How to assess potential hazardous properties of nanomaterials: an example of TiO$_2$

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Why a concern?

Unique properties

Positive and desirable

Negative and undesirable

Risks?

- greater production → greater exposure → potential release into the environment → increased concern
- our knowledge on their potential adverse effects on human and environmental health remains very limited
Sources of NPs

- Natural
  - Vulcanic ash
  - Erosion
  - Seaspray
  - Forest fires

- Man-made
  - Un-deliberately made
    - Welding
    - Construction works
    - Diesel exhaust
    - Fires/candles...
  - Deliberately made
    - Cosmetics
    - Food
    - Detergents
    - Textile materials (protective, water-proof)
    - Medical applications...

1 cm$^3$ of urban air: $1\text{--}5\times10^4$ NPs
Welding: $4\times10^6$ NPs
Smoker’s breath: $>1\times10^8$ NPs
Risk = f(hazard, exposure)
The pillars of risk assessment

HAZARD IDENTIFICATION and CHARACTERISATION
ADME, acute, subchronic & chronic toxicity
genotox, reprotox, immunotox, human studies,
mode/mechanism of action,
Dose-response assessment, NOAEL,
Limit values, mathematical modelling (BMD)

EXPOSURE ASSESSMENT
Sources of exposure,
levels of exposure,
groups with higher exposures,
time trends...

RISK CHARACTERISATION
Comparison of limit values and exposure
data: public health relevance

Vulnerable groups (children –
high level exposure)
Hazard identification

- Physical-chemical characterization
- Toxicokinetics (absorption, distribution, metabolism, elimination)
- Toxicodinamic (toxic effects)
Physical-chemical characterization

Determinants of NPs toxicity

- Particle size (small particles are absorbed and distributed differently than large)
- Surface area (NPs have high surface area to volume ratio, which can make them very reactive or catalytic)
- Surface chemistry (i.e. hydrophilic or hydrophobic, lipophilic or lipophobic, and catalytically active or passive),
- Charge (i.e. interaction of electron donor or acceptor active sites with $O_2$ : electron capture can lead to the formation of the superoxide radical, which through dismutation or Fenton chemistry can generate additional ROS)
- Crystallinity (i.e. anatase, rutile and amorphous TiO$_2$),
- Shape (i.e. some NPs have a fibre type shape $\rightarrow$ fibre-like toxicity: asbestos and CNTs)
- Agglomeration state (Tendency of NPs to agglomerate!!!)
- Solubility

- Depending on these properties NPs can show different cellular uptake, subcellular localization, and ability to catalyze the production of reactive oxygen species (ROS)

- Effect on external exposure: smaller particles in the air $\rightarrow$ longer time of settlement $\rightarrow$ increased inhalation exposure
How the surface area of a nano-object creates a greater biological response

“The same mass of 20 nm TiO$_2$ induced a much greater pulmonary-inflammatory neutrophil response than did TiO$_2$ (250 nm) when both types of particles were instilled at the same mass dose...”

“However, when dose was expressed as particle surface area, the inflammatory response for ultrafine and fine TiO$_2$ fitted the same dose–response curve.
Toxicokinetics

Toxicokinetics of nanoparticles ≠ toxicokinetics of bigger particles

ADME (Absorption, Distribution, Metabolism, Elimination)

• Inhalation exposure
  – Deposit in all regions of the respiratory tract
  – Can escape from specific defence mechanisms
  – May translocate out of the respiratory tract (endocytosis or transcytosis) and distribute to distant organs

• Dermal exposure
  – May penetrate through the skin
  – May be translocated via local lymphatic system to lymph nodes

• Oral exposure
  – Major part probably excreted by faeces
  – Can translocate from intestinal lumen via intestinal lymphatic tissue (Peyers patch)
  – May enter sub-mucosal tissue and enter blood stream
  – Captured by the liver, spleen, bone marrow, heart, etc…
Toxicodinamic

• The nano-structured materials can cause adverse effects per se, but beyond that, the interaction of these materials with cells and subcellular structures is different from larger particles.
• Depends multiple parameters: size, shape, chemistry, crystalline state, surface properties (area, porosity, charge, surface modifications, coating), agglomeration, bio-persistence and dose.
• These parameters modify the response and cellular interactions, leading to e.g.
  – greater inflammatory potential than larger particles / per mass,
  – acute lung injury,
  – induction of oxidative stress,
  – pro and antioxidant activity in the relevant kind to the environment,
  – binding proteins and receptors and
  – intra-nuclear, intra-mitochondrial location….
Routes of exposure, translocation to organs and potential health effects of NPs

Proposed mechanisms of NP-induced cytotoxicity.

Proposed mechanisms of NP-induced cytotoxicity.

• Common property – induction of Reactive Oxygen Species (ROS).

• Consequences of oxidative stress: Lipid peroxidation, damage to Cellular membranes, DNA, RNA, Protein damage

• Blocking of ion channels, pore formation, physical damage.

