thickening	SiO_2	<10	Sphere	-	No	-	-	-

Examples from literature

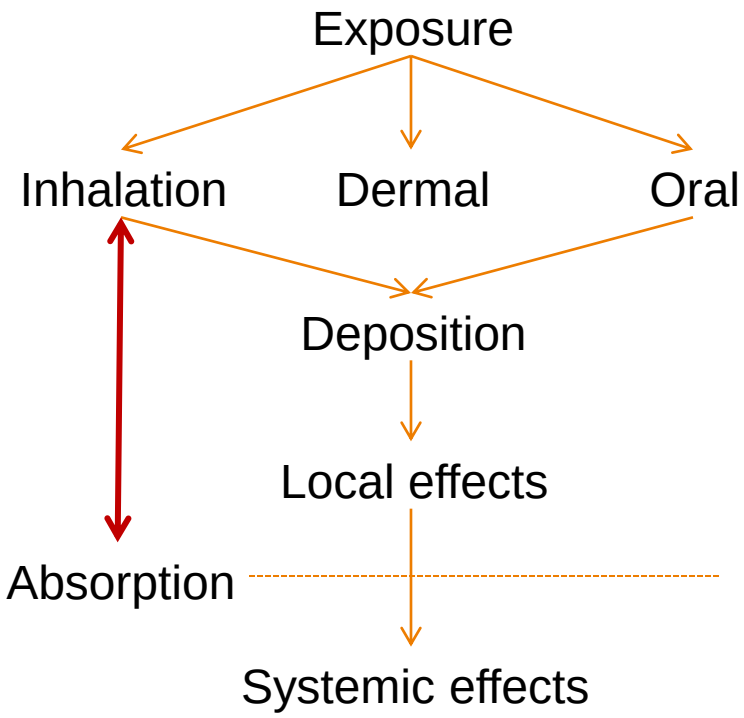

b) Relation phys/chem properties NP and *toxicity*

- Goal: Identify phys/chem factors associated with NP toxicity
- Method: literature and expert interviews
- Complicating factors:
  - NP may consist of core, coating, functionalization
  - Publications on toxicity tests often describe only core material
  - Transformation of NP after deposition in local matrix (like protein corona)
  - Probably transformed NP involved in membrane interaction, cell uptake and translocation

Fencing the effort

- Map the behaviour of NP after deposition and identify relations between phys/chem properties and single steps in the behaviour.

