Interaction of nanomaterials with biological systems

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1. Risk assessment of nanomaterials
2. Exposure assessment
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4. Considerations for *in vitro* studies
5. Extrapolation to *in vivo*
Why do we study interactions of nanoparticles with biological systems?

- Rapid development of new nanomaterials
- Uncertainties on the safety of applying nanotechnology exist
- Possibilities for extrapolations from one nanoform to another unknown
- Traditional risk assessments: time, money and animal lives
- Currently: case by case approach
What do we want to predict?

- Do the nanoparticles we use pose a risk for human health?
- How do we determine this?
  - \textit{In vivo} assays with animals according to OECD guidelines
    “black box” but at this moment more relevant for hazard identification
    (=toxicity) in risk assessment
  - \textit{In silico} by comparison and extrapolation, (Q)SAR
    Do we have enough knowledge to do this for nanomaterials?
  - \textit{In vitro} assays by incubation of cells with nanoparticles
    Good for generating a lot of comparative data and studying mechanisms

Can we use \textit{in silico} and \textit{in vitro} assays to predict risks for human health?
RISK = EXPOSURE x HAZARD

Why should nanomaterials be any different compared to bulk materials of the same chemical composition?
Sources and routes of exposure

• Sources of exposure
  ○ Consumer products
  ○ Food
  ○ Medical applications
  ○ Work
  ○ Environment

• Route of exposure
  ○ Inhalation
  ○ Skin
  ○ Ingestion
  ○ Intravenous
Example of exposure source: Consumer products
Extent of exposure

• External exposure: Dispersion in direct environment
  ○ Longer retention of nanopowders vs. micropowders in air?
  ○ In surface water: retention of particles in dispersion when associated with organic matter, or sink and end up in sediment vs. soluble compounds

• Form of exposure in different environments during life cycle:
  ○ (Dis)aggregation
  ○ Dissolution
  ○ Association with other substances
  ○ HETEROGENEOUS!!

How AND WHEN to measure exposure ??
RISK = EXPOSURE x HAZARD
Internal dose: transfer across barriers

Size and surface charge of gold nanoparticles determine absorption across intestinal barriers and accumulation in secondary target organs after oral administration

CARSTEN SCHLEH, MANUELA SEMMLER-BEHNKE, JENS LIPKA, ALEXANDER WENK, STEPHANIE HIRN, MARTIN SCHÄFFLER, GÜNTER SCHMID, ULRICH SIMON, & WOLFGANG G. KREYLING

Surface modification and size dependence in particle translocation during early embryonic development

Furong Tian, Daniel Razansky, Giovani Gomez Estrada, Manuela Semmler-Behnke, Andrea Beyerle, Wolfgang Kreyling, Vasilis Ntziachristos, and Tobias Stoeger

Effects of surface modification on delivery efficiency of biodegradable nanoparticles across the blood–brain barrier

Sneha Avinash Kulkarni & Si-Shen Feng
Internal dose: Spherical gold nanoparticles

- Most in liver, spleen
- 10 nm particles more widespread

De Jong et al., Biomaterials, 2008
Internal dose: Gold nanorods

- PEG-Au most in spleen
- CTAB-Au most in liver

Lankveld et al., Nanomedicine, 2011
Internal dose: cell uptake

Figure 8 Size dependence of uptake after decoupling equilibria data from Figure 1B. Decoupling refers to the subtraction of equilibrium data from exocytosis data. This leads to a more accurate indicator of NP uptake concentration and rate. (A) Size dependence on uptake process. (B) Kinetics of the uptake process for HeLa cells. (C) Table consists of HeLa cell uptake and removal half-life corresponding to transferrin-coated Au NPs with sizes 14, 50, and 74 nm.

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RISK = EXPOSURE x HAZARD

Exposure assessment

Internal dose

Hazard characterisation

Hazard identification

Dose response assessment

Risk characterisation

Dose Response Curve
Hazardous properties of nanomaterials

The properties of matter at the nano-scale can change dramatically from those at micro- or macro-scales
- Electrical conductivity
- Elasticity
- Increased strength
- Different light absorbance
- Greater reactivity

How does this affect the hazardous properties of the materials?
Which mechanisms may play a role?

