NANOPARTICLES AND THE SKIN - A HEALTH RISK FOR THE CONSUMER?

L’OREAL GLOBAL SAFETY EVALUATION
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Berlin, March 2006
SOURCES OF NANOMATERIALS

- Biological:
  - Ferritin
  - ATP synthase
  - Viruses, nanobacteria

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

- Man-made:
  - Diesel exhaust
  - Fires / toasters
  - Manufactured nanoparticles
  - One cm³ of urban air contains 10,000 – 50,000 nanoparticles
TWO PRINCIPAL FEATURES MAY AFFECT PHYSICAL AND TOXICOLOGICAL PROPERTIES OF NPs/NMs

- **Quantum effects**
  - important at the low end of nanoscale
  - may produce changes in optical, magnetic, thermal or conductivity properties

- **Increased surface area per unit mass**
  - 1 mL of nanoparticles (2.5 nm; 5 g/cm³) has a surface of **240 m²**
  - Surface may affect dissolution kinetics / bioavailability or increased surface activity

Supergel: nano-SiO₂ + water
DOES « NANO-TOXICOLOGY » EXIST?
NO OR LITTLE EVIDENCE FOR TYPICAL TOXICOLOGICAL PROPERTIES OF NPs

NOTE: Toxicological profiles of substances, bulk, micro, nano, vapour or solution, tend to be the same or similar (testing of chemicals, drugs, food ingredients).

Particle size of a substance has little impact on its toxicological profile, UNLESS:

- **Size effect**: absorption or absorption kinetics may be affected by particle size

- **Surface effects**: surface activity plays a role (relative surface increases with particle size)

- **Efect on external exposure**: smaller particles → longer time of settlement → increased inhalation exposure

- **New physical shape**: some NPs (SWCNTs) have a fibre-type shape. Since they are insoluble, they display fibre-like toxicity (asbestos)

Relative particle surface: mm-particles << microparticles << nanoparticles << solutions or vapours (individual molecules)
USE OF NANOTECHNOLOGY IN THE COSMETIC INDUSTRY: ISOLATED SINGLE SKIN CELL (Atomic Force Microscopy - AFM)
USE OF NANOTECHNOLOGY IN THE COSMETIC INDUSTRY: SURFACE FINE STRUCTURE OF HAIR (AFM)
NANO-SIZED COSMETIC FORMULATIONS

Examples of nanomaterials

Liposomes 100-300 nm (Caffeine)

Nanocapsule 100-600 nm (Vitamin A, E)

Nanoemulsion 50 nm (transparent)

Oleosomes 150-500 nm

Oil

Polymer

Lipid

Water

GJN, 3/2006
### Nanotechnology-based innovations

<table>
<thead>
<tr>
<th>Innovation</th>
<th>Product</th>
<th>Benefit of nanotechnology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanoemulsion</td>
<td>Hair conditioner</td>
<td>Unique texture, transparency</td>
</tr>
<tr>
<td>Nanocapsule</td>
<td>Skin care</td>
<td>Protects &amp; transports active ingredient</td>
</tr>
<tr>
<td>Nanopigment</td>
<td>Sunscreen</td>
<td>Filters UV rays, consumer compliance</td>
</tr>
</tbody>
</table>
A complete review on liposomes in cosmetics and drugs concluded that liposomes do not penetrate through the intact stratum corneum (SC) or enhance penetration of active ingredients (1995).

Joke Bouwstra (University of Leiden) published more than 30 articles on the percutaneous penetration of liposomes or similar formulations using $^{14}$C-labelled capsule membranes.

It was concluded that lipids from soft capsules penetrate into the deep layers of the SC, but were absent in the living skin. Lipids from hard capsules were found only in/on the superficial layers of the SC.

Intact capsules – hard or soft - were only found on the surface of the SC.

NANOTECHNOLOGY IN COSMETICS: SUNSCREENS

NANOPIGMENTS

Titanium dioxide is a mineral UV filter composed of micron-sized aggregates. The aggregates themselves are composed of grains that are nano-sized.

The aggregates are coated with a layer of silica: Image of Eusolex® T-AVO TiO2 UV filter (Merck, D).
MODE OF ACTION OF CHEMICAL AND PHYSICAL SUNSCREENS

ORGANIC UV FILTER (4-MBC)  

INORGANIC UV FILTER

IDEALLY, ORGANIC AND INORGANIC FILTERS ARE USED IN COMBINATION (SYNERGISTIC ACTIVITY)

1. Reflection
2. Light scattering

• Absorption
• Release of absorbed energy (heat)
RISK ASSESSMENT OF HUMAN DERMAL EXPOSURE TO NPs: SUNSCREENS

- Use / exposure: NPs used in cosmetics consist mainly of ZnO or TiO$_2$ (sunscreens)

- Systemic exposure: penetration of NPs into / through the skin, systemic exposure?

