National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Safety assessment of nanomaterials.

What about extrapolation between ENM? Read across and categorization.

Wim H De Jong, DVM, PhD.
Centre for Health Protection,
National Institute for Public Health and the Environment,
Bilthoven, The Netherlands.

24 October 2014

SCENIHR 2009 Opinion

Risk Assessment of Products of Nanotechnologies

The hypothesis that smaller means more **reactive** and thus more **toxic** **cannot be substantiated by the published data**. In this respect nanomaterials are similar to normal substances in that some may be toxic and some may not”

Although this may be considered comfortable that in principle there is no difference between “normal chemicals” and nanomaterials, it also has the implication that nanomaterials should be investigated on a **case-by-case** basis as the risks cannot be estimated beforehand.

Classical approach of risk assessment is applicable

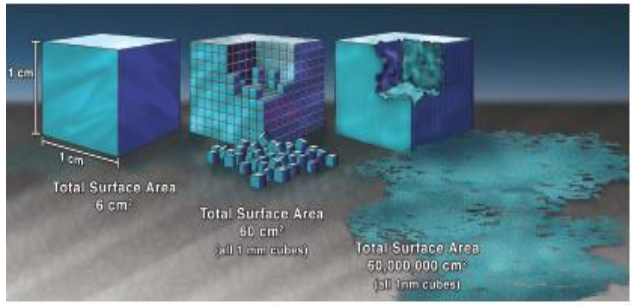
Exposure assessment/Hazard identification/
Hazard characterization/Risk characterization

Main question/concern for safety

Increase in surface area >> increase in surface activity, but also increase in possible contact with cells and tissues

Does this also result in increased toxicity?

In view of the multitude of nanomaterials expected to be developed, the question arises whether we do need to test all new (modified) nanomaterials for safety aspects or is extrapolation between nanomaterials possible?



Why is risk assessment of nanomaterials difficult?

- Knowledge gaps in risk assessment
  - Nanoparticle characterization, detection, and measurement
  - Dose responses of possible effects (including, what is the dose?)
  - Fate and persistence of NP in humans and environment
  - Aspects of (eco)toxicology (interaction at sub-cellular and molecular levels)

In view of uncertainties extrapolation from convential form of "large" particles considered not possible.

But what is possible within the nanomaterial domain itself?

What we already know/use (nanodefinition)

- SCENIHR Opinion on "Scientific basis for the definition of the term 'nanomaterial' " (2010)
  - Most physicochemical characteristics not useful for definition
  - Only size is appropriate characteristic to be used in definition

- EU Recommendation for a definition of nanomaterials (2011)
  - Sets NM apart as group particulates (1 – 100 nm)

- Auffan et al., 2009
  - Size not sufficient for definition
  - Novel size dependent properties
  - Inorganic nanoparticles (metal and metal oxide NP)
  - Properties affected/changing when size below 20 – 30 nm

What do we want to know for Risk Assessment?

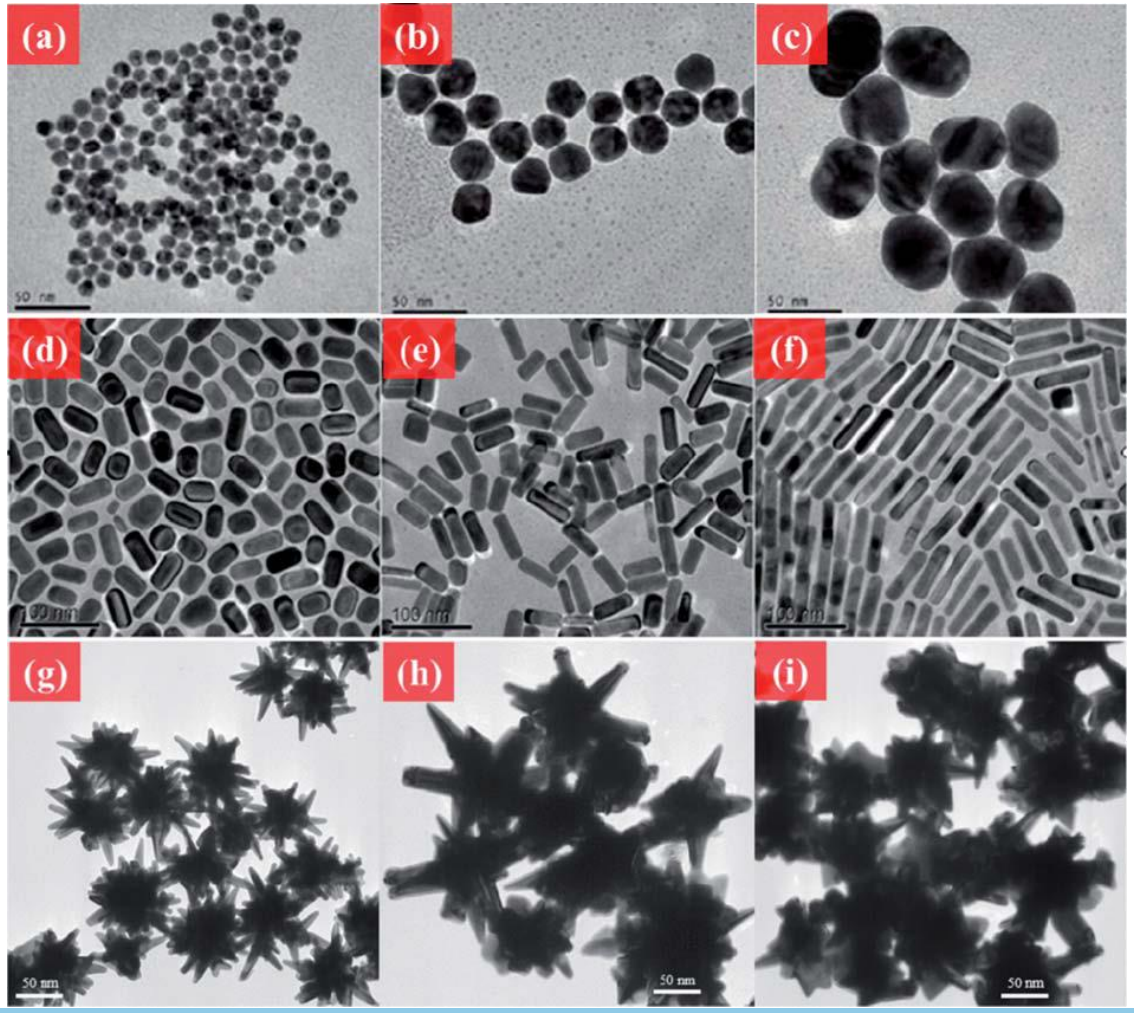
We really want to know which physico-chemical characteristics drive potential adverse (toxic) effects.