Prevention of absorption = exclusion of systemic effects



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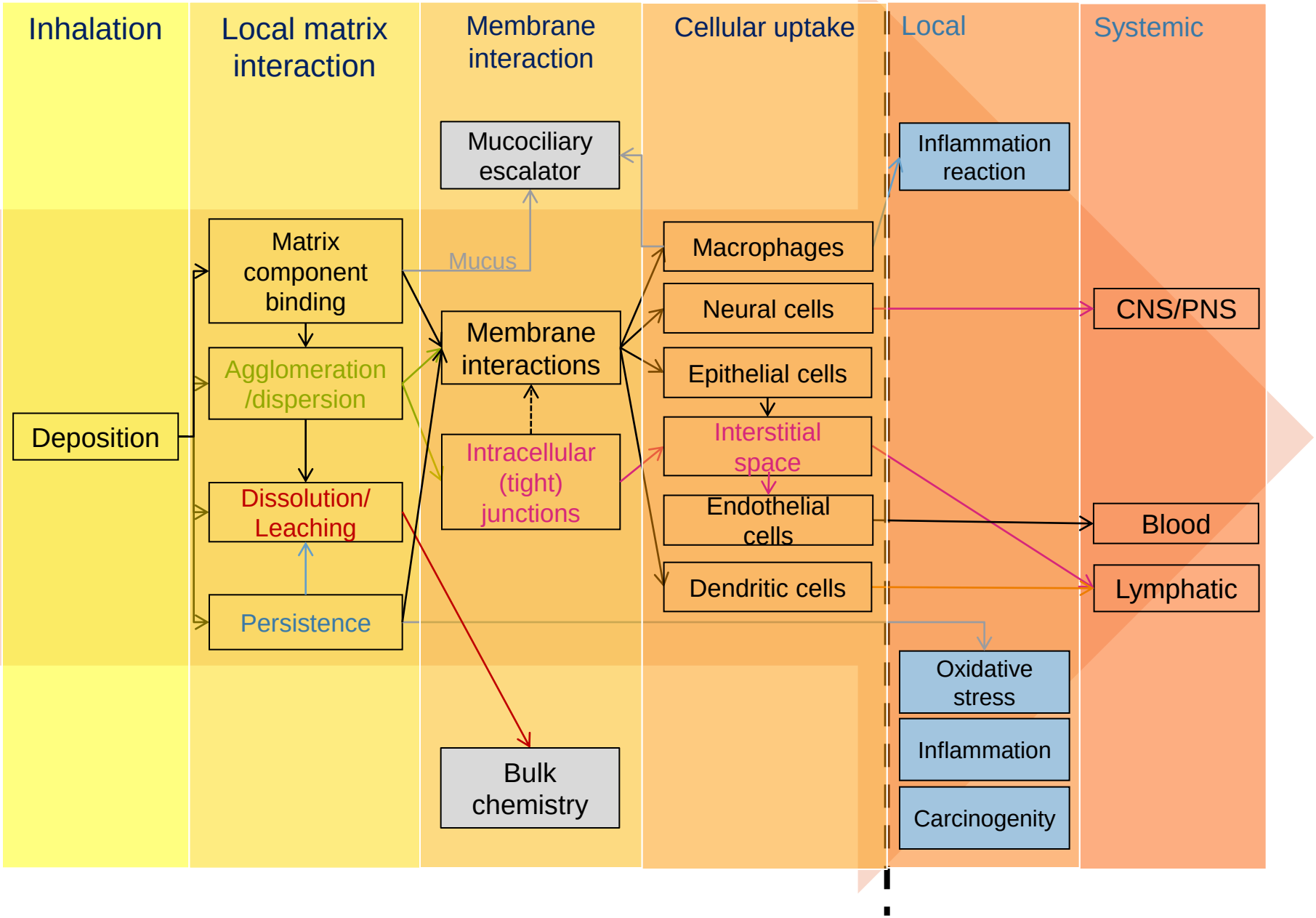


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Phys/chem properties influencing the behaviour of the ENP in the lung local matrix (examples)

ENP property	Matrix component binding	Agglomeration/ dispersion	Dissolution	Persistence	Functionality
Hydrophobicity ↑	+ protein				Polarity
Steric stabilition ↑	- protein	+ dispersion			Steric stabilisation
Particle size ↑		- agglomeration	- dissolution		Particle size
Particle number concentration ↑		+ agglomeration			
Absolute ζ-potential ↑		- agglomeration			Surface charge
Solubility components ↑			+ dissolution	- persistence	Water solubility


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On the agenda

- Membrane interaction and cell uptake
- Other exposure routes (oral, dermal)
- c) Exposure and release

2. Case study Quantum dots

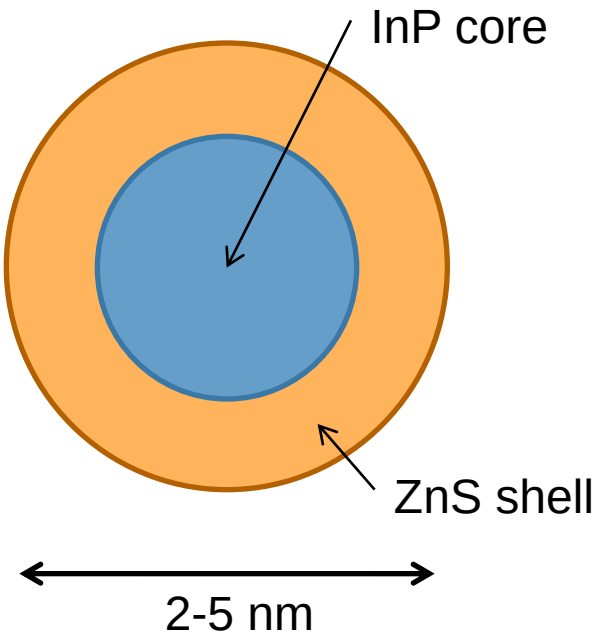
- Quantum dots are transition metal, that can absorb and emit light

- QDs are among others used for
  - Enlargement efficiency solar cells
  - Sensors (incl. biomedical)
  - LED lightning