• Several types of NPs are genotoxic: i.e. Fr/pt, Co/Cr, ZnO, TiO2, black carbon, carbon nanotubes...
Titanium dioxide (TiO$_2$)

- >96% titania is used in this form
- Main advantages: insoluble, chemical and thermal stability, inflammable, low price...
- Several crystalline forms:

- TiO$_2$ is considered as biologically inert, at least fine sized (>100 nm)
- The use of fine sized is more and more replaced by nanosized TiO$_2$ (<100 nm) and new applications are being developed.
In vitro studies TiO$_2$

Different cell lines, different sizes and crystalline structures of TiO$_2$ particles:

- Low cytotoxicity
- Oxidative stress
- Genotoxic effects: DNA damage, chromosomal aberrations
- Apoptosis

- The effects are size and crystalline structure dependent: anatase more active than rutile,
- Photoactivated TiO$_2$ is more toxic and genotoxic.
Our studies:

<table>
<thead>
<tr>
<th></th>
<th>Kristalna struktura (XRD)</th>
<th>Velikost delcev (FEG-SEM)</th>
<th>Oblika delcev (FEG-SEM)</th>
<th>Specifična površina (BET)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TiO₂-An</strong></td>
<td>Anataz</td>
<td>&lt;25 nm</td>
<td>okrogla</td>
<td>129.3 m²/g</td>
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<td><strong>TiO₂-Ru</strong></td>
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<td>&lt;100 nm</td>
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<td><strong>TiO₂-B</strong></td>
<td>Anataz</td>
<td>&gt;100 nm</td>
<td>okrogla</td>
<td>8.6 m²/g</td>
</tr>
</tbody>
</table>

50 000 x povečava 25 000 x povečava (FEG-SEM)

Oddelek za nanostrukturne materiale, IJS, Ljubljana
Nano TiO$_2$ : 25 nm anatase vs. 10x40 nm rutile

Particles aggregated and agglomerated: sizes in the range of 1000 nm.

In HepG2 cells anatase TiO$_2$ NP induced more ROS than rutile.

In HepG2 cells anatase TiO$_2$ NP induce more oxidative DNA damage than rutile.

In Hepg2 cells rutile TiO$_2$ NP induce higher upregulation of DNA damage responsive genes than anatase.
TiO$_2$ NP genotoxicity is oxidative stress mediated

Dose-dependent increase in the level of intracellular GSH content

In GSH depleted cells anatase TiO$_2$, but not rutile, induced DNA damage.

TiO$_2$ NPs cause activation of cellular antioxidant processes, with intracellular GSH playing a critical role in defense against TiO$_2$ NPs-induced DNA damage.
The content of GSH as well as the extent of DNA damage were significantly higher in TiO$_2$-An than in TiO$_2$-Ru exposed cells, while expressions were higher in TiO$_2$-Ru exposed cells - anatase and rutile crystalline type of TiO$_2$ NPs exhibit different toxicity mechanism.
Pulmonary toxicity of TiO$_2$

- TiO$_2$ particles deposit in lung (clearance of 20 nm slower than 250 nm)
- Inflammation and tissue damage $\rightarrow$ lung tumors
- Exposure to nano TiO$_2$ represents higher risk than exposure to fine TiO$_2$
- Nano TiO$_2$ can translocate to distant organs including central nervous system $\rightarrow$ systemic effects
- Immunologic response $\rightarrow$ increased risk of allergy
- Up regulation of lung neutrophins $\rightarrow$ increased risk of asthma
- Cardiovascular effects (microvessel dysfunction)
TiO$_2$ classified as carcinogen

- International Agency for Research on Cancer (IARC, 2006): TiO$_2$ is classified by the as an Group 2B carcinogen, "possibly carcinogenic to humans" - on inhalation exposure.

- National Institute for Occupational Safety and Health NIOSH recommended exposure limits:
  - 2.4 mg/m$^3$ for fine TiO$_2$ and
  - 0.3 mg/m$^3$ for ultrafine (including engineered nanoscale) TiO$_2$, as time-weighted average (TWA) concentrations for up to 10 hours per day during a 40-hour work week.

- Ultrafine TiO$_2$ is a potential occupational carcinogen but there are insufficient data at this time to classify also fine TiO$_2$ as a potential occupational carcinogen.

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Oral exposure TiO$_2$

TiO$_2$ is approved food additive (E171) – size and crystalline form are not defined.

- No acute toxicity even at very high dose 5 g/kg BW
- From gastrointestinal tract TiO$_2$ nano particles are distributed over the body and retained in liver, spleen, kidney, and lung.
- Experimental evidence that after oral exposure TiO$_2$ affects immune system, and may lead to allergy and asthma.
- Oral exposure of mice to TiO$_2$ NPs through drinking water induced clastogenicity, DNA strand breaks, oxidative DNA damage, and inflammation
  $\rightarrow$ Gentoxic *in vivo* after oral exposure?

- Should we limit ingestion of TiO$_2$ nanoparticles through nonessential drug additives, food colours, etc.? 

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Titanium Dioxide Nanoparticles Induce DNA Damage and Genetic Instability *in vivo* in Mice

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Dermal exposure to TiO$_2$

TiO$_2$ powder used in sunscreen preparations is predominantly but not exclusively rutile that is less photoreactive. Nano forms are preferred as they are transparent.