- Hazard: does nano-size increase the reactivity / toxicity of cosmetic NPs, such as ZnO or TiO$_2$?

- Risk management: can a chemical / photo-chemical / biological activity of ZnO or TiO$_2$ be modified (coating)?
Light scattering depends on particle size: too small is not useful!

CeO$_2$
5-10 nm

CeO$_2$
20 nm

CeO$_2$
50 nm

NOTE: NPs at 60 nm provide best UV protection

Ref.: http://www.apt-powders.com
PERCUTANEOUS PENETRATION ROUTES OF TOPICALLY APPLIED SUBSTANCES (Lademann, 2005)

N.B.: No evidence for follicular penetration into the living skin by NPs or microparticles
## Published in Vivo Studies on Dermal Absorption of Nanoparticles Show No Penetration

<table>
<thead>
<tr>
<th>Study</th>
<th>Material</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tan et al., 1996</td>
<td>Microfine TiO₂</td>
<td>No penetration into epidermis / dermis in vivo, man</td>
</tr>
<tr>
<td>Lademann, 1999</td>
<td>Microfine TiO₂</td>
<td>No penetration into epidermis / dermis in vivo, man</td>
</tr>
<tr>
<td>Pflücker et al., 2001</td>
<td>TiO₂, 10 and 100 nm</td>
<td>No penetration into epidermis / dermis, accumulation in the follicle orifice, but no penetration into living skin (pig)</td>
</tr>
<tr>
<td>Alvarez-Roman et al., 2004</td>
<td>Polystyrene NPs, 20 and 200 nm</td>
<td>No penetration into epidermis / dermis, accumulation in the follicle orifice, but no penetration into living skin (pig)</td>
</tr>
<tr>
<td>Howard, P, 2005 (SoT Meeting, 2005)</td>
<td>Fluorescent polymeric NPs</td>
<td>No penetration into epidermis / dermis in vivo, man</td>
</tr>
<tr>
<td>EU Nanoderm project (Tilmann Butz)</td>
<td>Fluorescent particles</td>
<td>No evidence for penetration into living skin (preliminary data, ECETOC, 11/2005)</td>
</tr>
</tbody>
</table>

**Conclusion:** No evidence that topically applied nanoparticles penetrate into normal skin (NPs will always penetrate less than a compound in solution!)
IN VITRO PERCUTANEOUS PENETRATION OF TiO₂ AND ZnO IN PIG SKIN (4 mg/cm², 24-hrs): ABSORBED DOSE = ZERO *

<table>
<thead>
<tr>
<th>TEST MATERIAL</th>
<th>SKIN WASH (%)</th>
<th>TAPE STRIPS (%)</th>
<th>SKIN (%)</th>
<th>RECEPT. FLUID (%)</th>
<th>ABSORBED DOSE (%)</th>
<th>RECOV. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiO₂, 30-60 x 10 nm, silica / methicone-coated O/W emulsion (10%)</td>
<td>97.7 to 100.2</td>
<td>0.1 to 0.2</td>
<td>0.1 to 0.3</td>
<td>0.0</td>
<td>0.0</td>
<td>98.2 to 100.4</td>
</tr>
<tr>
<td>TiO₂, 30-60 x 10 nm, methicone-coated O/W emulsion (10%)</td>
<td>85.4 to 92.9</td>
<td>0.0 to 0.3</td>
<td>0.1 to 0.5</td>
<td>0.0</td>
<td>0.0</td>
<td>86.1 to 93.0</td>
</tr>
<tr>
<td>ZnO, 80 nm, O/W emulsion (10.3%)</td>
<td>NP</td>
<td>98.6 to 102.3</td>
<td>1.4 to 1.5 **</td>
<td>0.8 to 1.4 **</td>
<td>0.0</td>
<td>102.3 to 106.8</td>
</tr>
</tbody>
</table>

**CONCLUSION:** NO EVIDENCE FOR PERCUTANEOUS PENETRATION

Gamer et al., *Toxicol. In Vitro*, BASF, 2005; ** values at or below background levels
Pseudo-penetration: nanoparticles in/on the hair follicle orifice may be mis-interpreted as penetration into the living epidermis.
SKIN PENETRATION OF SMALL MOLECULES IN SOLUTION VS. INSOLUBLE NPs*