Biological behaviour/toxicokinetics

Complexity of grouping of nanomaterials

- More variation in physchem parameters possible that can affect exposure, kinetics and hazard of a material than for 'normal chemicals'
- Physchem parameters that may affect exposure, kinetics and/or hazard
  - Size
  - Aggregation/agglomeration
  - Shape
  - Coating/surface functionalisation/surface chemistry
  - Surface charge
  - Dissolution rate
  - Composition
  - Reactivity
  - Photoreactivity
  - …

Various forms of Au nanomaterials



Cheng et al.,Nanoscale, 2013

Effect of shape on biological responses

nature nanotechnology | VOL 3 | JULY 2008 | 423

LETTERS

Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study

CRAIG A. POLAND[1], RODGER DUFFIN[1], IAN KINLOCH[2], ANDREW MAYNARD[3], WILLIAM A. H. WALLACE[1], ANTHONY SEATON[4], VICKI STONE[5], SIMON BROWN[1], WILLIAM MacNEE[1] AND KEN DONALDSON[1]*

The Journal of Toxicological Sciences (J. Toxicol. Sci.)
Vol.33, No.1, 105-116, 2008

Original Article

Induction of mesothelioma in p53+/– mouse by intraperitoneal application of multi-wall carbon nanotube

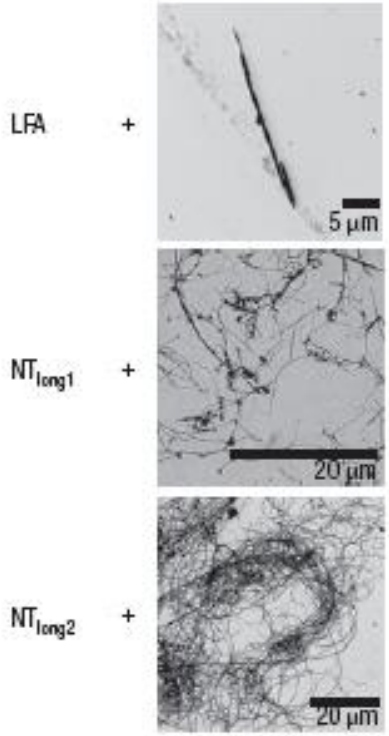
Atsuya Takagi[1], Akihiko Hirose[2], Tetsuji Nishimura[3], Nobutaka Fukumori[4], Akio Ogata[4], Norio Ohashi[4], Satoshi Kitajima[1] and Jun Kanno[1]

TOXICOLOGICAL SCIENCES 110(2), 442–448 (2009)
doi:10.1093/toxsci/kfp100
Advance Access publication May 8, 2009

Sakamoto Y, Nakae D, Fukumori N, Tayama K, Maekawa A, Imai K, Hirose A, Nishimura T, Ohashi N, Ogata A.
Induction of mesothelioma by a single intrascrotal administration of multi-wall carbon nanotube in intact male Fisher 344 rats.
The Journal of Toxicological Sciences, 34, 65-76, 2009

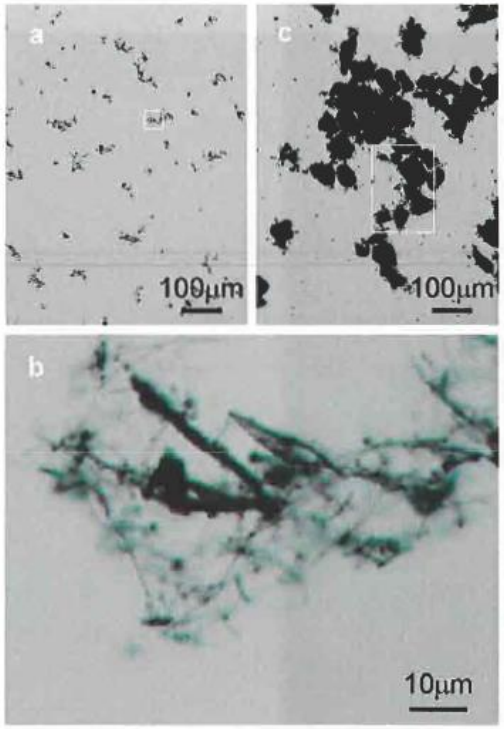
Absence of Carcinogenic Response to Multiwall Carbon Nanotubes in a 2-Year Bioassay in the Peritoneal Cavity of the Rat

Julie Muller,* Monique Delos,† Nadtha Panin,* Virginie Rabolli,* François Huaux,* and Dominique Lison*,[1]



Poland et al 2008

MWCNT induce chronic inflammation



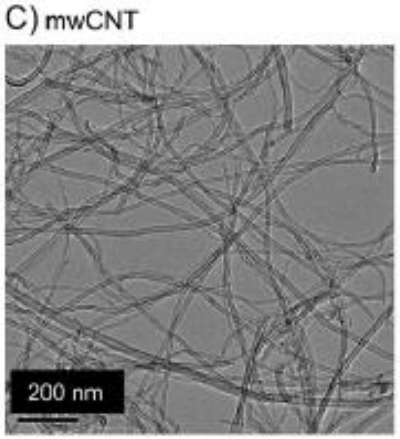
Takagi et al 2008, Sakamoto et 2009

MWCNT induce tumors in P53 mice

and F344 rats

Nygaard et al 2009

CNT act as adjuvant



Muller et al 2009

MWCNT do NOT induce tumors in 2 year study

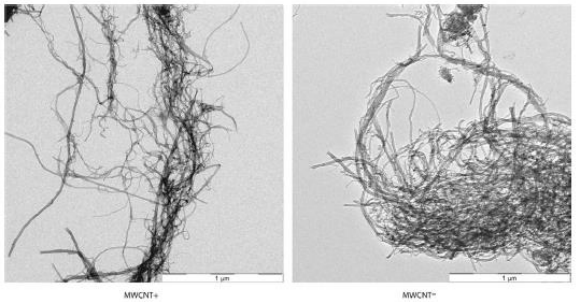
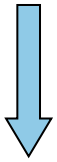


FIG. 1. Transmission electron microscopy images of the carbon nanotubes. MWCNT +, unheated multiwall carbon nanotubes and MWCNT–, multiwalled carbon nanotubes heated at 2400°C under argon. The images were obtained on a Leo 922 (Zeiss), 200 kV.

