Nanotechnology risk assessment and safety compliance in Thailand – Regulatory mechanisms, guidelines and best practices

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National Nanotechnology Center (NANOTEC), NSTDA
THAILAND

National Workshop on Nanosafety and Regulatory Aspects of Nanotechnology
Hotel Grand Bluewave, Shah Alam, Malaysia
29-30 October 2014
Overview

• Introduction

• Hazard Identification of Nanomaterials- Physical-Chemical Characterizations and Toxicological Testing

• Researches on Nanotoxicology- Our Experiences and Inter-Lab Comparison

• Testing of Nanoproducts- Our Experience and Best Practices

• Thailand’s Policies on Nanotechnology
• Non-engineered nanomaterials

• Engineered nanomaterials
Nanotechnology Life Cycle Perspective

Worker Exposure

- Raw Material Production
- Consumer Product Manufacturing
- Consumer Use
- End of Life

Consumer Exposure

- Human exposure
- Ecological exposure

Human Population and Ecological Exposure
Risk assessment framework

Risk = Hazard + Exposure

Adaptive Screening Risk Assessment Framework

Hazard Identification

Assess Exposure

Evaluate Toxicity

Raw Materials

Process

Product

Process

Use/Reuse/Disposal

Risk Characterization

Interim Mitigation Measures

(Adapted from: Shatkin JA. Nanotechnology: Health and Environmental Risks, 2nd ed.)
Hazard Identification of Nanomaterials
- Physical-Chemical Characterizations and Toxicological Testing -
Physicochemical characteristic of nanomaterials

Titanate Nanomaterials

Appearance: Naturally white opaque color
Crystalline forms: Anatase, Rutile, Brookite etc.
Applications: Pigment composition in Paint, Plastics, Food additives and Health care products

**TiO$_2$**

**Rutile**
- Cosmetics
- Sunscreen products
- Food additives

**Anatase**
- Photocatalytic air purification
- Self cleansing surface
- Solar energy conversion
- Self-sterilization (antimicrobial) on surface coating materials

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Titanate Nanomaterials

Photocatalytic activity

Ion-doped titanium dioxide

<table>
<thead>
<tr>
<th>Ion-doped TiO₂</th>
<th>Weight fraction of phase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anatase</td>
</tr>
<tr>
<td>N-TiO₂</td>
<td>100</td>
</tr>
<tr>
<td>C-TiO₂</td>
<td>71</td>
</tr>
<tr>
<td>Cr-TiO₂</td>
<td>20</td>
</tr>
</tbody>
</table>

Hinthong et al., Proceeding in IEEE Nano 2010
Silver nanoparticles (Sigma 576832)
Size: < 100 nm
Form: nanopowder

<table>
<thead>
<tr>
<th>Sonication time (min)</th>
<th>Mean diameter (nm)</th>
<th>Zeta potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>750.03 ± 223.15</td>
<td>-30.53 ± 1.74</td>
</tr>
<tr>
<td>1</td>
<td>185.58 ± 27.40</td>
<td>-31.32 ± 1.33</td>
</tr>
<tr>
<td>5</td>
<td>190.41 ± 22.86</td>
<td>-33.39 ± 2.11</td>
</tr>
<tr>
<td>10</td>
<td>182.65 ± 12.97</td>
<td>-34.14 ± 3.59</td>
</tr>
</tbody>
</table>

Dynamic light scattering (DLS)

TEM

50 nm
Nanomaterial characterizations

“The first step towards nanotoxicology studies”

- To ensure that the results are reproducible
- To provide basis for understanding the properties of nanoparticles that determine their biological effects

**Powder**
- Purity
- Morphology
- Particle size and distribution
- Crystallinity
- Coatings
- Type of aggregation/agglomeration
- Surface properties (charge, defects, etc.)

