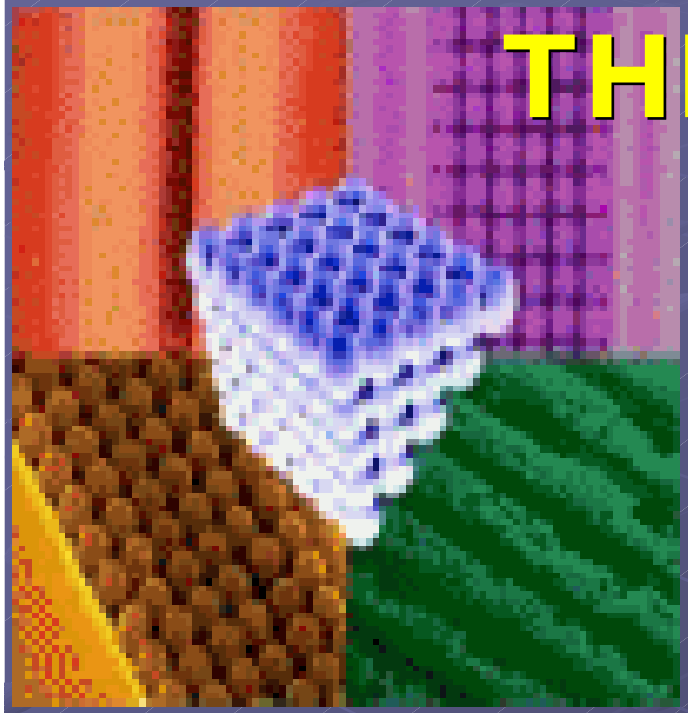
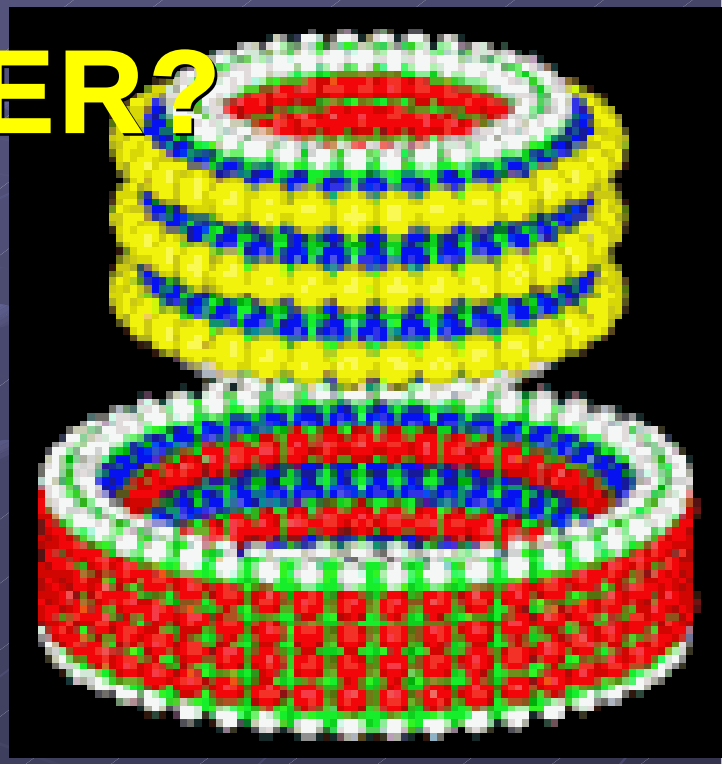
NANOPARTICLES AND THE SKIN - A HEALTH RISK FOR THE CONSUMER?

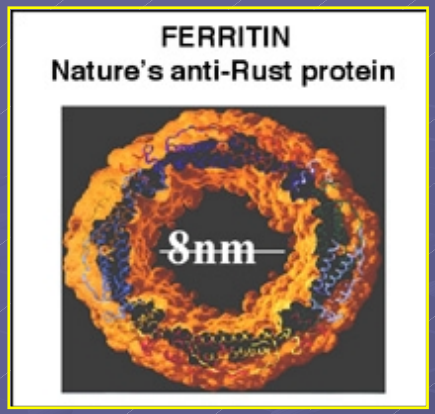


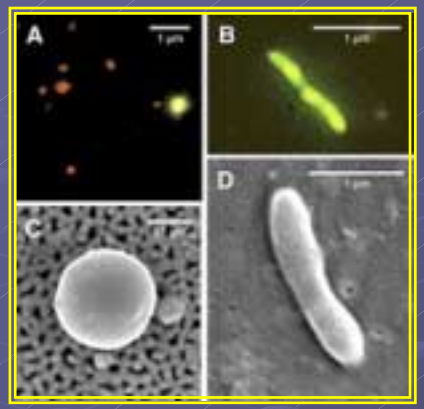


Gerhard J. Nohynek, M.Sc., Ph.D., D.A.B.T.
L'OREAL GLOBAL SAFETY EVALUATION
gnohynec@rd.loreal.com

Berlin, March 2006

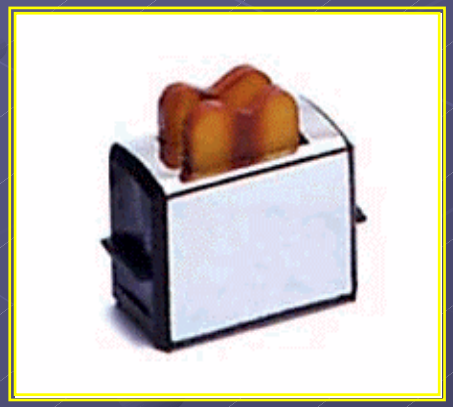
SOURCES OF NANOMATERIALS





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

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



- Man-made:
  - Diesel exhaust
  - Fires / toasters
  - Manufactured nanoparticles
  - One cm^3 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/cm^3) has a surface of 240 m^2
  - Surface may affect dissolution kinetics / bioavailability or increased surface activity

Supergel: nano-SiO_2 + water

DOES « NANO-TOXICOLOGY » EXIST? NO OR LITTLE EVIDENCE FOR TYPICAL TOXICOLOGICAL PROPERTIES OF NPs

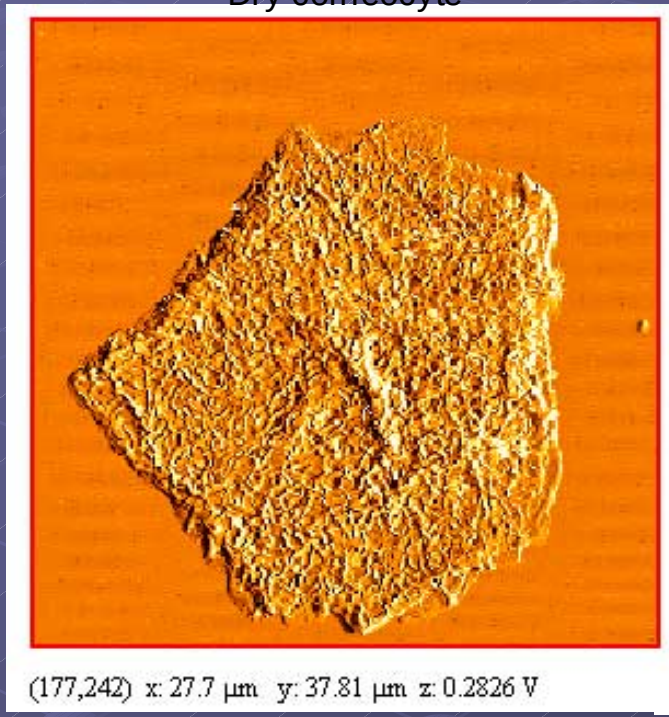
- NOTE: Toxicological profiles of substances, bulk, micro, nano, vapour or solution, tend to be the same or similar (testing of chemicals, drugs, food ingredeints)