Nanofibres, the CNT issue

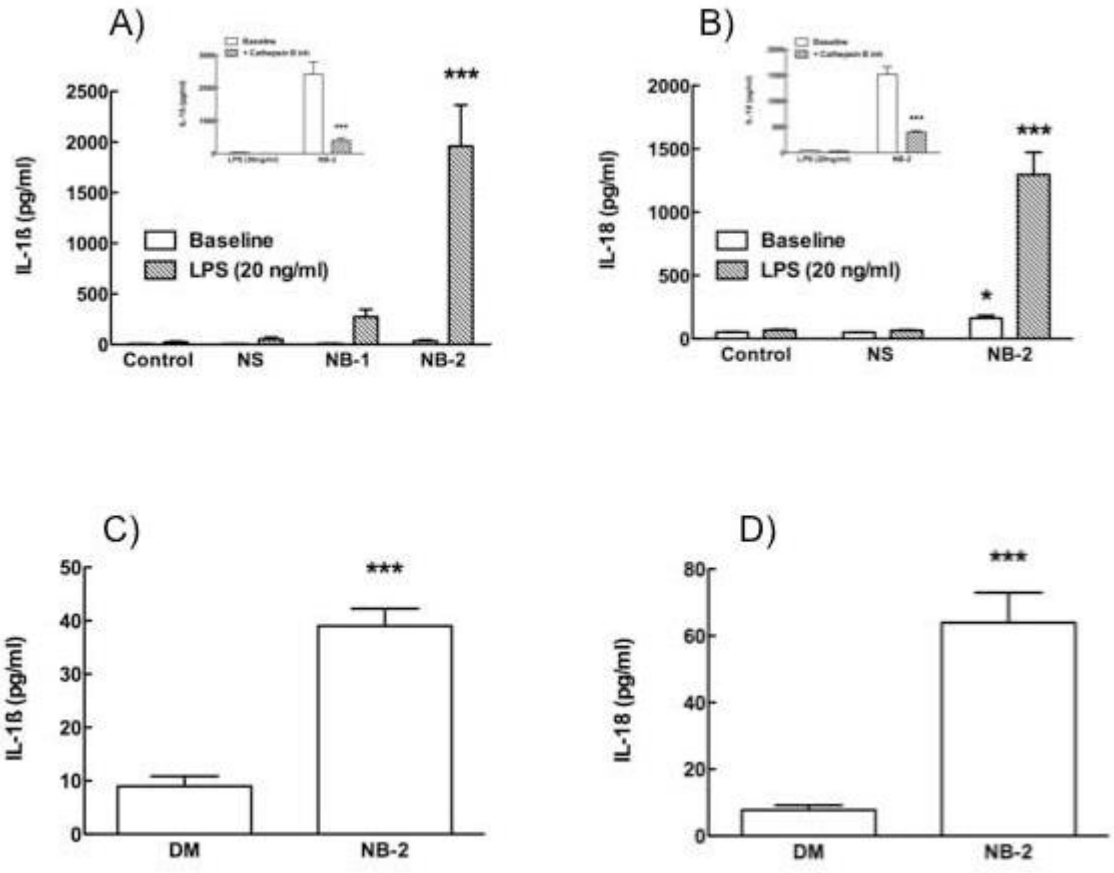
There are different types of MWCNT
"when a fibre has characteristics of brown/blue asbestos
(rigid, non degradable, length >20 µm)
it behaves like brown/blue asbestos"
(Poland et al., 2008, Donaldson et al., 2010)

Lesson is NOT
MWCNT behave like asbestos but.....
.....when producing and using MWCNT
or any fibre-like nanomaterial
Check for these specific characteristics
(rigidity, degradability, fibre length)



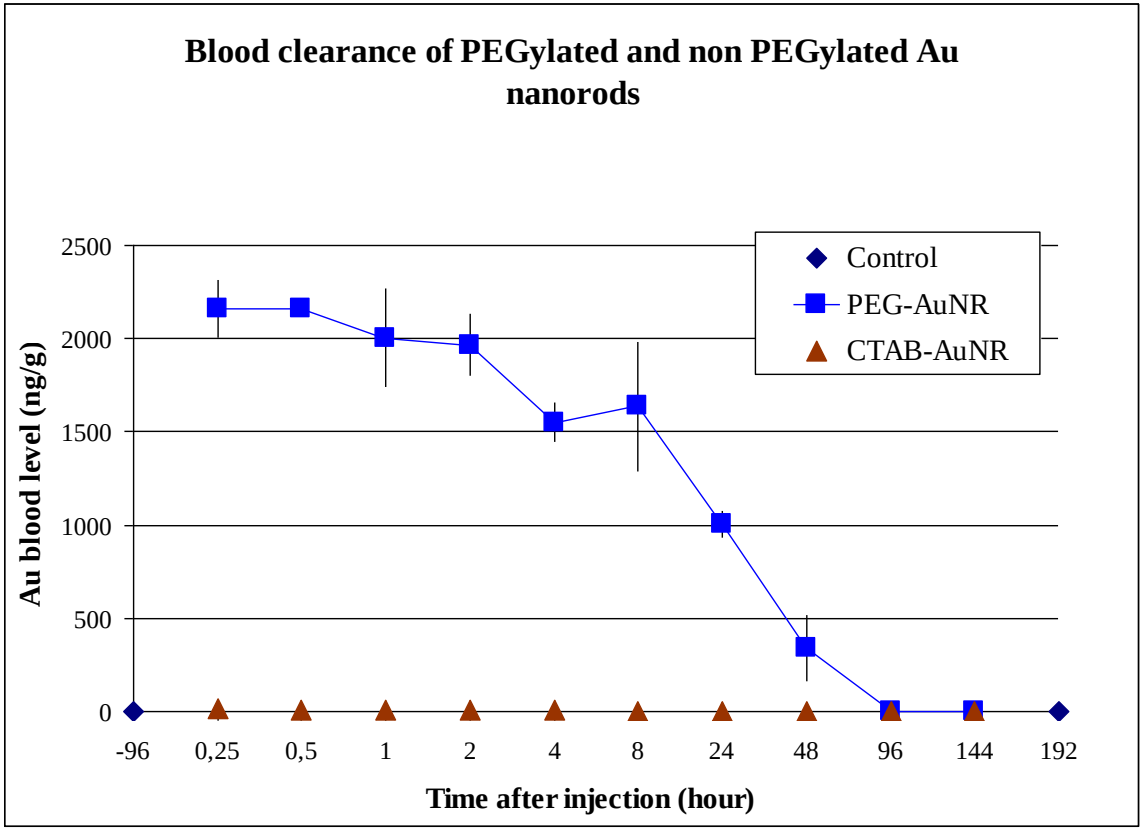
Perform proper safety evaluation to exclude this specific hazard associated with a certain types of fibres.
Including extensive characterization.