- Oxidative stress paradigm
- Steric hinder
- Trojan Horse effects
- Other pathways?

Oxidative stress

Skin
  - Dermatitis
  - Psoriasis

Reproductive organs
  - Sperm immotility
  - Infertility

Joints
  - Rheumatoid Arthritis

Lung
  - Asthma

Brain
  - Parkinson
  - Alzheimer

Vessels
  - Atherosclerosis

Liver
  - Liver necrosis

Multi organs
  - DNA damage
  - Aging
Selection of most relevant endpoints

- Oxidative stress
- Cytotoxicity
- Genotoxicity
- Immunotoxicity
- Hepatotoxicity
- Reproductive toxicity
- Carcinogenicity

Inflammation
Selection of read out system and incubation time

Cytotoxicity:
- Count cells
- Measure DNA
- Cell proliferation
- Metabolic activity
- Membrane integrity
Selection of cell type

- Many exposure routes -> many exposed cell types
- In vivo studies: nanoparticles are mainly taken up by the mononuclear phagocyte system which includes macrophages in liver, lung, spleen and bone marrow
- Choose most exposed, or most sensitive?
- Do cell lines from organs predict organ specific toxicity?
- In general: widely varying response between cell types (Park et al., Nanomedicine, 2009)
Potential assay artifacts

• Interference in assay
  ◦ Spectrophotometer, scattering or absorbance of (fluorescence) signal used in various assays
• Chemical interactions
  ◦ Interaction with assay substrates due to chemical properties of nanomaterials
• Depletion of cell culture medium
  ◦ Adsorbance of tissue culture components (nutrients, growth factors) due to adsorptive properties of nanomaterials (Casey et al., Tox Lett 2008)
• Inability of nanomaterials to enter prokaryotic cells (eg Ames test)
Potential artifacts

- Presence of contaminants:
  - Unknown surfactants
  - Production residues
  - Bacterial endotoxins

Black: NR as prepared using surfactant CTAB (cetyl trimethylamonium bromide, cetrimonium bromide)
Red: NR as prepared but filtered
Green: CTAB removed, NR coated with polyethylene glycol
Blue: CTAB removed, NR coated with polystyrene sulfonate

Gopal Rayavarapu and Petersen, University Twente, The Netherlands
What do you need to address in an assay?

Use appropriate controls, eg. for cytotoxicity with WST:

**Incubation Purpose**

- **Medium+cells+NP** Evaluation of test samples (toxic?)
- Medium+cells Negative control: normal level of cell growth
- Medium+cells+dispersant Toxicity of dispersant
- Medium+NP+WST Background: interaction NP and WST read out system
- Medium+formazan+NP Needed to investigate interaction NP with formazan
- Medium+cells+DMSO Positive cytotoxic control
- Medium+cells+? Positive control for specific assays (cytokine induction)
Dispersion methods

• Factors affecting nanoparticle dispersions (Murdock et al., Tox Sci, 2008):
  ○ Dispersion medium (eg. Ionic strength, with or without serum)
  ○ Ultrasound energy
  ○ Use of dispersing agents
  ○ Sequence of dispersion preparation steps
  ○ Time…

• Very few reports on effects of differently dispersed nanoparticles on *in vitro* toxicity outcomes
RISK = EXPOSURE x HAZARD

Exposure assessment

Internal dose

Hazard characterisation

Hazard identification

Dose response assessment

Risk characterisation

Dose Response Curve
Toxicity is determined by DOSE

- Water is non toxic
  - But high volumes in a short period results in organ edema, including in the brain, requiring hospitalization
- *In vitro* rather high doses can be obtained which may be irrelevant for the *in vivo* situation
  - Even when something is toxic *in vitro* this may not be so *in vivo*
- Paracelsus (1493 – 1541)
  - “Alle Ding sind Gift, und nichts ohn Gift; allein die Dosis macht, daß ein Ding kein Gift ist“.
  - "All things are poison and nothing is without poison, only the dose permits something not to be poisonous“
Dose response assessment

• Use dose-response modelling (eg. Proast) for better estimation of toxic concentrations

• Express dose in mass, surface area, number of particles?
• Nominal concentration versus dose at the cell... (Hinderliter et al., Part Fibre Toxicol 2010)
• Most responses seen at concentrations >> 1 μg/ml?
Incubation time: effect on cytotoxicity outcome

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) guidance documents for cytotoxicity studies (2001):
“…chemical exposure should last at least one full cell cycle.”