- 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)

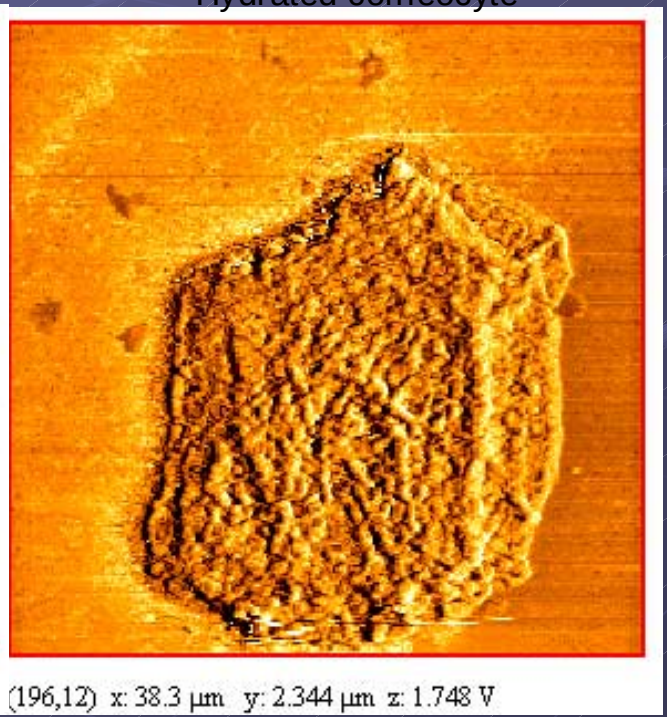
USE OF NANOTECHNOLOGY IN THE COSMETIC INDUSTRY: ISOLATED SINGLE SKIN CELL (Atomic Force Microscopy - AFM)

Dry corneocyte



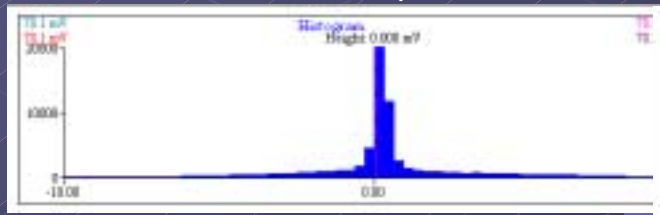
(177,242) x: 27.7 µm y: 37.81 µm z: 0.2826 V

Hydrated corneocyte

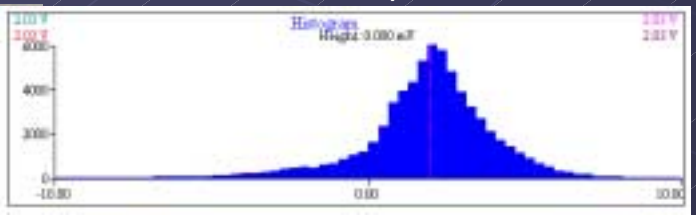


(196,12) x: 38.3 µm y: 2.344 µm z: 1.748 V

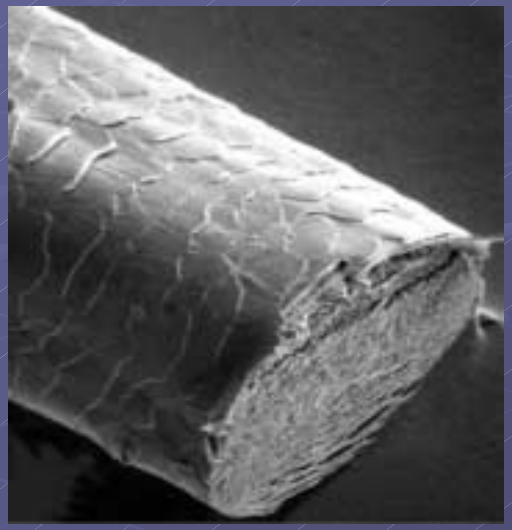
Friction profile

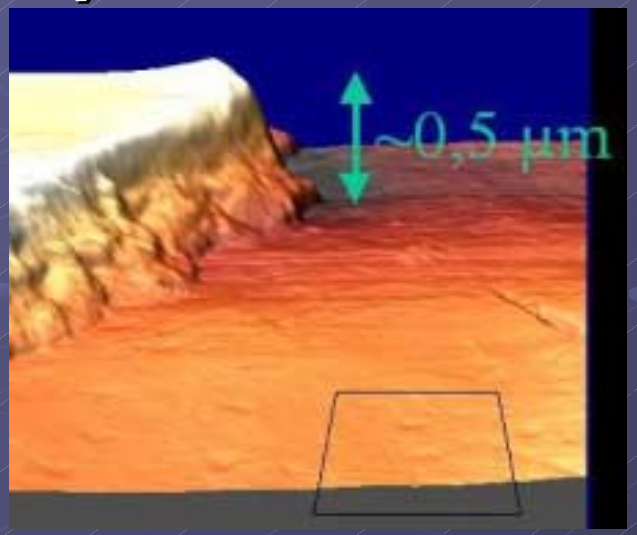


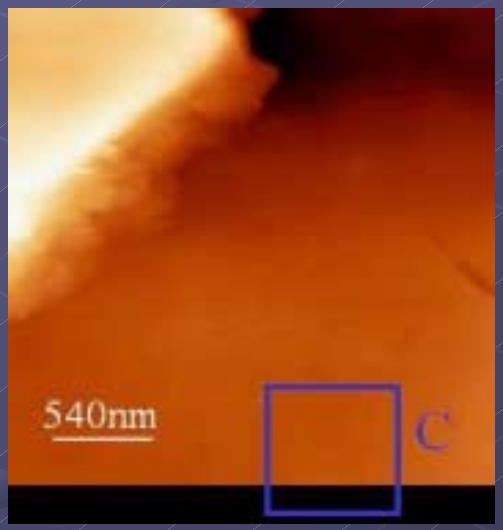
Friction profile

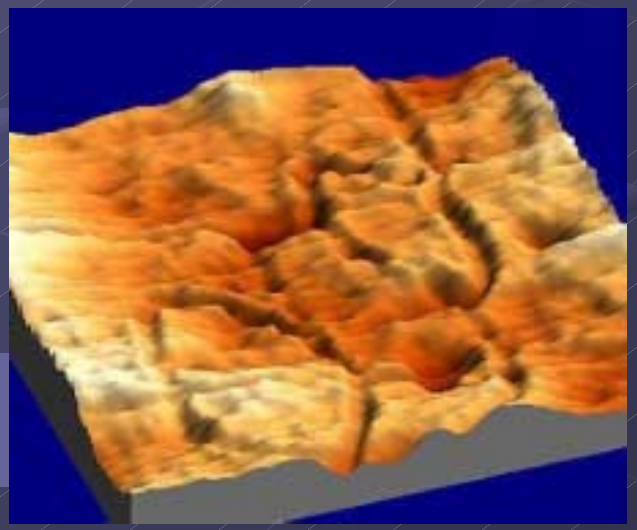


USE OF NANOTECHNOLOGY IN THE COSMETIC INDUSTRY: SURFACE FINE STRUCTURE OF HAIR (AFM)