- The majority of studies indicated that nano-TiO$_2$ particles do not penetrate stratum corneum of healthy skin.
- No clear conclusion if nanopTiO$_2$ can penetrate damaged or burned skin, what happens after long term and repeated exposure.
- *In vitro* studies with skin models showed that nano-TiO$_2$ particles are up taken by keratinocites, fibroblasts, melanocites.
- Different toxic effects were observed including cell death via apoptosis, intracellular formation of reactive oxygen species, adverse effects on cellular matrix, genotoxic effects and immune responses.
How nano-TiO$_2$ behaves in the presence of UV?

- Numerous *in vitro* studies showed that in the presence of UV TiO$_2$ (nano and submicron sized) induce more pronounced adverse effects: cytotoxicity, oxidative stress, genotoxicity.
- UV irradiated anatase and anatase + rutile were more toxic than rutile.
- In all the studies so far only concurrent exposure to TiO$_2$ and UV was explored and in one the cells were pre-exposed to UV and then exposed to nano TiO$_2$.
- In all studies only short therm exposures were applied.
- What happens if cells are exposed to pre irradiated TiO$_2$— are the properties and toxicity of pre irradiated TiO$_2$ different from non UV irradiated?
After UV pre-irradiation nanosized and submicron sized anatase TiO$_2$ become cytotoxic

- Nanosized (TiO$_2$-A) and submicron (TiO$_2$-B) were pre-irradiated for 24 h in UV-chamber simulated sun spectrum ($\lambda > 300$ nm). The HepG2 cells were exposed about 1 h later.

Nano: 18 nm

Submicron: 105 nm
After UV pre-irradiation nanosized and submicron sized anatase TiO₂ induce more oxidative DNA damage.

Nano: 18 nm

Submicron: 105 nm
Implications of these findings

• Our data change known facts about TiO$_2$ NPs toxicity and about »inertness« of larger TiO$_2$ particles.
• If they penetrate the skin the hazard of the use as sunscreen may be even higher as currently assumed.
• What happens with other applications, i.e. in the environment?
  – Possibilities of applications of TiO$_2$ photocatalytic properties are enormous → exposure of biological systems including humans to photocatalitically activated TiO$_2$ is highly probable.
What we know

• Nanomaterials are from toxicological point of view different from their larger counterpart and should be considered as new material.

• Nanomaterials are not uniform, thus safety assessment is required for each separately (i.e. soluble and biodegradable vs. insoluble or biopersistent, immobilized vs. free).

• The approaches for toxicity testing and risk assessment are the same as for the chemicals with some specific adaptations (i.e. metric for dose response, appropriate exposure conditions that would reflect real exposure…).

• For hazard evaluation most of the strategies propose:
  – Use of both in vivo and in vitro approaches
  – Need comprehensive physical/chemical characterizations
What we don’t know

• For many the nanomaterials that are already in use we do not have enough information to allow for the risk assessment.

• Many materials have been tested *in vitro*, however very often toxicokinetic data are missing, which prevents any conclusion on the potential risks.

• Information on human exposure is missing in particular for oral and dermal exposure.

• No information on susceptible individuals or population (i.e. children, elderly..)
Nanoparticle Risk
f(hazard, exposure)

- Embedded Nanostructures
  - (i.e. Nanoscaled tin-bismuth alloys embedded in aluminum matrix)
- Fixed on a matrix
  - (i.e. thin films on silica)
- Suspended in Liquids
  - (i.e. nanotubes in water)
- Dry dispersible, agglomerates or aggregates
  - (i.e. TiO$_2$, SWN… powders)
Risk/benefit

Benefit may justify the use despite unknown risk:

- Medical applications: i.e. cancer treatment, diagnostic tools, implants…
- Exposed populations are small and under medical control.

No such benefit to justify the unknown risk:

- In food industry: use of nano-additives just for “cosmetic” purposes for instance for food colouring.
- Use of nano TiO₂ sunscreens to prevent skin cancer is a manipulation. Safer and more effective is avoiding sun exposure.
- Exposed populations are big including potentially more susceptible individuals and groups (i.e. children).
Take home message

• Scientific basis of nanotoxicology and epidemiology (exposure assessment and risk evaluation) are lagging behind the fast development and application of new nanomaterials
  – Inherently slower: Long-term effects subject to long latency periods
• Bridging this gap will require new approaches for evaluating risk and making decisions in the face of potential risks where there is incomplete information on exposure, hazard, and response.
• Production could outpace protections.
  – “The possibilities are enormous but we know barely anything about the risks. …When the economic expectations are big, one tends to ignore the health risks,” Kai Savolainen (2009):
• Limited available science should not deter effective safety measures.
  – Application of precautionary principle; Conservative approach
• Interdisciplinary collaboration and better recognition of safety issues by industry is essential for achieving these goals.
“Guilty” or “Not Guilty”

Circumstantial evidence

We find him “not guilty”...but hey...we could be WRONG!
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April 6, 2011 Jana just after defending her PhD thesis:
Mechanisms of toxic and genotoxic activity of TiO2 nanoparticles.