Safety Assessment of Nanomaterials – BASF as an example of the chemical industry

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BASF Corporation
The Code of Conduct is a voluntary commitment to responsible action based on our Values & Principles:

- protection of employees, customers and business partners
- protection of the environment
- participation in safety research
- commitment to open communication and dialogue

The Code of Conduct is binding for all employees. It is published on the internet: www.basf.de/dialogue-nanotechnology
Other approaches

- Cefic published a position paper in 2006 on implementation of nanotechnology that included principles for occupational safety, consumer safety, and dialogue with the public.

- ED and DuPont collaborated in 2007 on a product stewardship and self-assessment process that included hazard evaluation.
ED-DuPont strategy

- Mammalian toxicity:
  - 28-day inhalation study with histopathology and 90-day recovery
  - Single-dose instillation study with 90-day recovery
  - Dermal sensitization/irritation
  - Skin penetration
  - Genetic toxicity

- Environmental toxicity
  - Acute aquatic toxicity to fish, daphnia, algae
  - Terrestrial toxicity as needed.
Alternate testing strategy

- Nanotechnology Panel of American Chemistry Council proposed similar testing strategy in 2006 using tiered testing, good characterization, and appropriate positive and negative controls. Each test has a description of the information need and rationale.

**Mammalian Tests**
- Eye and Skin Irritation
- Dermal Penetration
- Acute inhalation toxicity
- Repeat-dose toxicity
- *In vitro* mammalian gene mutation and cytogenetics

**Ecotox Tests**
- Acute toxicity to aquatic species
- Biodegradation
- Activated sludge respiration inhibition if release to POTW

- Similar testing strategies have been proposed by the European Centre for EcoToxicology and Toxicology of Chemicals (ECETOC) and the German Chemical Industry association (VCI).
## ACC Testing Strategy

<table>
<thead>
<tr>
<th>Test</th>
<th>Information needs</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye and Skin Irritation</td>
<td>This may be most applicable for dispersed or dry nanoscale materials.</td>
<td><em>In-vitro</em> methods should be considered; however, current experience is that some <em>in-vitro</em> screens are not appropriate for the water insoluble particles.</td>
</tr>
<tr>
<td>Dermal Penetration</td>
<td>If the exposure route indicates a potential for dermal contact or if the nanoscale material is dispersed in a liquid.</td>
<td>If exposure routes indicate that skin contact is likely it may be preferable to determine the skin penetration or absorption.</td>
</tr>
<tr>
<td>Acute inhalation</td>
<td>This may be most applicable for dry nanoscale materials or if inhalation exposure is a high potential. Lethality should not be the only endpoint considered, although lethality may be necessary for hazard and transportation classification. Additional endpoints such as bronchoalveolar lavage and histopathology should be considered, but will require modifications to standard acute toxicity testing protocols (e.g. appropriate control group(s))</td>
<td>When a toxicity evaluation of nanoscale particles delivered to the respiratory tract is to be made, a controlled test atmosphere would ideally be used that mimics the characteristics of the potential exposure. Creating high aerosol concentrations of discrete nanoscale particles in the laboratory or the work environment may not be possible due to issues with generation and/or coagulation.</td>
</tr>
</tbody>
</table>
BASF approach to testing

- Testing scheme based on a combination of appropriate tests and realistic approaches.

- Assessment of potential toxicity / hazard
  - Inhalation (i.e. short-term inhalation test)
  - Dermal penetration
  - Genotoxicity
  - Aquatic toxicity
  - Interpolation using “family approach”

- All include characterization of the neat particle and in the medium.
## Nanomaterials in the Body

**Nanomaterial**
- Powder
- Embedded in matrix or on surfaces

### Dispersion
- Aerosol
- Suspension

### Uptake in the body
- Deposition in the lung, Alveolar, intestinal, dermal penetration

### Modification in the body
- Surface coating changes
- Agglomeration, desagglomeration

### Distribution in the body
- Penetration of biological barriers
- Tissue distribution, Intracellular distribution

### Primary Effect
- Inflammation
- Catalysing formation of reactive compounds
- Direct interaction with cellular structures