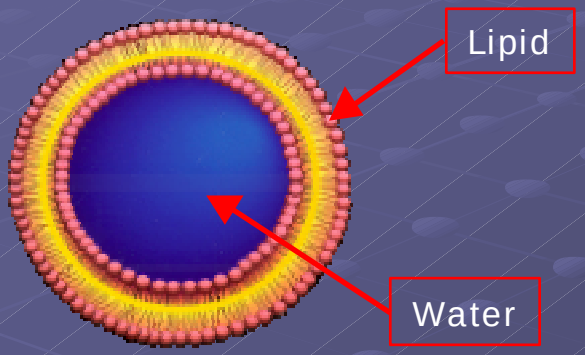




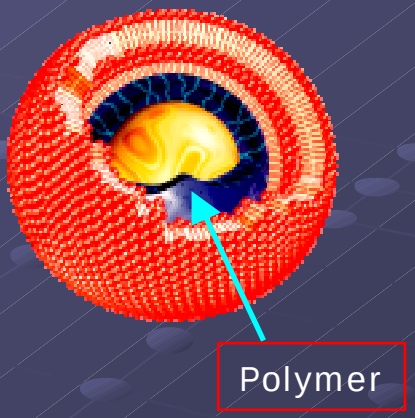
NANO-SIZED COSMETIC FORMULATIONS

Examples of nanomaterials

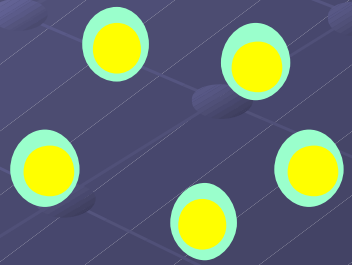
Liposomes
100-300 nm
(Caffeine)



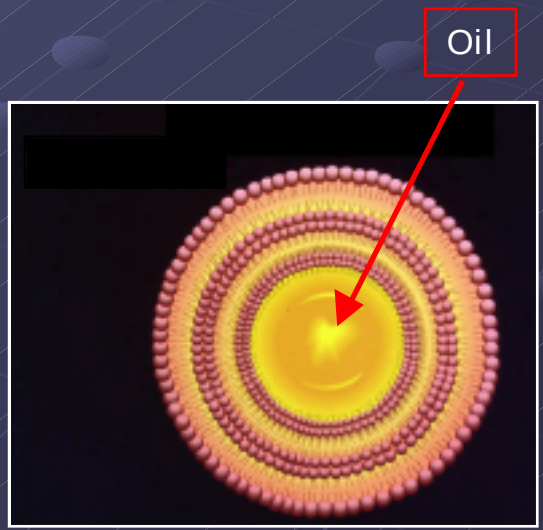
Nanocapsule
100-600 nm
(Vitamin A, E)



Nanoemulsion
50 nm
(transparent)



Oleosomes
150-500 nm



NANOTECHNOLOGY IN COSMETICS

Nanotechnology-based innovations

Innovation	Product	Benefit of nanotechnology
Nanoemulsion	Hair conditioner	Unique texture, transparency
Nanocapsule	Skin care	Protects & transports active ingredient
Nanopigment	Sunscreen	Filters UV rays, consumer compliance

NANO-SIZED COSMETIC FORMULATIONS DO NOT PRODUCE PENETRATION INTO OR THROUGH THE LIVING SKIN

- A complete review on liposomes in cosmetics and drugs concluded that liposomes do not penetrate through the intact stratum cormeum (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 form hard capsules were found only in / on the superficial leyers of the SC.

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

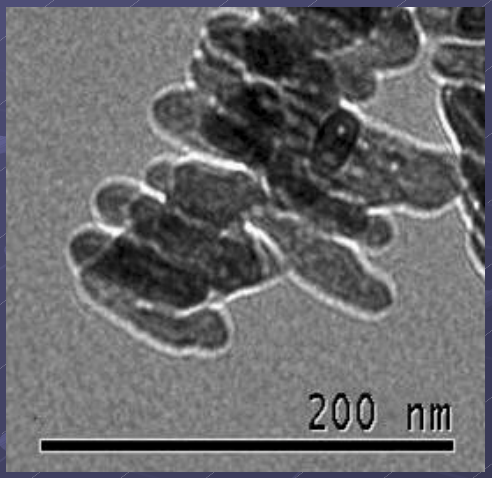
- Imbert D and Wickett R: Topical delivery with liposomes. *Cosmetics and Toiletries magazine*. 1995; **111**:32-45.
- Honeywell-Nguyen P et al.: Quantitative assessment of the transport of elastic and rigid vesicle components and a model drug from these vesicle formulations into human skin in vivo. *Journal of Investigative Dermatology*. 2004; 123(5):902-10.
- Van den Bergh B et al.: Interactions of elastic and rigid vesicles with human skin in vitro: electron microscopy and two-photon excitation microscopy. *Biochimica and Biophysica Acta*. 1999; **1461**:155-173.

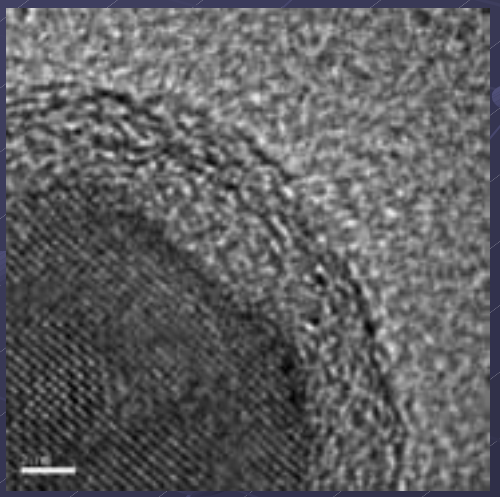
NANOTECHNOLOGY IN COSMETICS: SUNSCREENS

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).