Traditionally CdSe is used for many applications: other QD are explored nowadays

We use Indium Phosphide

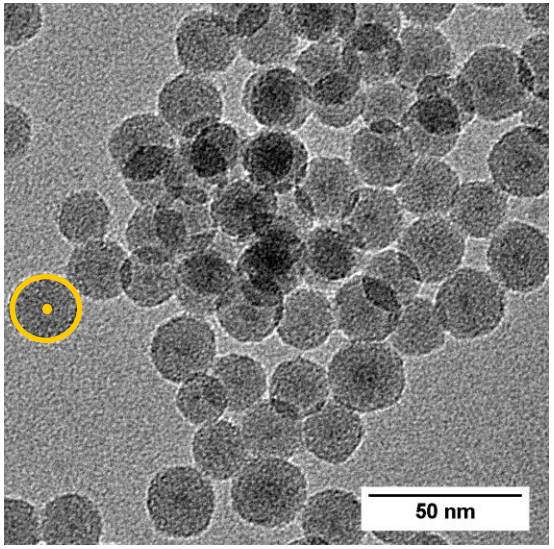
Indium phosphide



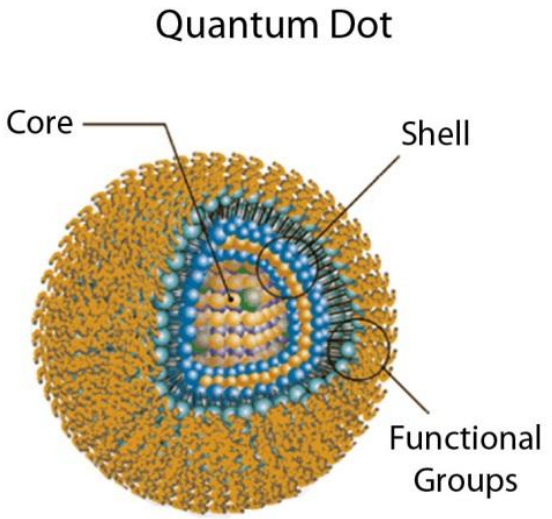
- InP/ZnS: ZnS equalizes the surface area to improve efficiency
- InP/ZnS may cause radical formation → associated with oxidative stress followed by inflammation;

- Analogue data from InP:
  - Causes **damage to lungs** through prolonged or repeated inhalation exposure → local effect associated with oxidative stress by **radical formation**
  - May cause **cancer** → local effect associated with local **cell uptake**
  - Suspected of **damaging fertility** or the unborn child →systemic effect associated with **ion-leakage**

Safe design solutions



- Encapsulation with silica
  - Reduction of radical formation potential
  - Reduction of Indium leaching
  - Size tuning



- Steric stabilisation with long PEG chains
  - Reduction of cell-uptake
  - Reduction of radical formation potential?

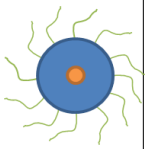
Luminescence of capped/functionalised QD