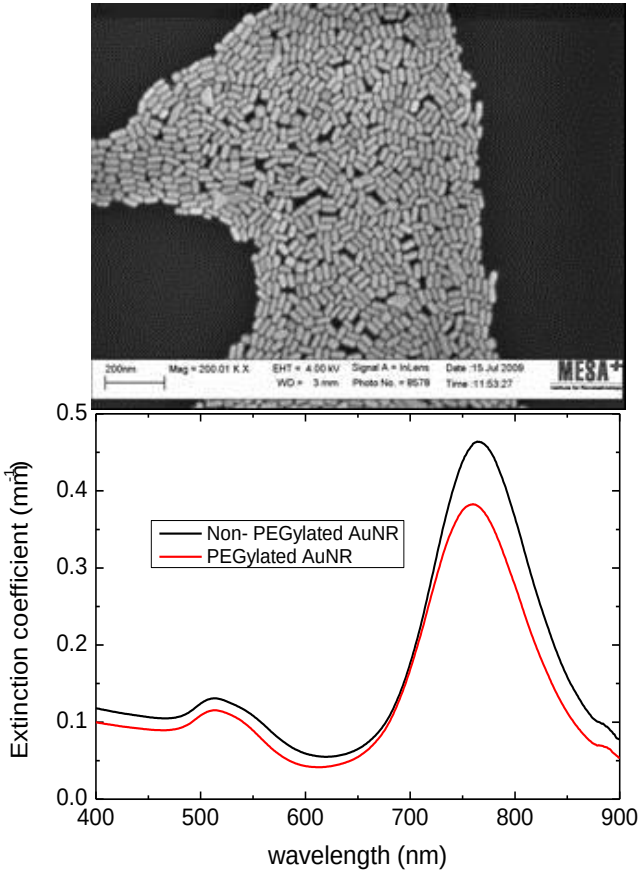
TiO_2 nanobelts induce inflammatory cytokines



Hamilton et al., 2009

Effects of surface (PEG coating) of gold nanorods on toxicokinetics





Lankveld et al., Nanomedicine, 2011

Present at the moment

- Broad, *general groups* based on physchem, i.e.
  - Carbon based nanomaterials
  - Metals and metaloxides
  - Nanotubes/wires

- Efforts to gain *detailed insights* in relationships between physicochemical characterization and a specific effect with aim to determine groups
  - High throughput screening

- The purpose of grouping/categorization can be different
  - To steer the testing strategy: where to focus on (prioritization)?
    - › Broad, general groups can give valuable input
  - To fill in datagaps by info from other materials (read-across for risk assessment)

Some characteristics already known.

- Nanoparticle charge
- Nanoparticle solubility
  - Release of toxic ions
- Composition
  - Impurities, **coatings**
- Shape
  - Carbon nanotubes, rigidity

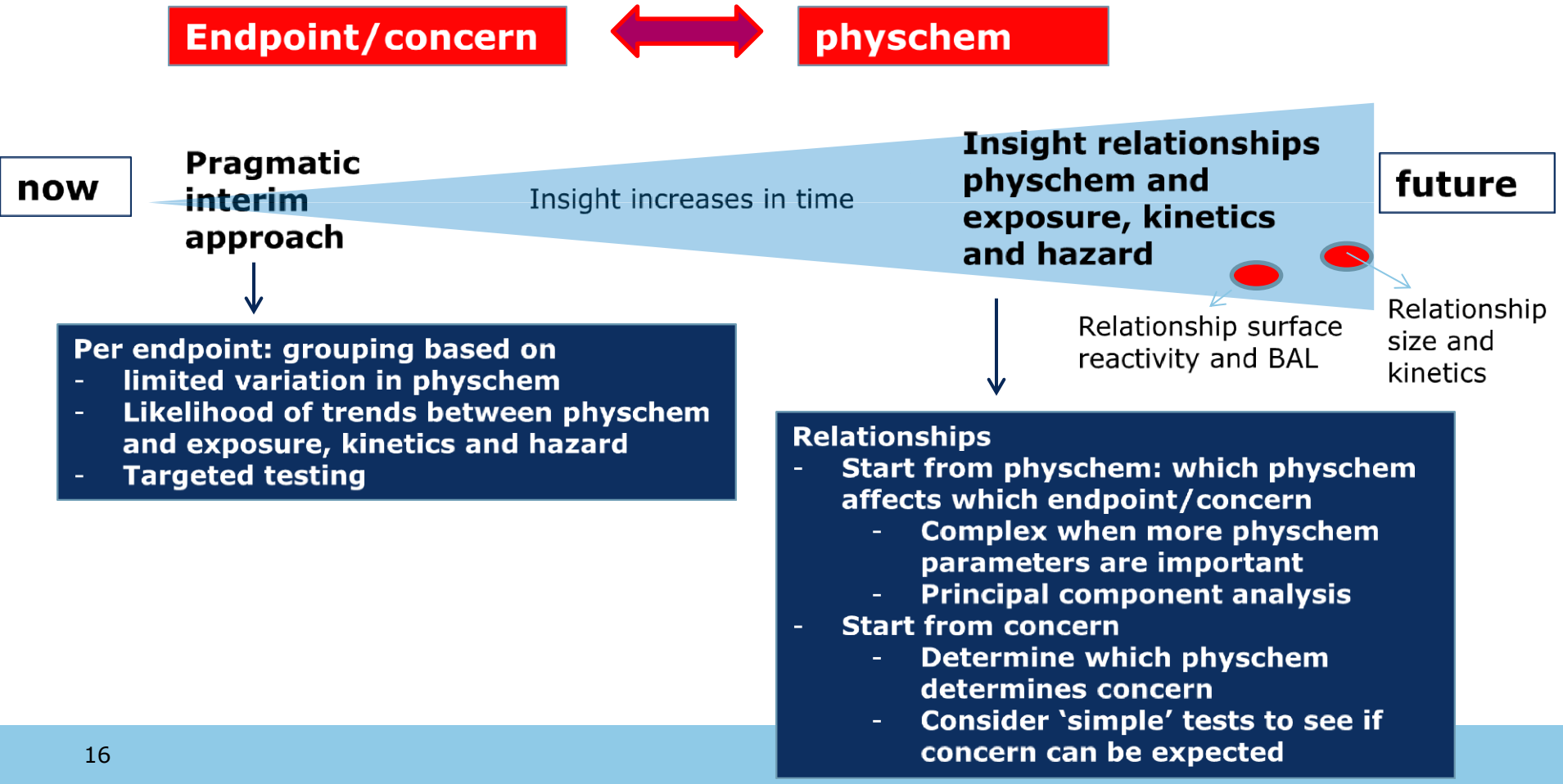
- Biological behaviour
  - **Toxicokinetics**
  - Ability to cross biological barriers (size, coating)