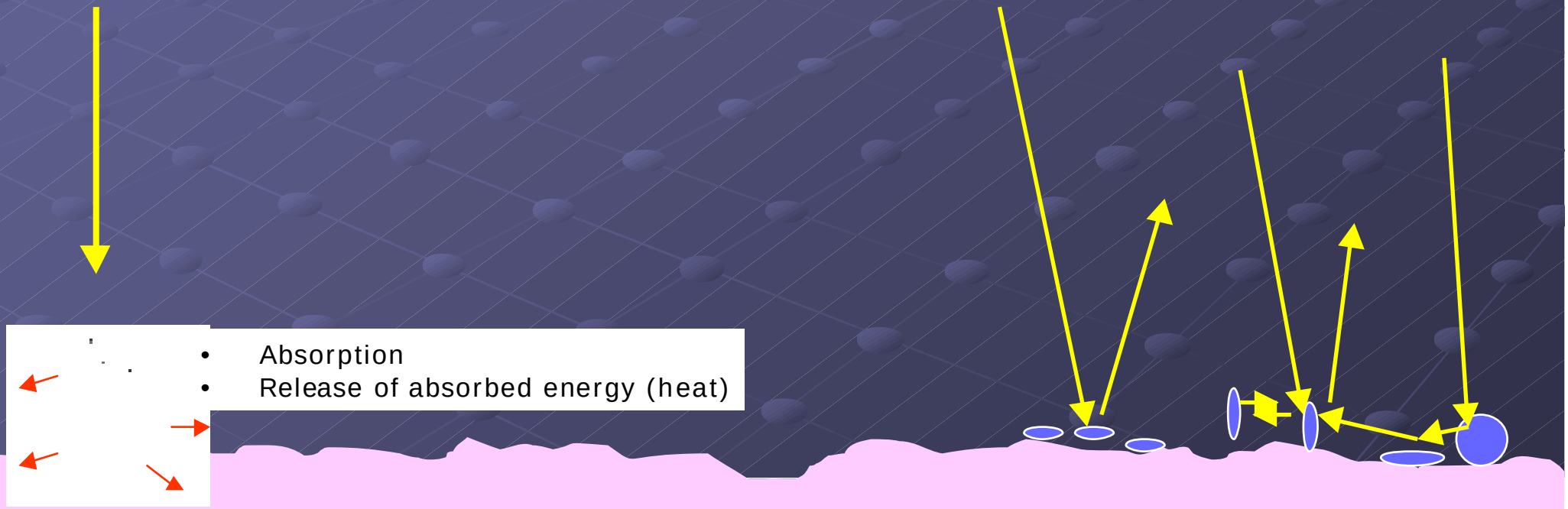
MODE OF ACTION OF CHEMICAL AND PHYSICAL SUNSCREENS

ORGANIC UV FILTER (4-MBC)

INORGANIC UV FILTER

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

LIGHT



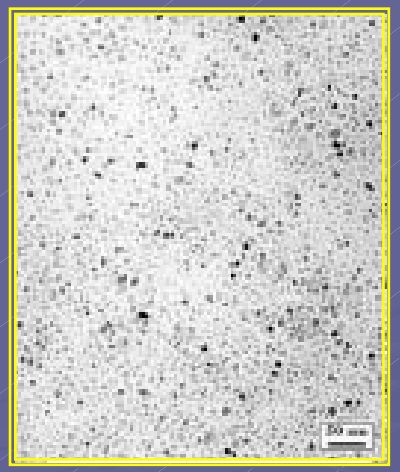
SKIN

GJN, 3/2006

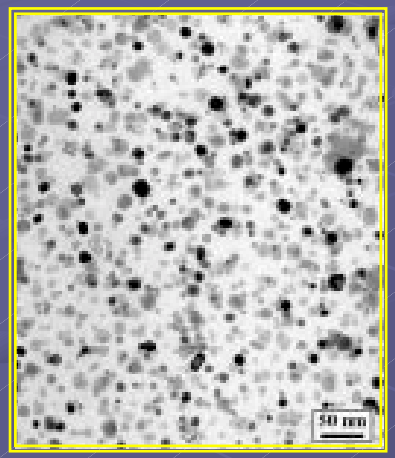
RISK ASSESSMENT OF HUMAN DERMAL EXPOSURE TO NPs: SUNSCREENS

- 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)?

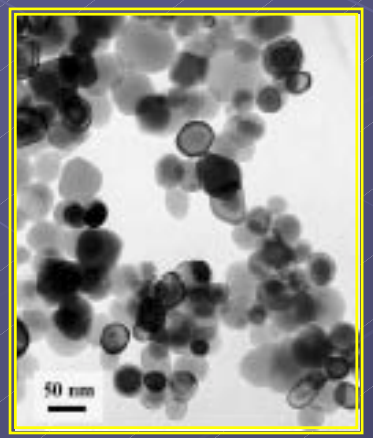
Light scattering depends on particle size: too small is not useful!