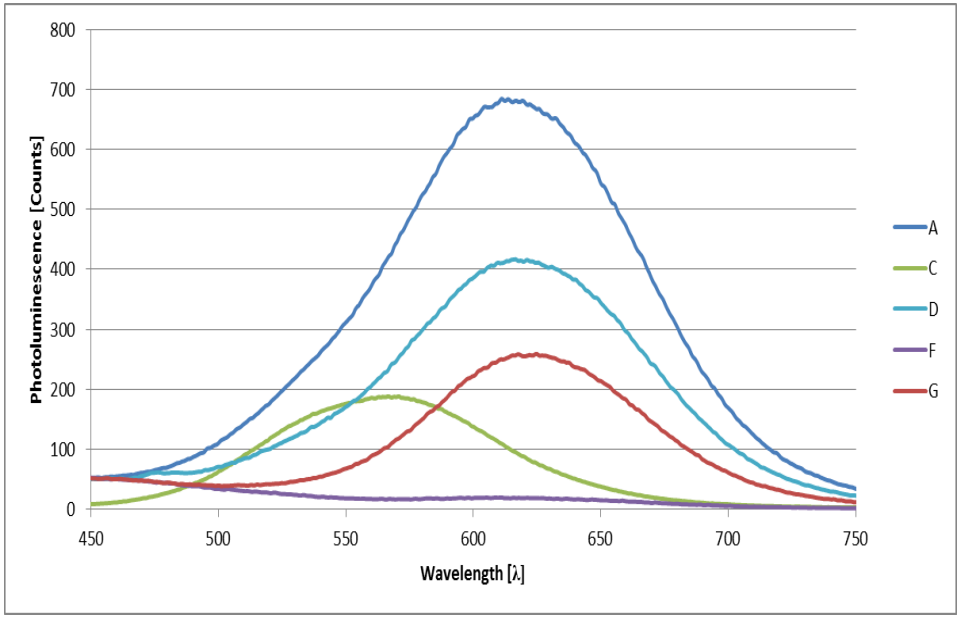
A  QD

D  PEG-5000 capped QD
SH group bonded to QD surface

G  PEG-2000 micelle capped QDs hydrophobic phospholipid end group adhered to QD

C  Silica coated QDs

F  PEG-2000 micelle capped silica-coated QDs



Obtained NON-reproduced results

Increasing cytotoxicity

Decreasing functionality

Theoretical explanation of lower toxicity of QD PEG-5000

- Damage to lungs (associated with radical formation potential):
  - Effect PEG chains on radical formation potential not clear; PEG chains could enlarge the diffusion layer, resulting in less ‘free’ radicals (speculation)
- Carcinogenic effects (require cell uptake):
  - Functionalization with PEG chains is known to prevent cell uptake.
  - Hydrophilic steric stabilisation reduces protein binding; leads to reduction in receptor mediated cell uptake
- Negative effect on fertility (associated with leakage):
  - Effect PEG chains on leakage not clear: could be similar as for radicals
  - Covalently bound PEG chains could reduce surface area

In vitro toxicity testing of QD-PEG 5000 (1)

- **RAW- 264.7 (macrophage cells)**

- Demonstration of:
  1. Reduced radical formation
  2. Reduced cell uptake
  3. Reduced leakage

1. Reduced radical formation

- Radical formation and oxidative stress tests are on the agenda
- Inflammation (TNF-α) is tested in RAW cells: no elevations compared to control

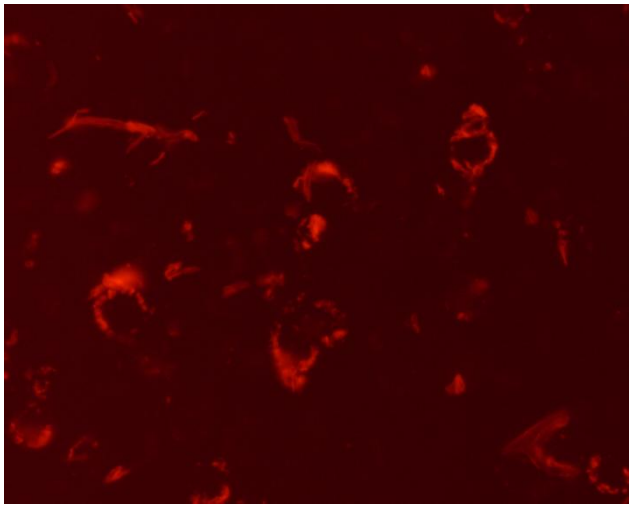
In vitro toxicity testing of QD-PEG 5000 (2)

- **RAW- 264.7 (macrophage cells)**

2. Reduced cell uptake

- Fluorescense microscopy: QD-PEG 5000 appears to be taken up by macrophage cells.

Fixated RAW cells; dead cells and free QD removed; QD concentration =125 µg/ml; 11% cell death; green exitation