DIFFUSION OF MOLECULES INTO THE SKIN IS LIKE A BREAKING DAM

NPs MOVE BY MECHANICAL FORCE: WHY SHOULD A ROCK MOVE ONLY IN ONE DIRECTION? (NO MECHANISM TO DRIVE ACTIVE PENETRATION)

* Prof. T. Butz, Chairman Nanoderm Task Force, 11/2005
GENOTOXICITY / PHOTOTOXICITY / PHOTOTOXICITY OF TiO$_2$ AND ZnO NANOPARTICLES
PHOTO-GENOTOXICITY / PHOTO-CARCINOGENICITY OF TiO₂: PROTECTION

<table>
<thead>
<tr>
<th>AUTHORS</th>
<th>MATERIAL</th>
<th>STUDY TYPE</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bestak and Halliday, 1996</td>
<td>TiO₂, no particle size given</td>
<td>Photo-carcinogenicity in DMBA pre-treated mice</td>
<td>Protection</td>
</tr>
<tr>
<td>Suzuki, 1987</td>
<td>TiO₂, no particle size given</td>
<td>Photo-carcinogenicity in hairless mice, pre-treated with Croton oil</td>
<td>Protection</td>
</tr>
<tr>
<td>Greenoak et al., 1993</td>
<td>TiO₂, no particle size given</td>
<td>DNA damage in mouse skin</td>
<td>Protection</td>
</tr>
</tbody>
</table>

N.B.: what do in vitro positive « photo-genotoxicity » results mean in practice, when a compound has been shown to protect against UV-induced cancer IN VIVO?
PHOTO-GENOTOXICITY OF 10 TiO_2-POWDERS USED IN COSMETICS *

- Upon request of the SCCNFP (1999), an industry consortium (TiO_2 producers and cosmetic industry) investigated
  - Cytotoxicity
  - Genotoxicity and photo-genotoxicity (Ames, CHO)

- Test material: TiO_2
  - NPs and microparticles
  - Rutile and anatase (photo-active) TiO_2-crystalline forms
  - Coated and non-coated particles

- Program performed by COVANCE / UK

* SCCNFP, 24 October, 2000
<table>
<thead>
<tr>
<th>NAME</th>
<th>CRYSTALLINE FORM</th>
<th>MEAN PARTICLE SIZE (nm)</th>
<th>COATING</th>
<th>Ames / Photo-Ames</th>
<th>CHO / Photo-CHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rutile</td>
<td>14</td>
<td>Al$_2$O$_3$/Dimethicone</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>2</td>
<td>Anatase</td>
<td>60</td>
<td>Al$_2$O$_3$/SiO$_2$</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>3</td>
<td>Anatase</td>
<td>60</td>
<td>Uncoated</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>4</td>
<td>Anatase</td>
<td>200.000</td>
<td>Uncoated</td>
<td>negative</td>
<td>negative *</td>
</tr>
<tr>
<td>5</td>
<td>Rutile</td>
<td>20</td>
<td>Al$_2$O$_3$/Dimethicone</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>6</td>
<td>Rutile</td>
<td>17</td>
<td>Al$_2$O$_3$/Stearic acid</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>7</td>
<td>Rutile</td>
<td>20</td>
<td>Uncoated</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>8</td>
<td>Rutile</td>
<td>15</td>
<td>Al$_2$O$_3$/Stearic acid</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>9</td>
<td>Rutile</td>
<td>15</td>
<td>Uncoated</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>10</td>
<td>Rutile</td>
<td>11-28</td>
<td>Al$_2$O$_3$/SiO$_2$</td>
<td>negative</td>
<td>negative</td>
</tr>
</tbody>
</table>

* Some inconclusive / positive results at high cytotoxic levels; overall rated negative
CYTOTOXICITY AND PHOTO-GENOTOXICITY OF TiO$_2$ (NANO/MICRO/COATED/UNCOATED/RUTILE/ANATASE) PARTICLES *

- All TiO$_2$ materials showed minimal cytotoxicity in the absence and presence of UV
- All TiO$_2$ materials were negative in the Ames-, photo-Ames and CHO-, and photo-CHO tests
- UV, particle size or crystalline form had no effect on cytotoxic or genotoxic potential of TiO$_2$ materials
- In vivo (hairless mice), TiO$_2$ protects against genotoxic and carcinogenic activity of UV light

**Conclusion:** fine particle TiO$_2$ is not expected to present a genotoxic or photo-genotoxic risk for humans under normal conditions of use