For grouping much more information is needed.

What could be used as interim solution?

Donaldson and Poland 2013

Grouping that allows for datagap filling

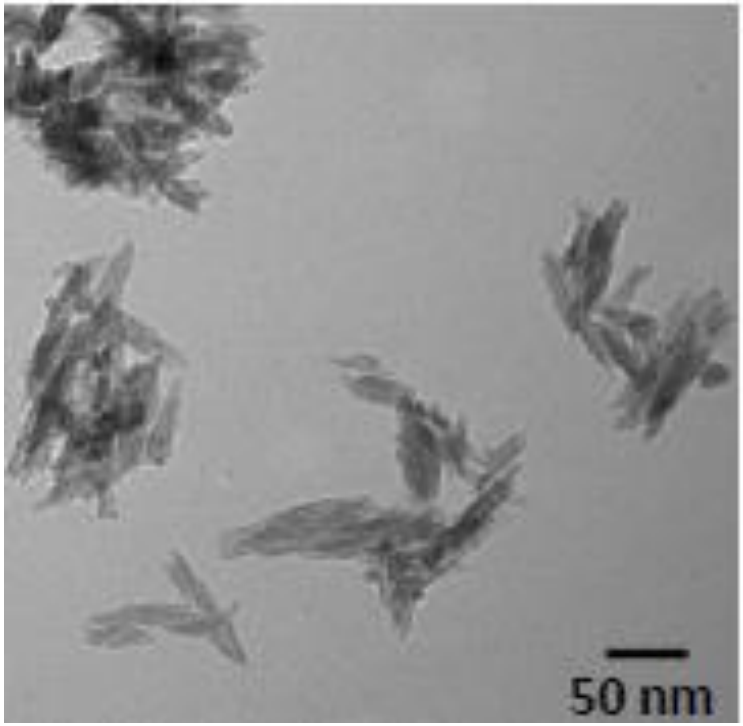


Example: SCCS TiO_2 evaluation

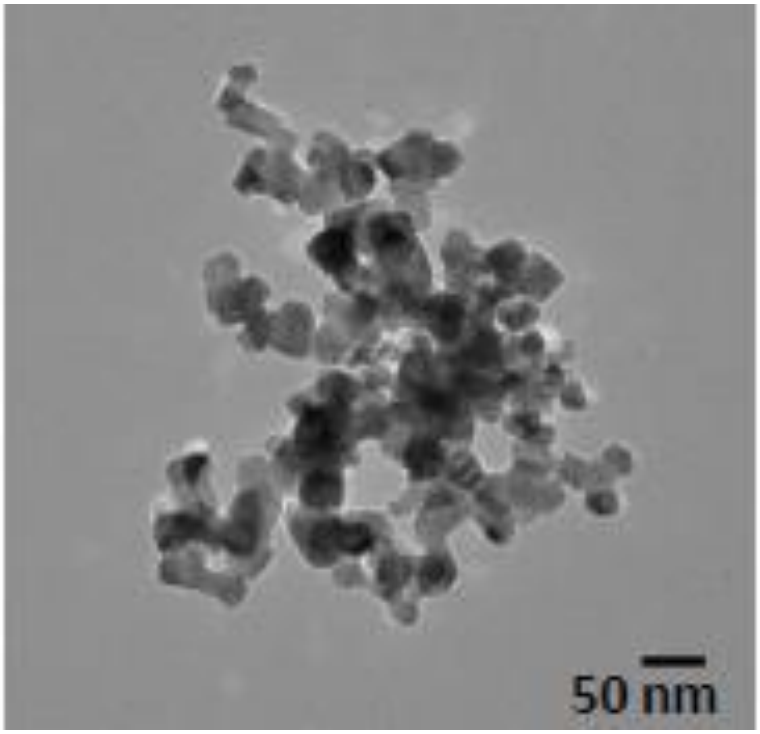
Material code	TiO2 crystaline form	Coating material	Doping material	Form	Bulk density (g/cm3)	VSSA (m2 cm-3)
S75-A	> 99.5% Rutile	6% silica, 16% alumina	None	Oil dispersion	0.35	460
S75-B	> 99.5% Rutile	6% silica, 16% alumina	None	Aqueous dispersion	0.35	460
S75-C	> 99.5% Rutile	7.5% alumina, 9,5% aluminium stearate	None	Oil dispersion	0.31	220
S75-D	> 99.5% Rutile	10% alumina, 13.5% stearate	None	Oil dispersion	0.58	300
S75-E	> 99.5% Rutile	10% alumina, 13.5% stearate	None	Aqueous dispersion	0.58	300
S75-F	Anatase 85%, Rutile 15%	7.5% trimethoxycaprylylsilane	None	Hydrophobic powder	0.2	192
S75-G	Anatase 85%, Rutile 15%	None	None	Hydrophilic powder	0.13	213
S75-H	> 99,5% Rutile	6% alumina, 1% glycerin	None	Hydrophilic powder	0.31	260
S75-I	> 99,5% Rutile	7% alumina 10% stearic acid	None	Hydrophobic powder	0.28	300
S75-J	> 99,5% Rutile	6% alumina 1% dimethicone	None	Hydrophobic powder	0.31	260
S75-K	> 94% Rutile	6-8% aluminium hydroxide, 3.5-4.5% dimethicone/methicone copolymer	None	Hydrophobic powder	0.12-0.28	426
S75-L	> 94% Rutile	6.5-8.5% hydrated silica, 2.5-4.5% aluminium hydroxide, 4.5-6.5% dimethicone/methicone copolymer	None	Hydrophobic powder	0.07-0.2	426
S75-M	> 98% Rutile, <2% anatase	17% silica	None	Hydrophilic powder	0.09	260
S75-N	> 95% Rutile, <5% anatase	Alumina 10% simethicone 2%	1000 ppm Fe	Amphiphilic powder	0.16	400
S75-O	100% Anatase	Simethicone 5%	None	Hydrophobic powder	0.75	400