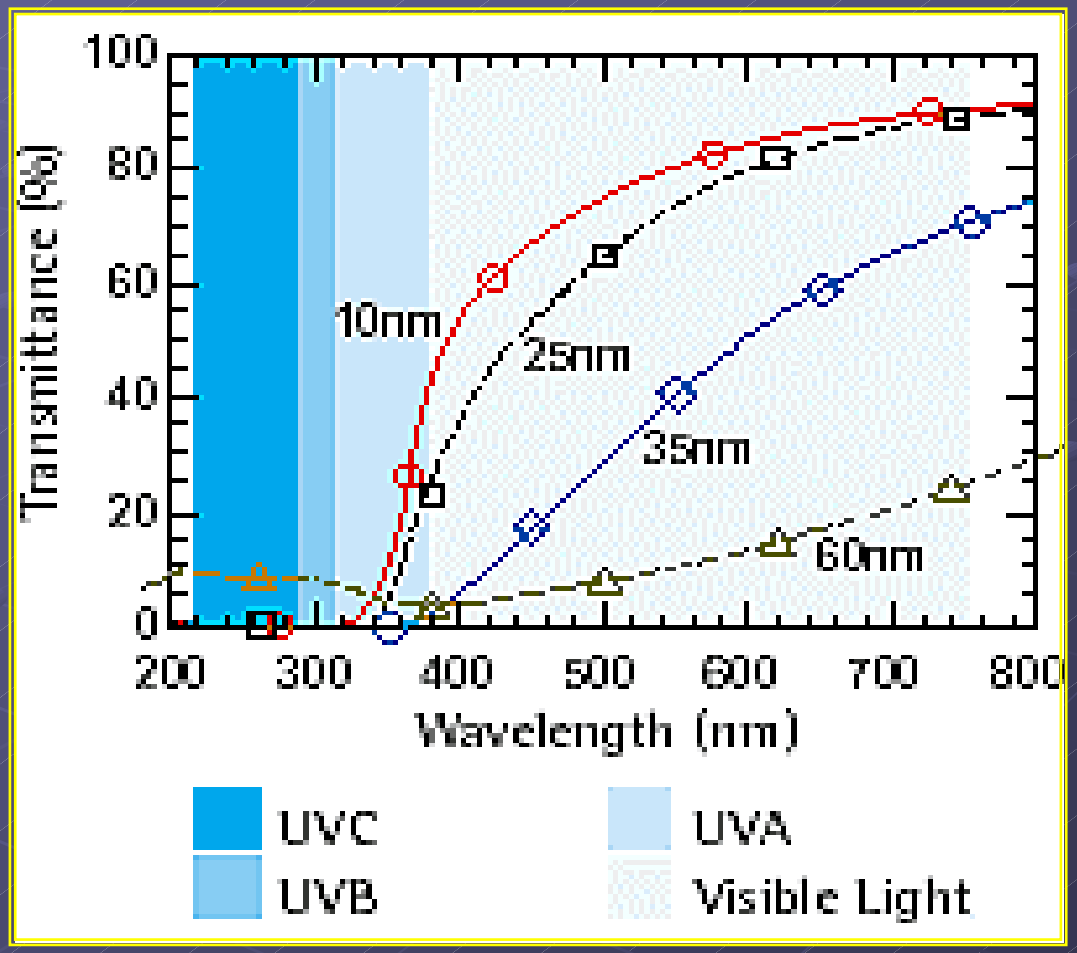
CeO_2 5-10 nm



CeO_2 20 nm



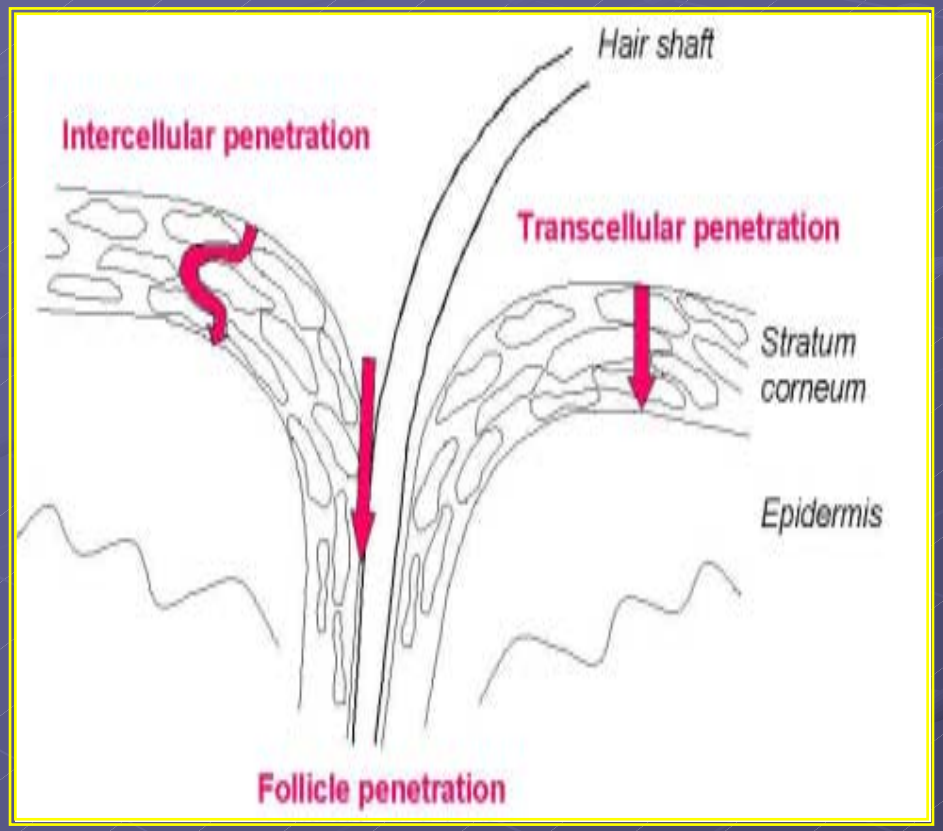
CeO_2 50 nm

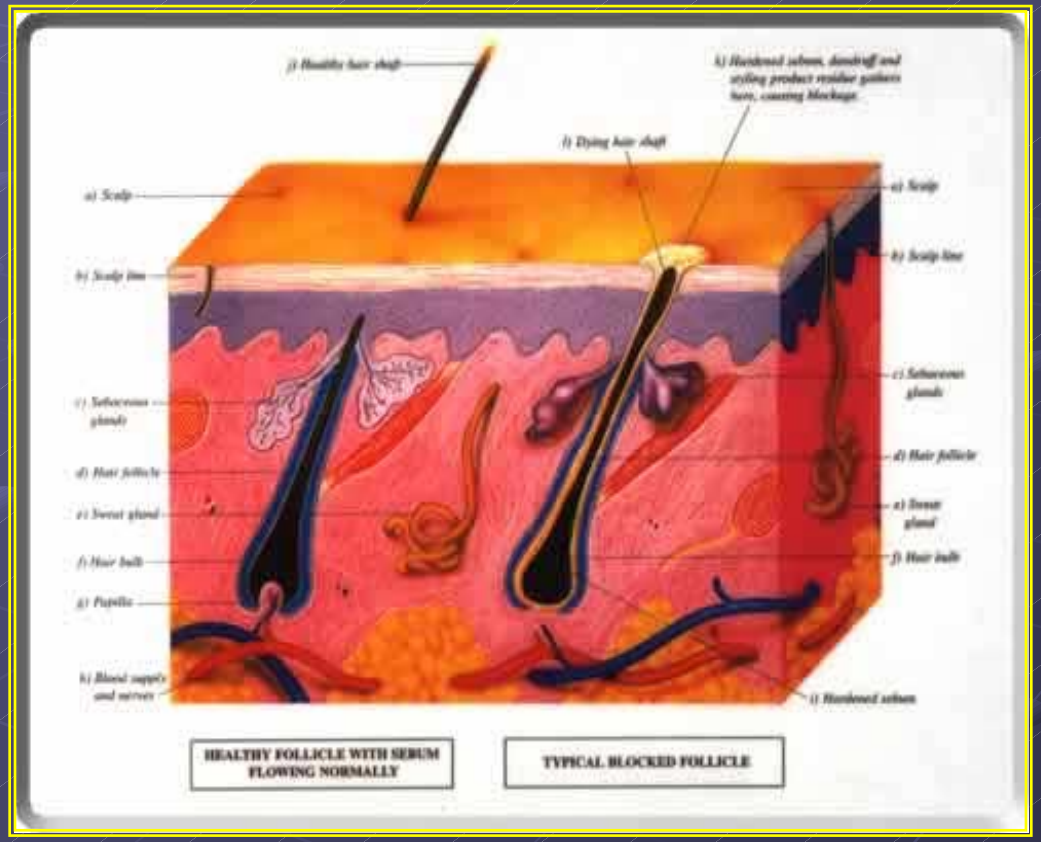


NOTE: NPs at 60 nm provide best UV protection

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

STUDY	MATERIAL	RESULTS
Tan et al., 1996	Microfine TiO_2	No penetration into epidermis / dermis in vivo, man
Lademann, 1999	Microfine TiO_2	
Pflücker et al., 2001	TiO_2, 10 and 100 nm	
Alvarez-Roman et al., 2004	Polystyrene NPs, 20 and 200 nm	No penetration into epidermis / dermis, accumulation in the follicle orifice, but no penetration ibto living skin (pig)
Howard, P, 2005 (SoT Meeting, 2005)	Fluorescent polymeric NPs	No penetration into epidermis / dermis in vivo, man
EU Nanoderm project (Tilmann Butz)	Fluorescent particles	No evidence for penetration into living skin (preliminary data, ECETOC, 11/2005)

CONCLUSION: NO EVIDENCE THAT TOPICALLY APPLIED NANOPARTICLES PENETRATE INTO NORMAL SKIN
(NPs will always penetrate less than a compound in solution!)