**In experimental condition**
- Aggregation/ agglomeration
- Surface
- Surface coating
- Solubility
- etc.

**Challenges**: development of new equipment and methodology
### Physico-chemical characterizations

#### Characterization of Nanomaterials

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Instrument</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology and compositions</td>
<td>SEM-EDX, TEM-EDX</td>
</tr>
<tr>
<td>Primary size</td>
<td>TEM</td>
</tr>
<tr>
<td>Hydrodynamic size, size distribution</td>
<td>DLS</td>
</tr>
<tr>
<td>Surface charge</td>
<td>Zeta potential analyzer</td>
</tr>
<tr>
<td>Specific surface area</td>
<td>BET</td>
</tr>
<tr>
<td>Metal contaminants/ dissolution</td>
<td>ICP, AA</td>
</tr>
</tbody>
</table>

#### Characterization of Nanomaterials in Products

Require additional sample preparation steps such as digestion, extraction and purification etc., + advanced instruments.
Equipment for “Nano” characterization

- Scanning Electron Microscope
- Transmission Electron Microscope
- Atomic Force Microscope
- Nanosizer

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# Models for toxicological studies

<table>
<thead>
<tr>
<th></th>
<th><strong>In vitro</strong> Cell-based models</th>
<th><strong>In vivo</strong> Animal models</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro</strong></td>
<td>Fast</td>
<td>Provide complementary data</td>
</tr>
<tr>
<td><strong>In vitro</strong></td>
<td>Easy</td>
<td>Can be designed for exposure routes</td>
</tr>
<tr>
<td><strong>In vitro</strong></td>
<td>Inexpensive</td>
<td>Slow</td>
</tr>
<tr>
<td><strong>In vitro</strong></td>
<td>Exhibit abnormal behaviors</td>
<td>Expensive</td>
</tr>
<tr>
<td><strong>In vitro</strong></td>
<td>Usually aneuploid</td>
<td>Labor-intensive</td>
</tr>
<tr>
<td><strong>In vitro</strong></td>
<td>Do not ideally represent</td>
<td>Ethically questionable</td>
</tr>
<tr>
<td><strong>In vitro</strong></td>
<td>phenotypes and mechanisms</td>
<td></td>
</tr>
</tbody>
</table>

**Response:**
- Cell death
- Metabolism
- Gene and protein expression
- Genotoxicity

![Counts](histogram.png)  

**Response:**
- Death
- Pathology (tissue, organ)
- Clinical blood chemistry
- Behavior

![Genotype](genotype.png)
Advanced *in vitro* models

3R principal:
- Reduction
- Refinement
- Replacement

Reconstructed human Epidermis (RhE)
- Skin corrosion (OECD TG431)
- Skin irritation (OECD TG439)
Zebrafish model

Zebrafish, *Danio rerio*

- Morphology
- Mortality
- Angiogenesis
- Erythropoiesis

**Zebrafish Embryo Toxicity Testing**

- Nanomaterial and chemical toxicity testing, pharmaceutical screening
- Demonstrating similarities to mammalian models and humans
- Recommended by US FDA
- Toxicity testing in biological systems
- Eco-toxicity testing
- OECD TG 203, TG 204, TG 210, TG 212, TG 215, TG 229, TG 230, TG 234, and TG 236
Researches on Nanotoxicology
- Our Experiences and Inter-Lab Comparison -
Biological Effects of Nanomaterials

- **Cell viability**
- **Cellular uptake**
- **ROS generation**

**AgNPs**

- IC<sub>50</sub> = 18.74 ± 2.38 µg/ml

**Cell cycle**

- Sub G1
- G1
- S
- G2/M

**Activity of CYP enzymes**

- CYP1A
- CYP2C
- CYP2D
- CYP2E1
- CYP3A

- Genotoxicity
- Immunotoxicity
- Expression of mRNA and protein
The conceivable interaction of insoluble particles with submersed cells grown at the bottom of a well, filled with an appropriate medium of height h. (A) Previously employed picture, (B) more appropriate concept discussed in this study. The number of particles in (A) and (B) is the same.


Appropriate experimental design:

- 96-well (0.32 cm²) 
- 6-well (9.5 cm²) 
- Petri dish (55 cm²)

**Volume adjustment for insoluble materials**

- Concentration (w/v)
- Particles per area
Our Publications on Nanosafety:


A number of research gaps are awaiting to be explored!
Inter-Lab Comparison: Round Robin Project

Scope of the project:
“Inter-lab comparison of MTS cytotoxicity assay”

### NANOTEC’s International Advisory Board

- **Prof. Harald F. Krug**
  - Visiting Professor
  - (Dec, 2012 - March, 2013)

#### Inter-Lab Comparison: Round Robin Project

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<th>4</th>
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<th>6</th>
<th>7</th>
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</table>