SCCS Opinion on TiO2, 2014

Titanium Dioxide Nanopowder



TEM image anatase 80%/rutile
15x45 nm Titanium Dioxide Powder
www.nanocomposix.com



TEM image rutile
25 nm Titanium Dioxide Powder
www.nanocomposix.com

TiO_2 catalytic activity

Material code	Crystal size	Aspect ratio	UV Absorption (Extinction coefficient)			Zeta potential	Photo-catalytic activity*		Photo-stability	Coating stability
	(XRD)	(L /W)	E308	E360	E400	(IEP)	ΔE	% to Reference		
S75-A	15	3.8	44	20	11	7	3	9	Photo-stable	Stable
S75-B	15	3.8	51	22	12	N/A	3	9	Photo-stable	Stable
S75-C	15	3.7	54	16	7	N/A	7.8	23	Photo-stable	Stable
S75-D	9	4.5	48	7	3	N/A	7.2	21	Photo-stable	Stable
S75-E	9	4.5	50	10	4	N/A	7.2	21	Photo-stable	Stable
S75-F	21	1.2	45	15	8	N/A	11.8	35	Photo-stable	Stable
S75-G	21	1.2	38	16	9	7	25.1	74	Photo-stable	NA
S75-H	21	1.7	30	17	9	7	0.3	1	Photo-stable	Stable
S75-I	15	3.2	38	14	6	N/A	0.8	2	Photo-stable	Stable
S75-J	21	1.5	36	16	9	N/A	0.6	2	Photo-stable	Stable
S75-K	15	3.9	60	12	1	N/A	2.3	7	Photo-stable	Stable
S75-L	15	4.3	55	14	2	N/A	0.8	2	Photo-stable	Stable
S75-M	20	2.6	26	12	5	2	0.6	2	Photo-stable	Stable
S75-N	13	4.1	45	13	5	9	0.7	2	Photo-stable	Stable
S75-O	18	1.2	20	8	5	N/A	15.7	46	Photo-stable	Stable

Conclusion on TiO_2 nanomaterials used as UV-filter in sunscreens based on submitted data

On the basis of the available evidence, the SCCS has concluded that the use of TiO_2 nanomaterials with the characteristics as indicated below, at a concentration up to 25% as a UV-filter in sunscreens, can be considered not to pose any risk of adverse effects in humans after application on healthy, intact or sunburnt skin.

This, however, does not apply to applications that might lead to inhalation exposure to TiO_2 nanoparticles (such as powders or sprayable products).

Rationale for conclusions

Based on provided information and open literature it was concluded that skin exposure was found to be unlikely to lead to:

- Skin penetration and thus systemic exposure
- Acute toxicity via dermal application or oral ingestion
- Skin irritation, eye irritation or skin sensitization when applied on healthy skin
- Reproductive effects when applied on healthy skin

However

- Positive genotoxic effects were reported in open literature
  - also negative effects were reported, so it is overall inconclusive
- Inhalation toxicity observed (inhalation exposure to be avoided)
- Penetration in outer layer of stratum corneum
  - limitation of acceptable photo-catalytic activity

TiO_2 nanomaterial photo-catalytic activity

Three groups could be distinguished in photo-catalytic activity as % of control:

High photo-catalytic activity	35% – 74%	(n=3)
Medium photo-catalytic activity	21% – 23%	(n=3)
Low photo-catalytic activity	<10%	(n=9)

Conclusions on TiO_2 nanomaterials

- Also other TiO_2 nanomaterials are considered not to pose a risk of adverse effects in humans when applied as 25% concentration in sunscreens after application on healthy, intact or sunburnt skin.