### Toxic Effect
- Organ toxicity,
- Genotoxicity

From Schulze *et al.*, 2009
Research Areas in Toxicology and Ecology

- **Skin**: Penetration of nanomaterials through skin
- **Lung**: Uptake and effects of nanomaterials in the lung
- **Biokinetics**: Distribution and excretion
- **Genotoxicity**: Interaction of nanomaterials with DNA
- **Environment**: Distribution and effects of nanomaterials in (aquatic) environment
- **Test methods**: Which test systems are suitable for (regulatory) testing of nanomaterials?
Dermal absorption

*in vitro* dermal penetration study with nano ZnO and TiO$_2$ on pig skin

EM micrograph of particle in formulation
Gamer et al. (2006) reported that

- nearly all the ZnO was recovered in the top 5 tape-stripped layers associated with stratum corneum of pig skin. A small amount was associated with hair follicles.
- Most TiO$_2$ was washed off the surface.

Similar results reported by Cross et al. (2007) for permeation of ZnO through human skin over 24 hrs. Electron microscopy showed that all the Zn was associated with the stratum corneum.

Similar results were reported by Lademann et al. (1999) and Pflücker et al. (2001) for localization of TiO$_2$ in human skin.
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Inhalation Exposure
Aerosol Generation

- Head/Nose only
- Brush-Generator, cyclone separator used to focus on small particles
- Analysis of concentrations
- Particle size measurement (Impactor, OPC (optical particle counter) and SMPS (scanning mobility particle sizer))
# Inhalation Exposure Study Design

- **Male Wistar rats**

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9 - 20</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td></td>
<td></td>
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<td></td>
<td>R</td>
<td></td>
<td></td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>E</td>
<td></td>
<td></td>
<td>E + e</td>
<td>e</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>E + e</td>
</tr>
</tbody>
</table>

X: Head-nose exposure to aerosols for 6 hours per day on 5 consecutive days
R: Recovery period
E: Evaluations:
- Organ burden (lung, mediastinal lymph nodes, liver, kidney, spleen and basal brain with olfactory bulb)
- Particle size distribution within the lung
- Histology of selected organs, cell proliferation / apoptosis

e: Cytological and biochemical parameters in the bronchoalveolar lavage fluid
# Inhalation Exposure
## Organ Burden

<table>
<thead>
<tr>
<th>Target concentration (mg/m³)</th>
<th>lung burden day 5</th>
<th>lung burden day 21/29</th>
<th>lymph nodes day 5</th>
<th>lymph nodes day 21/29</th>
<th>other organs* day 5</th>
<th>other organs* day 21/29</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TiO₂</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>118</td>
<td>93</td>
<td>&lt; 0.5 **</td>
<td>&lt; 0.5 **</td>
<td>&lt; 0.5 **</td>
<td>&lt; 0.5 **</td>
</tr>
<tr>
<td>10</td>
<td>545</td>
<td>400</td>
<td>&lt; 0.5 **</td>
<td>0.6</td>
<td>&lt; 0.5 **</td>
<td>&lt; 0.5 **</td>
</tr>
<tr>
<td>50</td>
<td>1635</td>
<td>1340</td>
<td>2.1</td>
<td>11</td>
<td>&lt; 0.5 **</td>
<td>&lt; 0.5 **</td>
</tr>
<tr>
<td><strong>ZnO</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>349</td>
<td>25</td>
<td>0.34</td>
<td>0.34</td>
<td>409.1</td>
<td>503</td>
</tr>
<tr>
<td>2.5</td>
<td>123</td>
<td>26</td>
<td>0.47</td>
<td>0.34</td>
<td>440.1</td>
<td>484.6</td>
</tr>
<tr>
<td>12.5</td>
<td>428</td>
<td>28</td>
<td>0.34</td>
<td>0.47</td>
<td>432</td>
<td>548.2</td>
</tr>
<tr>
<td><strong>CeO</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>42</td>
<td>36</td>
<td>&lt; 0.3</td>
<td>&lt; 0.3</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>2.5</td>
<td>135</td>
<td>128</td>
<td>&lt; 0.3</td>
<td>&lt; 0.3</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>10</td>
<td>340</td>
<td>393</td>
<td>0.3</td>
<td>2.1</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