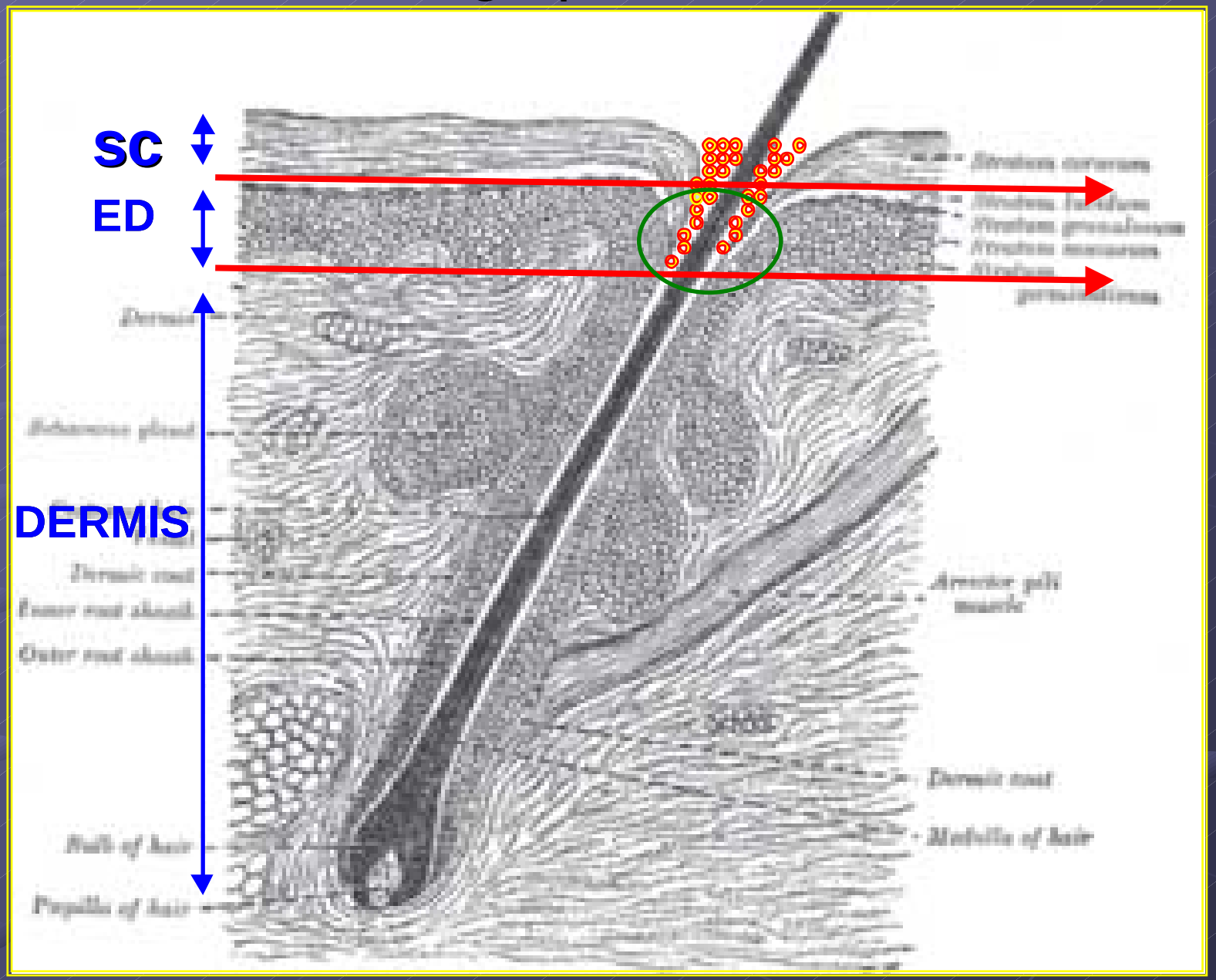
IN VITRO PERCUTANEOUS PENETRATION OF TiO_2 AND ZnO IN PIG SKIN (4 mg/cm², 24-hrs): ABSORBED DOSE = ZERO *

TEST MATERIAL	SKIN WASH (%)	TAPE STRIPS (%)	SKIN (%)	RECEPT. FLUID (%)	ABSORBED DOSE (%)	RECOV. (%)
TiO_2, 30-60 x 10 nm, silica / methicone-coated O/W emulsion (10%)	97.7 to 100.2	0.1 to 0.2	0.1 to 0.3	0.0	0.0	98.2 to 100.4
TiO_2, 30-60 x 10 nm, methicone-coated O/W emulsion (10%)	85.4 to 92.9	0.0 to 0.3	0.1 to 0.5	0.0	0.0	86.1 to 93.0
ZnO, 80 nm, O/W emulsion (10.3%)	NP	98.6 to 102.3	1.4 to 1.5 **	0.8 to 1.4 **	0.0	102.3 to 106.8

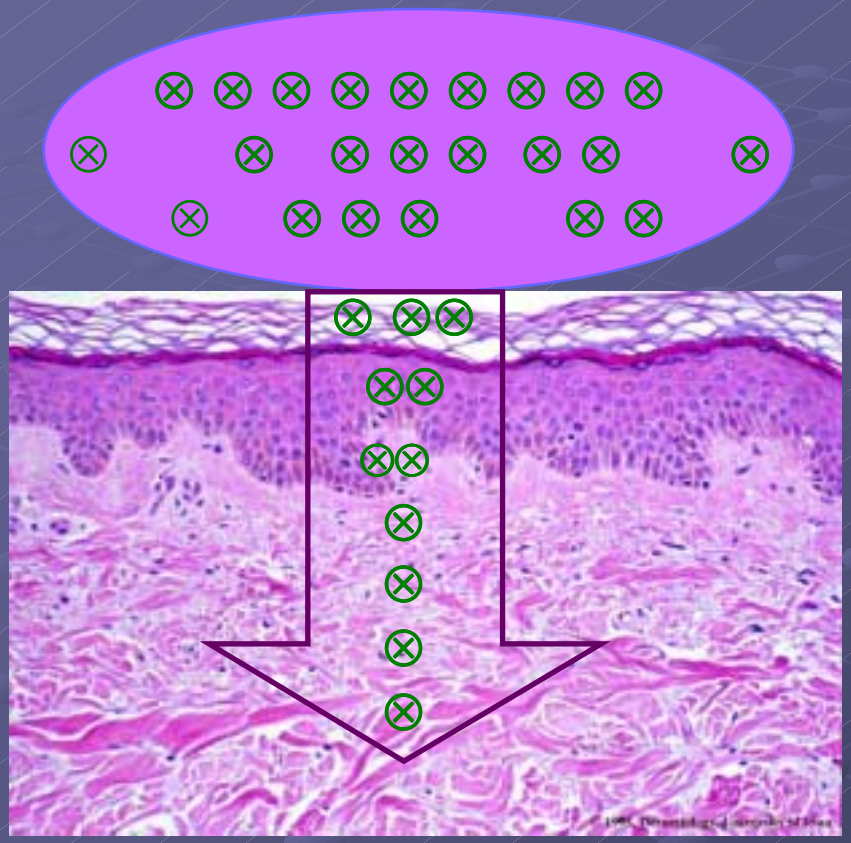
CONCLUSION: NO EVIDENCE FOR PERCUTANEOUS PENENTRATION