- Based on the data and information provided on the potential hazards common **characteristics** were identified in the evaluated TiO_2 nanomaterials to form a "group" of TiO_2 nanomaterials

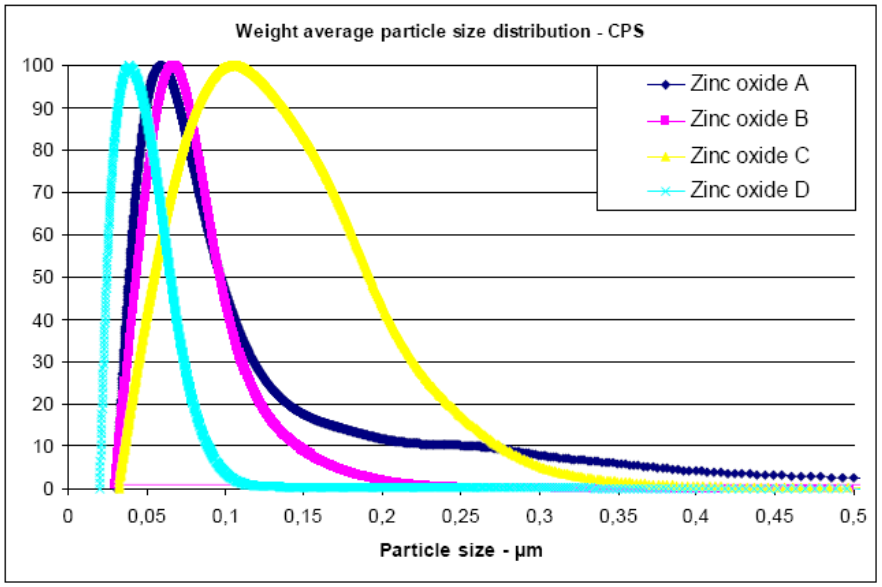
SCCS 2014, for other TiO_2 naomaterials to be used as UV-filter in sunscreens the following applies: (1)

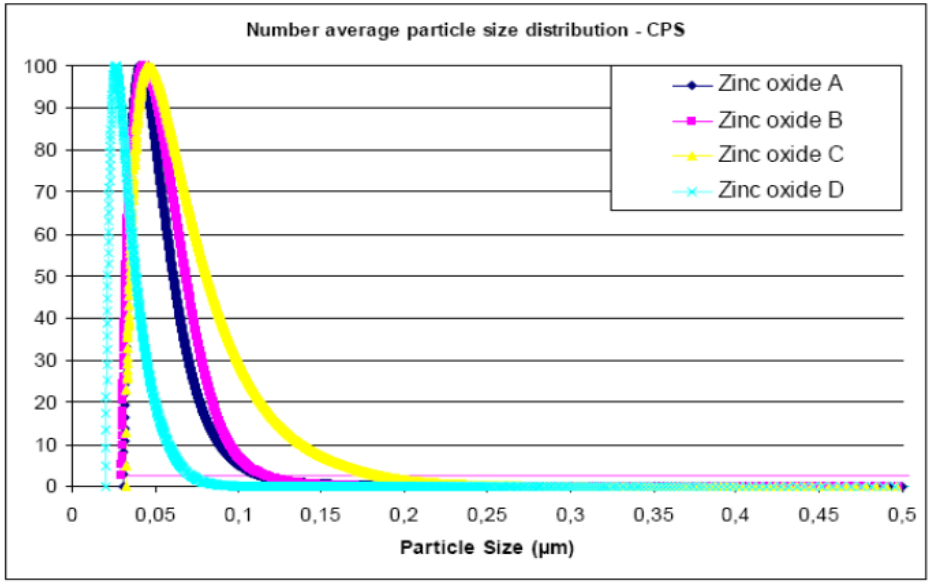
- have TiO_2 purity of ≥99%, or in case of a lesser purity, the impurities must be demonstrated to be safe for use in cosmetic formulations;
- are composed of mainly the rutile form, or rutile with up to 5% anatase, with crystalline structure and physical appearance as described in the current submission, i.e. clusters of spherical, needle, or lanceolate shapes;
- have a median particle size based on number size distribution of 30 to 100 nm (measured by different methods) as submitted in the dossier, or larger. Thus whilst primary particle size may be smaller (around 10 nm), the median particle size of TiO_2 nanomaterials in a cosmetic formulation must not be smaller than 30 nm in terms of number based size distribution;
- have an aspect ratio from 1.0 and up to 4.5, and volume specific surface area up to 460 m^2/cm^3;

SCCS 2014, for other TiO_2 naomaterials to be used as UV-filter in sunscreens the following applies: (2)

- are photostable in the final formulation;
- do not have photo-catalytic activity. However, the SCCS considers up to 10% photo-catalytic activity compared to corresponding non-coated or non-doped reference as acceptable.
- are coated with one of the coating materials described in Table 1, and the coatings are stable in the final formulation and during use. Other cosmetic ingredients applied as stable coatings on TiO_2 nanomaterials can also be used, provided that they can be demonstrated to the SCCS to be safe and the coatings do not affect the particle properties related to behaviour and/or effects, compared to the nanomaterials covered in this opinion;