* liver, kidney, spleen, basal brain, blood

** detection limit

* detection limit
Inhalation Exposure
Effects in the Lung (and Serum)

**Histopathology**

**Proliferation and Apoptosis**

**Clinical chemistry**
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**
Carboxymethyllysine (CML)
Malondialdehyde (MDA)
8-OHdG

**Cytokines et al.**

1. Apolipoprotein A1 24. IL-1α 47. MDC
2. β-2 Microglobulin 25. IL-1β 48. MIP-1α
3. Calbindin 26. IL-2 49. MIP-1β
4. CD40 27. IL-3 50. MIP-1γ
5. CD40L 28. IL-4 51. MIP-2
6. Clusterin 29. IL-5 52. MIP-3β
7. C-Reactive Protein 30. IL-6 53. MMP-9
8. Cystatin 31. IL-7 54. Myoglobin
9. EGF 32. IL-10 55. OSM
10. Emodethlin-1 33. IL-11 56. Osteopontin
11. Eotaxin 34. IL-12p70 57. RANTES
12. Factor VII 35. IL-17 58. SCF
13. FGF-basic 36. Insulin 59. Serum Amyloid P
14. FGF-9 37. IP-10 60. SGOT
15. Fibrinogen 38. KC/GROα 61. TIMP-1
17. GM-CSF 40. LIF 63. TNF-α
18. Growth Hormone 41. Lipocalin-2 64. TPO
19. GST-α 42. MCP-1 65. VCAM-1
20. GST-1 Yb 43. MCP-2 66. VEGF
21. Haptoglobin 44. MCP-3 67. von Willebrand Factor
22. IFN-γ 45. MCP-5
23. IgA 46. M-CSF
Inhalation Exposure
Effects of TiO₂

Concentration-Effect Diagram

- Rats exposed to 2, 10 or 50 mg/m³ nano-TiO₂
- Immediately after the last exposure
- Relative increase vs. control

→ Reflect different levels of local inflammation in inhalation studies
Inhalation Exposure
Effects of TiO₂

Time-Effect Diagram

- Rats exposed to 50 mg/m³ nano-TiO₂
- Relative increase vs. control

Most prominent change occurred 2 days after the last exposure.
**Research Areas in Toxicology and Ecology**

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Distribution

- Male Wistar rats (3 rats/group and time point)
- Single injections of a suspension of TiO\textsubscript{2} in serum (5 mg/kg body weight)
- The content of Ti was determined 1, 14, and 28 days after treatment in:
  - blood cells
  - plasma,
  - brain
  - lymph nodes (mediostinal, mesenteric, poploteal)
  - liver
  - spleen
  - lung
  - kidney
TiO$_2$ particle size distribution in the application preparation (rat serum)

Differential (blue line) and integral (black line, right Y-axis) particle size distribution.
Distribution and Elimination

From Fabian et al., 2007.
Results:

- Distribution: liver > spleen >> lung >> kidney
- Sequestration of particles in reticuloendothelial system; slow clearance from liver or spleen demonstrated over 90 days

Similar results obtained by others with TiO$_2$ (Sugibayashi et al., 2008) and quantum dots (Choi et al., 2007) in which particles observed only in liver, spleen, kidneys, and lung.
Research Areas in Toxicology and Ecology

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In vitro Methods

Nanoparticles (dry powder)

Dispersion in medium

- $H^+$
- Tyr
- Alb
- Met
- $PO_4^-$

pH, ions, amino acids, proteins, etc

A - single dispersed nano particles
B - aggregated nanoparticles
C - agglomerated nanoparticles into micron sized particles
D - nanoparticles with a corona

From Boverhof and David, 2009
# Genotoxicity Testing
## Dispersion of Nanomaterials

<table>
<thead>
<tr>
<th>Substance</th>
<th>Dispersed in Water/DMSO</th>
<th>Dispersed in Serum</th>
<th>Dispersed in Lung surfactant</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiO$_2$ (hydrophilic)</td>
<td>Fines</td>
<td>NPs</td>
<td>Aggl</td>
</tr>
<tr>
<td>TiO$_2$ nanopowder</td>
<td>Fines</td>
<td>NPs</td>
<td>-</td>
</tr>
<tr>
<td>ZnO</td>
<td>Aggl</td>
<td>Fines</td>
<td>Aggl</td>
</tr>
<tr>
<td>Fe$_2$O$_3$</td>
<td>Aggl</td>
<td>NPs</td>
<td>Aggl</td>
</tr>
<tr>
<td>Carbon nanopowder</td>
<td>Some Fines</td>
<td>Some Fines</td>
<td>Aggl</td>
</tr>
<tr>
<td>MWCNT</td>
<td>Aggl</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Genotoxicity