Gamer et al., *Toxicol. In Vitro*, BASF, 2005; ** values at or below background levels

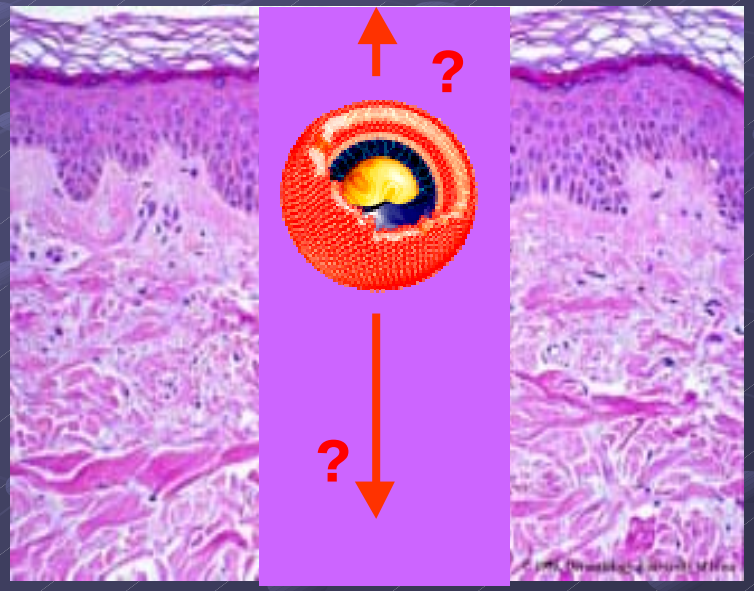
Pseudo-penetration: nanoparticles in/on the hair follicle orifice may be mis-interpreted as penetration into the living epidermis



SKIN PENETRATION OF SMALL MOLECULES IN SOLUTION VS. INSOLUBLE NPs*

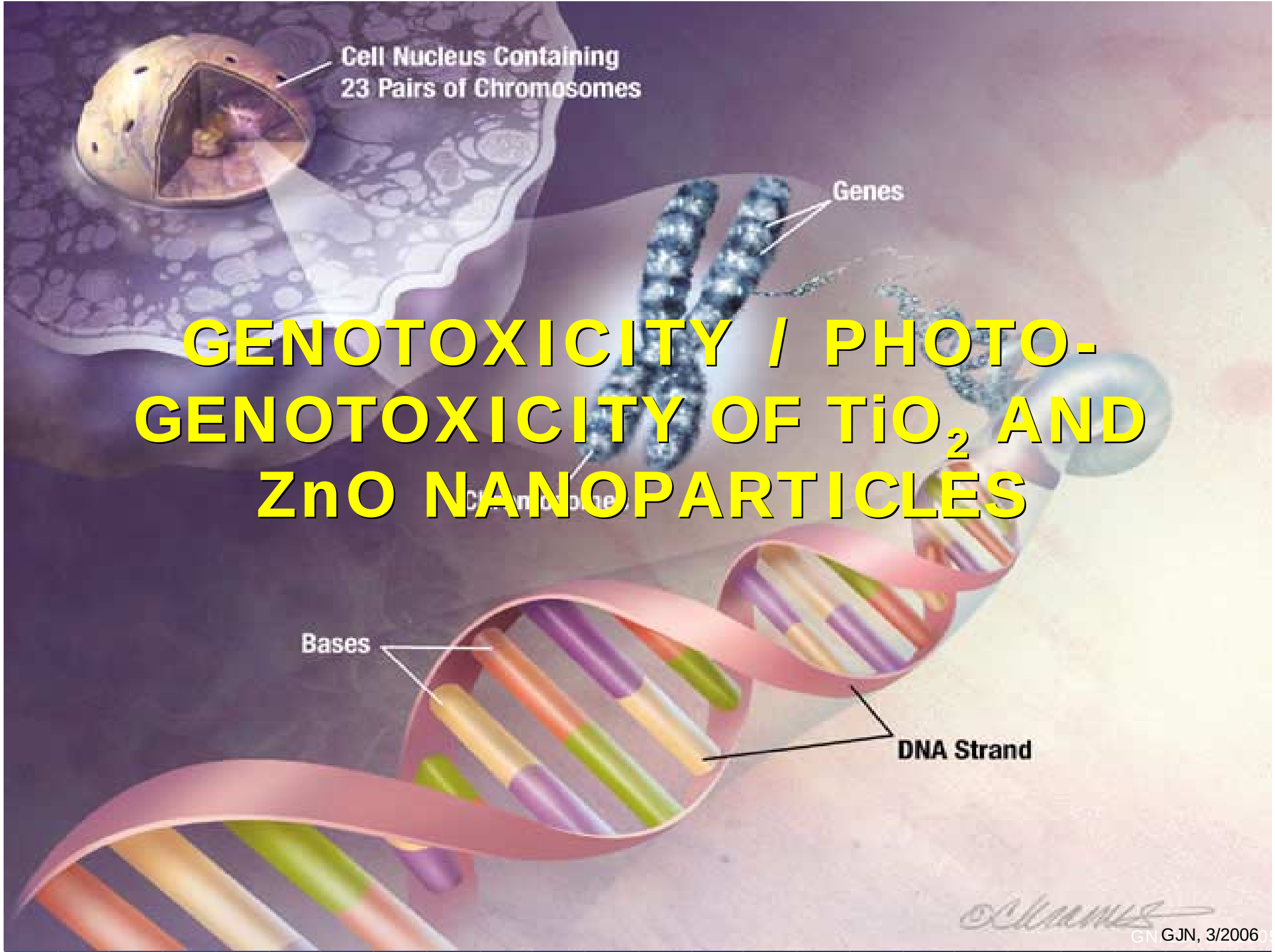


DIFFUSION OF MOLECULES INTO THE SKIN IS LIKE A BREAKING DAM



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 / PHOTO-GENOTOXICITY OF TiO_2 AND ZnO NANOPARTICLES

PHOTO-GENOTOXICITY / PHOTO-CARCINOGENICITY OF TiO_2: PROTECTION

AUTHORS	MATERIAL	STUDY TYPE	RESULTS
Bestak and Halliday, 1996	TiO_2, no particle size given	Photo-carcinogenicity in DMBA pre-treated mice	Protection
Suzuki, 1987	TiO_2, no particle size given	Photo-carcinogenicity in hairless mice, pre-treated with Croton oil	Protection
Greenoak et al., 1993	TiO_2, no particle size given	DNA damage in mouse skin	Protection

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

GENO- AND PHOTO-GENOTOXICITY OF TiO_2 PARTICLES (AMES TEST, CHO CELLS)