- **Chemical Ctrl**
- **NP Test**

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**Materials:**
- Polystyrene nanoparticles
- Positive control
- Reagents
- A549 cells

**Infrastructure:** cell culture room facility, microplate reader and other equipment

**Manpower:** with adequate skill

---

**SOP:**

SOP – MTS cell viability assay with A549

<table>
<thead>
<tr>
<th>Version</th>
<th>Date</th>
<th>Author</th>
<th>Comment</th>
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<tbody>
<tr>
<td>0.7</td>
<td>1/30/12</td>
<td>rm/Empa</td>
<td>First draft</td>
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<td>0.8</td>
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<td>Add flow chart, clarify some details</td>
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<tr>
<td>0.85</td>
<td>2/23/12</td>
<td>rm/Empa</td>
<td>Implementing parts of the suggestions by hic/274</td>
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<td>Corrections provided by KRISS</td>
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<td>0.95</td>
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<td>0.98</td>
<td>5/03/12</td>
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<td>Adopting changes proposed by Empa</td>
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</table>

Distribution list: Nam Woong Song/KRISS, John Elliott/NIST, Matthias Roesslein/Empa
Testing of Nanoproducts
- Our Experiences and Best Practices -
Silver nanoproducts available in Thailand

Everyday used products claimed to be “nano-silver”

- Face powder
- Detergent
- Soap/Shampoo
- Spray
- Deodorants
- Toothpaste
- Cream
- Textiles
Cosmetics

Analysis of silver in nanoproducts: 20 items

- By using SEM, silver nanomaterials can be detected in only few products
- Total silver concentration was determined by GFAAS
- How much silver can be exposed and penetrated into the skin? —on going research—
Silver release from textile nanoparticles into artificial sweat

Tested samples

- Laboratory textiles were prepared by pad-dry-cure method (A0, A1, A2, A3, A4)
- Six commercial claimed nanosilver shirts were purchased (B, C, D, E, F and G)

Physical-chemical analysis

Kulthong et al., (2010) Particle and Fibre Toxicology, 7:8
## Measurement of antibacterial properties

<table>
<thead>
<tr>
<th>Sample</th>
<th>Percent reduction of bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>A0</td>
<td>-</td>
</tr>
<tr>
<td>A1</td>
<td>98.04</td>
</tr>
<tr>
<td>A2</td>
<td>99.02</td>
</tr>
<tr>
<td>A3</td>
<td>97.30</td>
</tr>
<tr>
<td>A4</td>
<td>99.83</td>
</tr>
<tr>
<td>B</td>
<td>98.23</td>
</tr>
<tr>
<td>C</td>
<td>98.56</td>
</tr>
<tr>
<td>D</td>
<td>-</td>
</tr>
<tr>
<td>E</td>
<td>-</td>
</tr>
<tr>
<td>F</td>
<td>99.85</td>
</tr>
<tr>
<td>G</td>
<td>99.99</td>
</tr>
</tbody>
</table>

Kulthong et al., (2010) *Particle and Fibre Toxicology*, 7:8
## Release of silver into artificial sweat

### Table: Silver released into artificial sweat (mg/kg)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial silver content (mg/kg)</th>
<th>AATCC pH 4.3</th>
<th>ISO pH 5.5</th>
<th>ISO pH 8.0</th>
<th>EN pH 6.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>36.12 ± 22.42</td>
<td>21.01 ± 4.13</td>
<td>15.53 ± 3.62</td>
<td>34.27 ± 2.88</td>
<td>35.83 ± 19.68</td>
</tr>
<tr>
<td>A2</td>
<td>56.57 ± 34.28</td>
<td>33.39 ± 15.80</td>
<td>28.81 ± 10.34</td>
<td>66.54 ± 46.29</td>
<td>77.96 ± 23.80</td>
</tr>
<tr>
<td>A3</td>
<td>95.12 ± 33.12</td>
<td>70.15 ± 37.29</td>
<td>72.69 ± 11.99</td>
<td>82.22 ± 26.99</td>
<td>152.20 ± 36.54</td>
</tr>
<tr>
<td>A4</td>
<td>425.21 ± 93.73</td>
<td>217.61 ± 81.32</td>
<td>177.13 ± 57.13</td>
<td>268.31 ± 131.15</td>
<td>322.21 ± 87.00</td>
</tr>
<tr>
<td>E</td>
<td>15.16 ± 9.90</td>
<td>0.08 ± 0.05</td>
<td>0.01 ± 0.01</td>
<td>0.05 ± 0.30</td>
<td>0.36 ± 0.10</td>
</tr>
<tr>
<td>F</td>
<td>1.22 ± 0.87</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.05 ± 0.00</td>
</tr>
<tr>
<td>G</td>
<td>0.99 ± 1.53</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

* The amount of silver released was dependent on: **Initial amount of silver**, **Quality of the fabrics**, **Artificial sweat formulations** and **pH**.