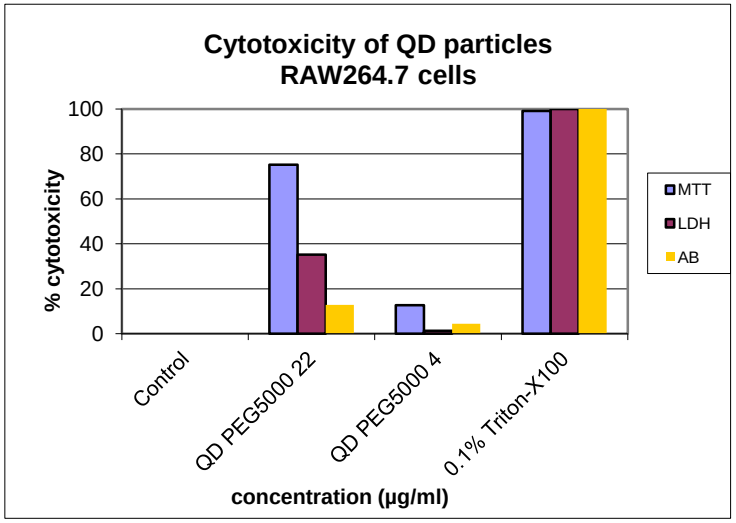


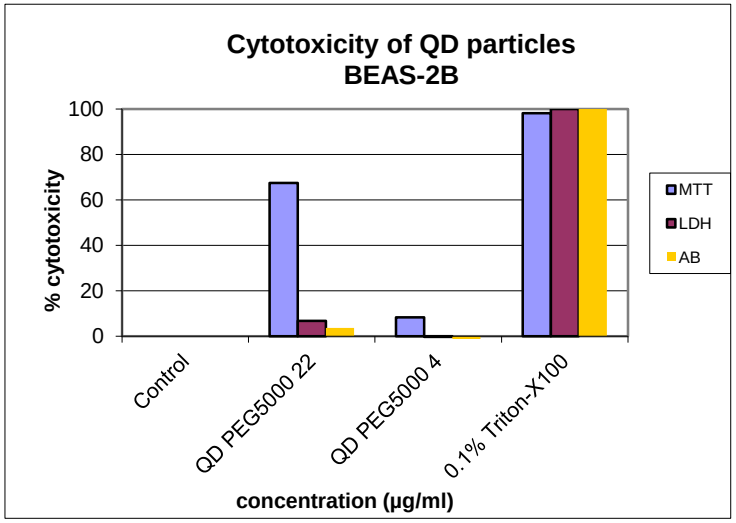
- EM must demonstrate real uptake or just attachment to membrane.
- Comparison with bare QD on the agenda

In vitro toxicity testing of QD-PEG 5000 (3)

- **RAW- 264.7 (macrophage cells)**

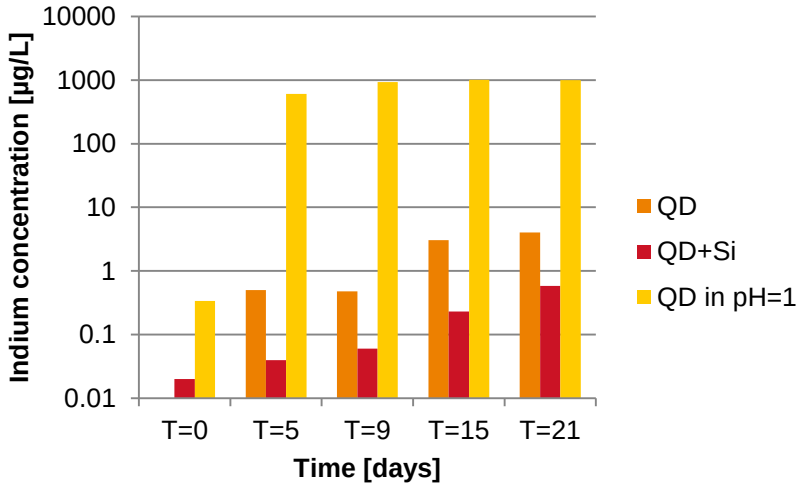
  - Comparison with lung epithelial cells on the agenda
  - Cytotoxicity is tested in RAW and epithelial cells → RAW cells appear to be slightly more sensitive





3. Reduced leakage

- Leakage is tested for QD, QD+Si and QD in acetous environment
  - Indium concentration: 1000 µg/ml
  - Acetous environment increases solubility of InP enormously
  - QD probably agglomerate (reduced surface area)
  - Si-shell is porous (still water contact)



- Leakage test for QD-PEG 5000 is on agenda


Advantages of general framework for safe design

- Understanding relation (phys/chem) properties of ENP and functionality, release and/or exposure and toxicity may facilitate:
  - Product improvement (less release means also more effective)
  - More targeted safe design approaches, less trial and error
  - Faster innovation processes
  - Development costs reduction
  - Safe design may lead to better image, more acceptance
- Potentially: arguments for grouping → reduction of regulatory burden (facilitates cross-reading)


Conclusion

- A general safe design framework can help to focus the safe design of ENP on the relevant phys/chem properties
- Only realisable in a multidisciplinary setting (at least material science, toxicology, toxicokinetics exposure assessment a.o.)
- Additional work on other exposure routes needs to be done
- New case studies are necessary to apply and improve the model

Thank you for your attention!