Cytotoxicity studies with nanoparticles:
- ranging from 1 hour to 6 days
- Up to 96 hours: cytotoxicity generally increases

HABER’s rule: Toxicity = dose x time
- BUT: sometimes recovery after exposure >48 hours
RISK = EXPOSURE x HAZARD
### What nanomaterial characteristics may determine *in vitro* toxicity?

<table>
<thead>
<tr>
<th>Type of nanoparticle</th>
<th>Factor(s) determining toxicity</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low solubility low toxicity</td>
<td>Surface area</td>
<td>SiO2, polystyrene</td>
</tr>
<tr>
<td>Low solubility high reactivity</td>
<td>Surface area and reactivity</td>
<td>Quartz, CdSe quantum dots</td>
</tr>
<tr>
<td>(Relatively) high solubility</td>
<td>Ion release rate</td>
<td>Ag, ZnO</td>
</tr>
<tr>
<td>All types</td>
<td>Structural morphology</td>
<td>Carbon black vs. carbon nanotubes Anatase vs. rutile TiO2</td>
</tr>
</tbody>
</table>

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**Trend:** It all happens at the surface!!
Importance of nanomaterial characterisation

- Quartz particles induce oxidative stress and cytotoxicity, but not when coated with aluminum lactate (Duffin et al., Inhal Toxicol 2007)
- Silica nanoparticles induce cytotoxicity, but not when coated with the surfactant Pluronic 127 (Dutta et al., Toxicol Sci 2007)
- Presence of surfactants on commercial nanomaterials not always reported (Murdock et al., Toxicol Sci 2008)
Minimum particle characterisation

- CONCENTRATION and Purity
- Crystal structure
- Surface coating
- Nanomaterial preparation methods
- Presence of contaminants/endotoxins
- Hydrodynamic size DISTRIBUTION and ‘state’ of aggregation
- Preferably in relevant dispersant!

Park et al., Tox Appl Pharmacol 2009
Considerations for extrapolation to *in vivo*

- Relevant concentrations:
  Worst case: 1 µg/ml *in vitro* / extracellular fluid *in vivo* requires exposure to 5 mg/person, but probably more:
  - The absorption of particles across barriers is not 100%
  - Particles bind to proteins, are taken up rapidly by macrophages
- Distribution to various organs
- Interaction between cell types, eg. in inflammation reaction
- Nanomaterials may aggregate in different dispersion media (cell culture medium versus blood/lung surfactant)
- Nanomaterials absorb different proteins *in vitro* than *in vivo*... resulting in different effects?
QNTR considerations

- Priority list of nanomaterials based on anticipated/modelled exposure levels?
- Define expectations for QNTR: full toxicity profile or screening?
- If we decide to generate a lot of data *in vitro*
  - Define relevant test systems
  - Avoid artefacts
  - Report data in harmonized database
  - Think about how to extrapolate to *in vivo*
- Most particles end up in lysosomes of cells – what does that mean for the differences in their toxicity?
- Most effects studied to date are short term – for persistent nanomaterials long term effects may be relevant
- Most likely toxicity is determined by a COMBINATION of INTRINSIC properties that are interlinked with DYNAMIC EXTRINSIC properties that depend on direct environment (temperature, ionic strength, etc.)

**CAN WE CAPTURE THIS IN A QNTR? AND IS IT WORTH IT….**
References

The status of *in vitro* toxicity studies in the risk assessment of nanomaterials

Park, Lankveld, van Loveren & de Jong

References (continued)

Thank You!