- **Ames Test**
  - TA 98, TA 100, TA 102, TA 1535 and TA 1537
  - 10 substances tested including Carbon nanopowder, MWCNT to induce reverse mutations at the histidine locus
  - Modified test substance preparation and characterization
  - **No mutagenicity detected**
Genotoxicity

- Chromosomal Aberration
  - *in vitro*: V79 Chinese Hamster cells: agglomerated TiO$_2$ and ZnO particles hampered test at high concentrations
  - *in vivo*: ZnO tested by IP injection
  - No aberrations detected
Genotoxicity

- Comet assay
  - Assessment of DNA damage in the lung both *in vivo* and *in vitro* exposure.
  - Optimization of single cell isolation
  - No genotoxicity detected with TiO$_2$
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Aquatic Fate and Toxicity

Nanoparticle (Powder)

Dispersion in water
- pH, ions, other substances...
- Mechanics, Light, ...

Characterization in Water

Exposed aquatic organisms

Aquatic Effects?
Environmental Effects

- Aquatic toxicity
  - Acute aquatic toxicity
  - Chronic aquatic toxicity
- Behaviour of nanoparticles in water
- Mobility in soil
Environmental Effects

- **Preparation of the dispersions/Different media**
  - Stirring + standard M4 medium. Surface water and pond water also tested.
  - Ultrasonication + natural surface water (with high and low TOC content)
  - Stirring and ultrasonication + natural surface water plus sediment

- **Acute toxicity tests with Daphnia magna (OECD Test Guideline 202, part 1)**
  - Single animal exposure or in groups of five individuals (10-20 per group). Exposure for 48h in glass tubes with flat bottom (10 mL), daily inspection of immobile animals
  - Tested concentrations: 0 (untreated control), 0.01 to 100.0 mg/L
## Toxicity to *Daphnia*

<table>
<thead>
<tr>
<th>Substance</th>
<th>Median diameter (nm)</th>
<th>Acute EC&lt;sub&gt;50&lt;/sub&gt; (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoated TiO₂</td>
<td>1500</td>
<td>&gt;100</td>
</tr>
<tr>
<td>T- Lite™ SF (coated TiO₂)</td>
<td>5000</td>
<td>&gt;100</td>
</tr>
<tr>
<td>T-Lite™ SF-S (coated TiO₂)</td>
<td>80</td>
<td>&gt;100</td>
</tr>
<tr>
<td>T-Lite™ Max (coated TiO₂)</td>
<td>7500</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Non-nanoscale ZnO</td>
<td>&lt;1000</td>
<td>1.0</td>
</tr>
<tr>
<td>Z-COTE® (coated ZnO)</td>
<td>2400</td>
<td>7.5</td>
</tr>
<tr>
<td>Z-COTE® Max (coated ZnO)</td>
<td>1300</td>
<td>1.0</td>
</tr>
</tbody>
</table>

From Wiench *et al.*, 2009
All data presented are available on the BASF website.


Collaboration

nanoSAFE

Safe production and use of nanomaterials

NanoCare

supported by

Cell_Nano_Tox

NANODERM
Quality of Skin as a Barrier to ultra-fine Particles
QLK4-CT-2002-02678

HESI
ILSI Health and Environmental Sciences Institute
The Chemical Industry has taken a pro-active position in testing nanomaterials in the absence of regulatory guidance.

Many of the study designs follow standard guidelines with modifications to accommodate the nature of the particles tested and how they behave.

Characterization of the neat particle and particle in the medium applied to the test system is an integral part of the testing strategy.

Standard protocols used for soluble chemicals are not applied to nanomaterials unless it can be demonstrated that they are appropriate.

Collaborative efforts to develop information can be well-suited to rapidly answering questions in a transparent atmosphere.