Kulthong et al., (2010) *Particle and Fibre Toxicology*, 7:8

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## Our safety testing capability

<table>
<thead>
<tr>
<th>Method</th>
<th>International recommendation / standard</th>
<th>Develop protocol</th>
<th>Research</th>
<th>BD/TT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Skin Corrosion test/ Skin irritation test</strong></td>
<td>OECD TG 431, 439</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td><strong>Photo-toxicity</strong> 3T3 NRU test</td>
<td>OECD TG 432</td>
<td>●</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Skin Sensitization</strong></td>
<td>- OECD / SPSFs approved</td>
<td>●</td>
<td></td>
<td>●</td>
</tr>
<tr>
<td>- <em>In vitro</em> skin sensitization assays (h-CLAT, DPRA, MUSST)</td>
<td></td>
<td></td>
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<tr>
<td>- <em>In vitro</em> skin sensitization assay KeratinoSens</td>
<td></td>
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<tr>
<td>- <em>In vitro</em> skin sensitization assay (IL-8 Luc assay)</td>
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<tr>
<td><strong>Genetic toxicity test</strong></td>
<td>- OECD TG 487</td>
<td>●</td>
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<tr>
<td>- <em>In vitro</em> micronucleus test</td>
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<tr>
<td>- <em>In vitro</em> comet assay</td>
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<tr>
<td><strong>Cytotoxicity test</strong></td>
<td>ISO 10993-5</td>
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<tr>
<td><strong>Toxicokinetic</strong> (for drug screening)</td>
<td>US FDA draft guidance 2012</td>
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<tr>
<td>- <em>In vitro</em> CYP induction</td>
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<tr>
<td>- <em>In vitro</em> CYP inhibition</td>
<td></td>
<td></td>
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<tr>
<td><strong>Acute toxicity in embryo</strong> (Zebrafish model)</td>
<td>OECD TGs (203, 210, 236)</td>
<td>●</td>
<td>●</td>
<td></td>
</tr>
</tbody>
</table>
Thailand’s Policies on Nanotechnology
Vision: “Safe-nano for Thailand’s Sustainable Development”

“3” KPIs in 5 years

1. **Knowledge management:** Thailand has an effective management system of nanosafety and ethics.

2. **Product labeling:** Nanoproducts in Thailand market have labels displaying nanomaterials components and safety information based on scientific evidence.

3. **Public awareness:** The public has knowledge, understanding, and awareness of nanosafety and risk and are able to select, store, and handle nanoproducts by themselves.
NanoSafety “Consortium”

**Activities**

- **Policy and coordination at national/international level**
  ISO/TC 229, OECD WPMN, OECD WPN, UNEP SAICM, UNITAR

- **Nanosafety Information Center of Thailand (NICT), "knownano.org"**
  belongs to the collaborative effort of Chulalongkorn University and NANOTEC.

- **R&D and Safety Investigations of Nanoproducts on issues important for Thailand and ASEAN**

- **Standards and Regulations**
  - Food and Drug Administration Ministry of Public Health
  - Thai Industrial Standards Institute Ministry of Industry
  - National Institute of Metrology (Thailand)

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Nano Labeling (Nano Q)

Nano Q is a certified mark for nanoproducts (Functional Textiles, Coating Materials, Household Products) which are certified by Nanotechnology Association of Thailand.

The aseptic ambulance of Supremeproducts Co., Ltd has received the first NanoQ label in Thailand.
SRA Research Team
Dr. Rawiwan Maniratanachote
Dr. Sasitorn Aueviriayavit
Dr. Suwimon Boonrungsiman
Dr. Wittaya Pimtong
Dr. Nawin Viriya-empikul
Miss Kornphimol Kulthong
Miss Apiwan Rosena
All research students

Thank you!