* Expert report, Dr. David Kirkland, COVANCE, 16 September, 1999; also see opinion of the SCCNFP, 24 October 2000, http://europa.eu.int/comm/health/ph_risk/committees/sccp/sccp_opinions_en.htm
## Genotoxicity / Photo-Genotoxicity of Zinc Oxide (ZnO) NPs: Results of GLP Studies

<table>
<thead>
<tr>
<th>Test</th>
<th>Test Organism</th>
<th>Result</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ames test</td>
<td><em>S. typhimurium</em></td>
<td>Negative</td>
<td>Non-photo-genotoxic</td>
</tr>
<tr>
<td>Photo-Ames test</td>
<td><em>S. typhimurium</em> TA98, 100, 1537</td>
<td>Negative</td>
<td>Non-photo-genotoxic</td>
</tr>
<tr>
<td>In vitro clastogenesis</td>
<td>CHO</td>
<td>Positive at =814 µg/mL</td>
<td>Clastogenic</td>
</tr>
<tr>
<td>Photo-clastogenesis *</td>
<td>CHO</td>
<td>Positive at =195 µg/mL</td>
<td>Photo-clastogenic?</td>
</tr>
<tr>
<td>In vitro clastogenesis</td>
<td>V-79</td>
<td>Positive at 10.0 or 20.0 µg/mL</td>
<td>Clastogenic</td>
</tr>
<tr>
<td>Photo-clastogenesis *</td>
<td>V-79</td>
<td>Positive at 2.5 or 3.0 µg/mL</td>
<td>Photo-clastogenic?</td>
</tr>
<tr>
<td>Comet test / photo-comet</td>
<td>Human keratinocytes, V-79 cells</td>
<td>Negative (kc), slightly positive (V-79)</td>
<td>Equivocal</td>
</tr>
</tbody>
</table>

**4-Fold Increase in Clastogenic Potency: « Photo-Clastogenic »?**

GJN, 3/2006
PSEUDO-PHOTO-CLASTOGENICITY of ZnO

ZnO-induced incidence (%) of chromosome aberrations (CA) in non-irradiated CHO cells (dark), pre-irradiated (PI) or simultaneously-irradiated (SI) CHO cells (high UV dose: 700 mJ/cm²) *

<table>
<thead>
<tr>
<th>HYPE</th>
<th>FACT</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPs are carcinogenic after inhalation</td>
<td>Inert NPs (TiO₂, CB) were carcinogenic in rats after chronic lung overload: irrelevant for man under normal exposure conditions</td>
</tr>
<tr>
<td>Inhalation of NPs produces new toxicities. NPs more toxic than MPs</td>
<td>Some NPs (TiO₂, CB) were somewhat more toxic than MPs, others (SiO₂, ZnO) equally or less toxic</td>
</tr>
<tr>
<td>Inhaled NPs are systemically absorbed and affect the CV system and the brain.</td>
<td>Some evidence for syst. absorption (lung overload), no evidence for adverse systemic effects, translocation to the brain needs to be confirmed.</td>
</tr>
<tr>
<td>Inhalation of NPs produces asbestos-like pulmonary toxicity</td>
<td>SWCNTs are μ-sized, insoluble fibres and produce toxicity typical for fibres</td>
</tr>
<tr>
<td>Oral uptake of NPs produces systemic exposure of the organism</td>
<td>Evidence unclear: minor syst. exposure after oral uptake of some NPs, no evidence for others. No evidence for adverse systemic effects.</td>
</tr>
<tr>
<td>NPs penetrate through the skin and produce systemic exposure</td>
<td>No evidence for penetration into or through the living skin. Damaged skin needs confirmation, but no mechanism suggesting active penetration.</td>
</tr>
<tr>
<td>NPs have distinct toxicological properties (NANO-TOXICOLOGY!)</td>
<td>LITTLE OR NO EVIDENCE FOR COMMON TOXICITIES OF NPs</td>
</tr>
</tbody>
</table>
HEALTH RISK OF NANOPARTICLES IN COSMETICS: CONCLUSION

- Available data suggest that use of NPs in cosmetic preparations poses no health risk to the consumer.

- This view is consistent with the conclusion of the recent ECETOC Conference, 7-9 Nov., 2005 (Chairman Prof. Helmut Greim): Concern level = Inhalation > oral uptake >> dermal exposure.

- NB: absent or insufficient bioavailability is the biggest obstacle for the development of new drugs – the pharmaceutical industry would pay billions for NPs that are systemically available after inhalation, oral or topical administration.

Today, we hardly have intravenous NP drug formulations.
COSMETIC APPLICATIONS OF NANOBOTS...

Barber saucers

Hair jacks

THANK YOU FOR YOUR ATTENTION!