NAME	CRYSTALLINE FORM	MEAN PARTICLE SIZE (nm)	COATING	Ames / Photo-Ames	CHO / Photo-CHO
1	Rutile	14	Al_2O_3/Dimethicone	negative	negative
2	Anatase	60	Al_2O_3/SiO_2	negative	negative
3	Anatase	60	Uncoated	negative	negative
4	Anatase	200.000	Uncoated	negative	negative *
5	Rutile	20	Al_2O_3/Dimethicone	negative	negative
6	Rutile	17	Al_2O_3/Stearic acid	negative	negative
7	Rutile	20	Uncoated	negative	negative
8	Rutile	15	Al_2O_3/Stearic acid	negative	negative
9	Rutile	15	Uncoated	negative	negative
10	Rutile	11-28	Al_2O_3/SiO_2	negative	negative

* 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 cytoxic 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 opnion of the SCCNFP, 24 October 2000, http://europa.eu.int/comm/health/ph_risk/committees/sccp/sccp_opinions_en.htm

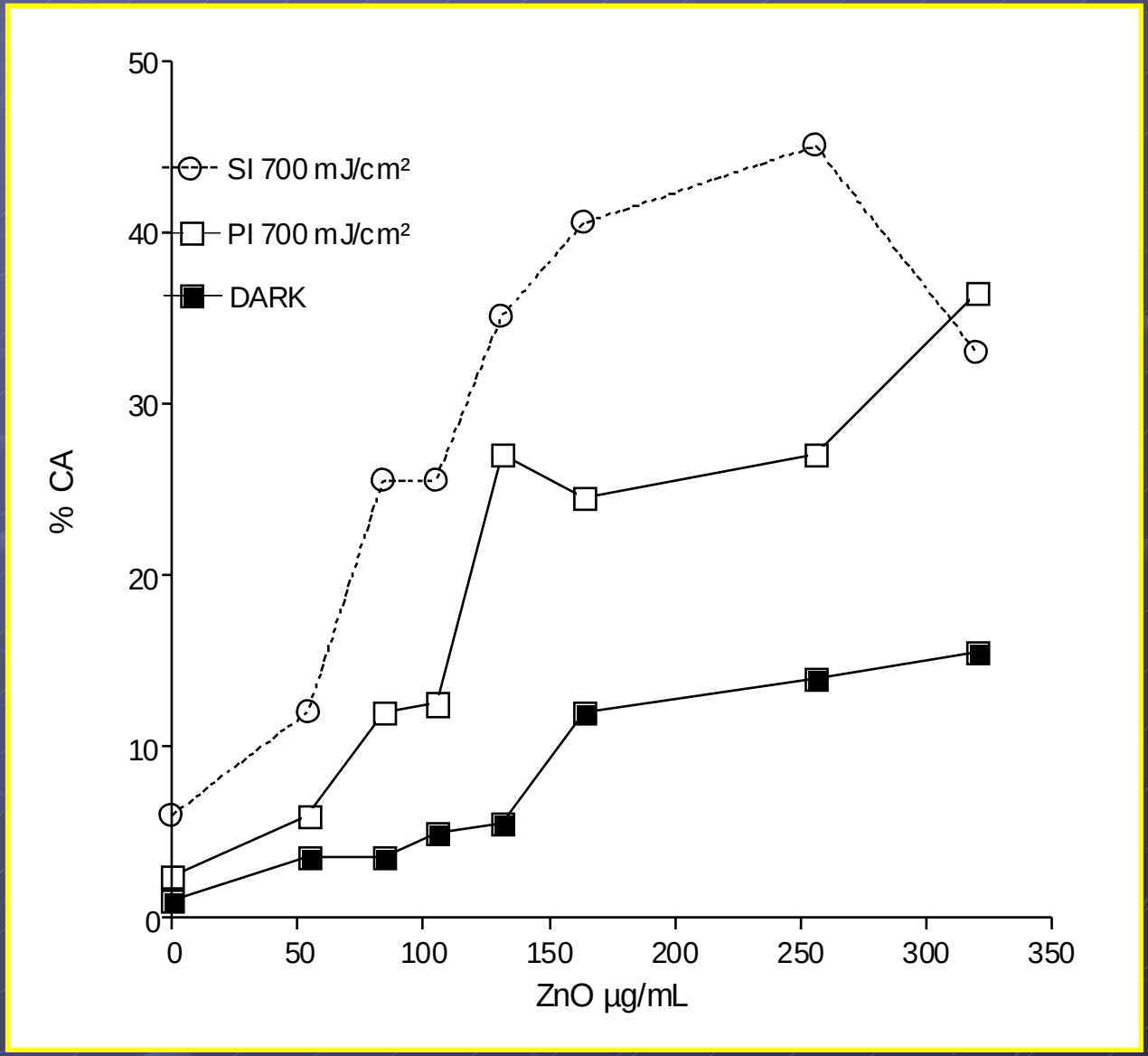
GENOTOXICITY / PHOTO-GENOTOXICITY OF ZINC OXIDE (ZnO) NPs: RESULTS OF GLP STUDIES

TEST	TEST ORGANISM	RESULT	COMMENT
Ames test	*S. typhimurium*	Negative	Non-photo-genotoxic
Photo-Ames test	*S. typhimurium* TA98, 100, 1537	Negative	Non-photo-genotoxic
In vitro clastogenesis	CHO	Positive at =814 µg/mL	Clastogenic
Photo-clastogenesis *	CHO	Positive at =195 µg/mL	Photo-clastogenic?
In vitro clastogenesis	V-79	Positive at 10.0 or 20.0 µg/mL	Clastogenic
Photo-clastogenesis *	V-79	Positive at 2.5 or 3.0 µg/mL	Photo-clastogenic?
Comet test / photo-comet test	Human keratinocytes, V-79 cells	Negative (kc), slightly positive (V-79)	Equivocal

4-FOLD INCREASE IN CLASTOGENIC POTENCY: « PHOTO-CLASTOGENIC »?

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) *



* Dufour et al., *Mut. Res* 2006 (in print)

NANO-HYPE and NANO-FACTS

HYPE	FACT
NPs are carcinogenic after inhalation	Inert NPs (TiO_2, CB) were carcinogenic in rats after chronic lung overload: irrelevant for man under normal exposure conditions
Inhalation of NPs produces new toxicities. NPs more toxic than MPs	Some NPs (TiO_2, CB) were somewhat more toxic than MPs, others (SiO_2, ZnO) equally or less toxic
Inhaled NPs are systemically absorbed and affect the CV system and the brain.	Some evidence for syst. absorption (lung overload), no evidence for adverse systemic effects, translocation to the brain needs to be confirmed.
Inhalation of NPs produces asbestos-like pulmonary toxicity	SWCNTs are µ-sized, insoluble fibres and produce toxicity typical for fibres
Oral uptake of NPs produces systemic exposure of the organism	Evidence unclear: minor syst. exposure after oral uptake of some NPs, no evidence for others. No evidence for adverse systemic effects.
NPs penetrate through the skin and produce systemic exposure	No evidence for penetration into or through the living skin. Damaged skin needs confirmation, but no mechanism suggesting active penetration.
NPs have distinct toxicological properties (NANO-TOXICOLOGY!)	LITTLE OR NO EVIDENCE FOR COMMON TOXICITIES OF NPs

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!

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