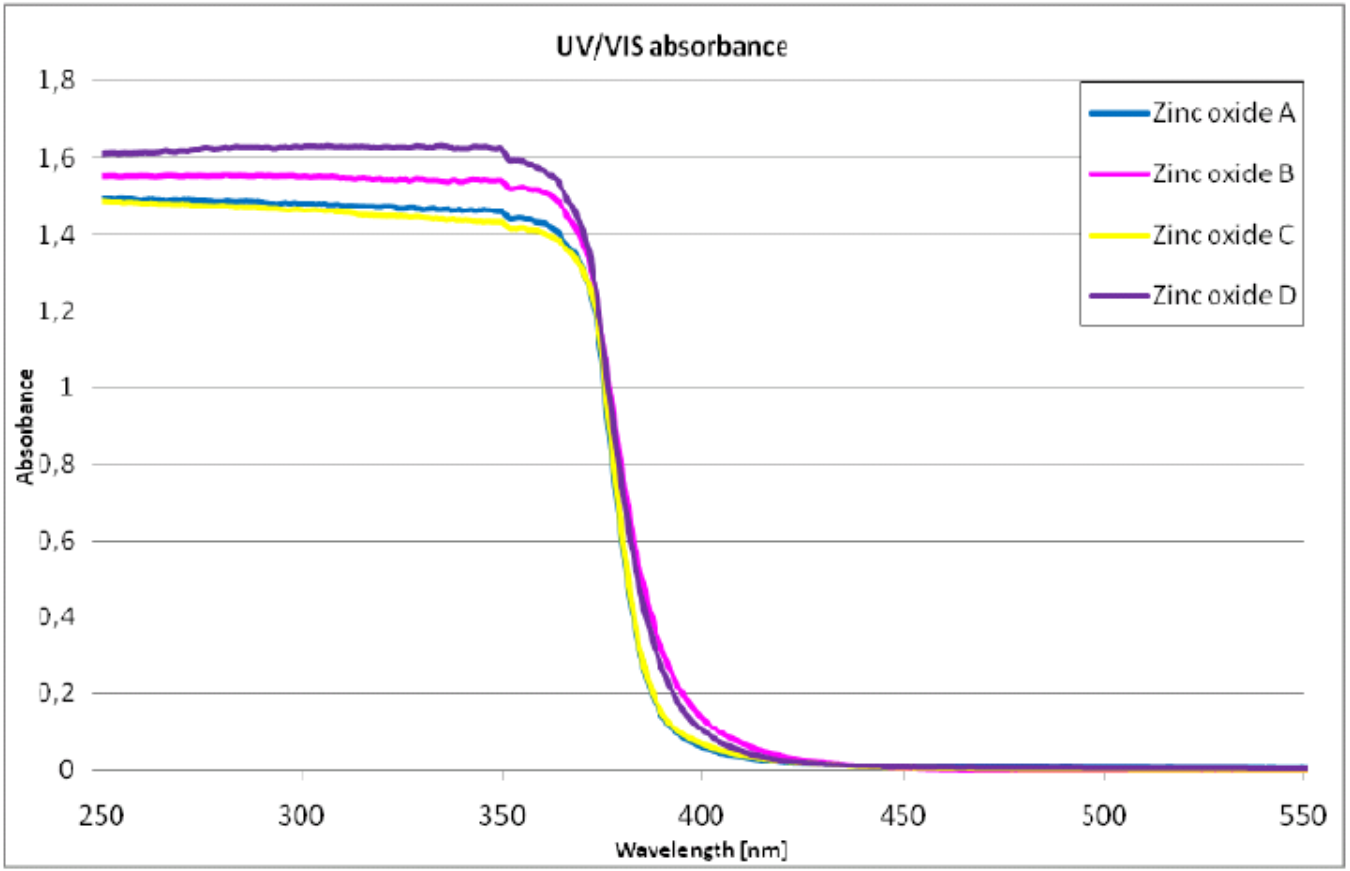
Example:
SCCS ZnO evaluation





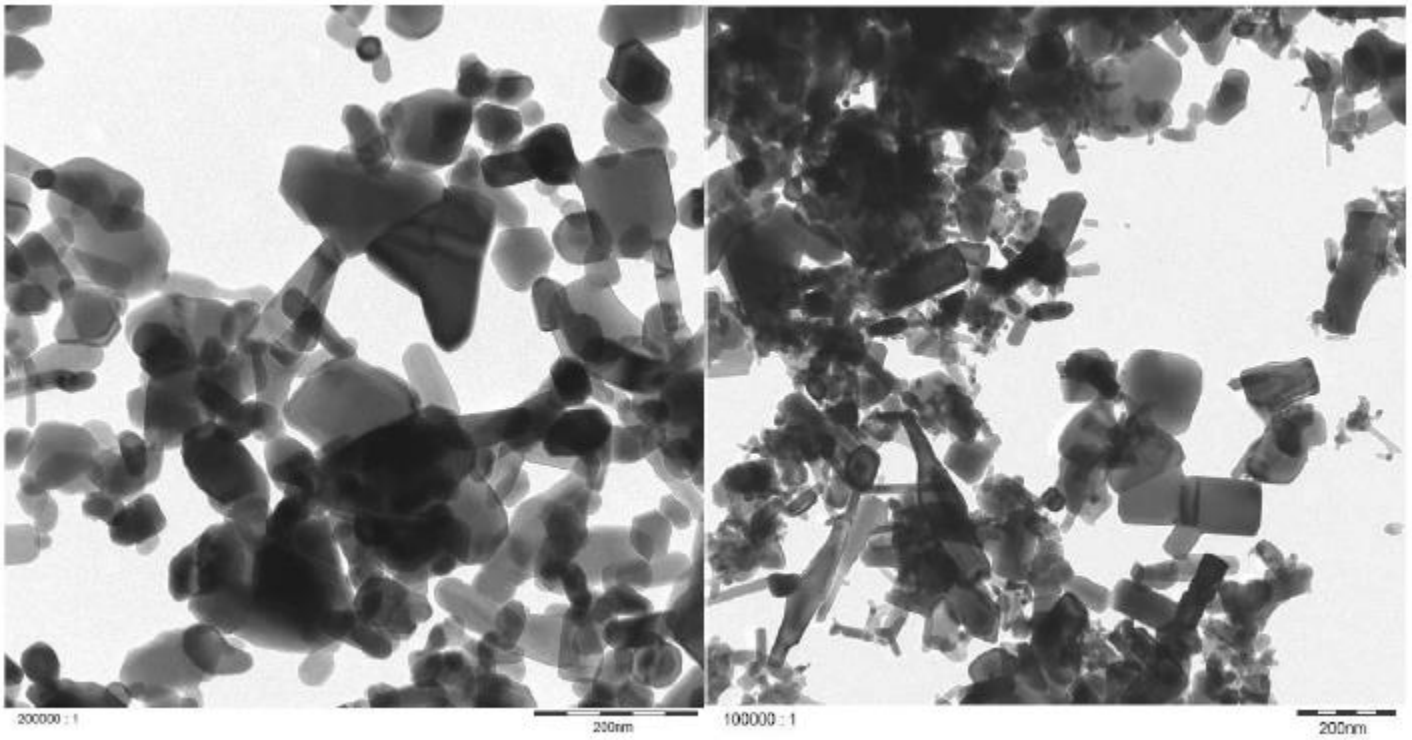
The four ZnO nanomaterials are different or similar depending on the metrics used (mass based vs number based size distribution).

Example:
SCCS ZnO evaluation



The four different ZnO nanomaterials show similar UV absorbance

Example:
SCCS ZnO evaluation



Both coated (left) and uncoated (right) ZnO of a manufacturere show similar morphology including different shapes (rod-like, star-like and isometric forms).

SCCS Opinion on ZnO, 2012

Overall conclusions on ZnO nanomaterial for sunscreen applications

In summary, it is concluded on the basis of available evidence that the use of ZnO nanoparticles with the characteristics as indicated below, at a concentration up to 25% as a UV-filter in sunscreens, can be considered not to pose a risk of adverse effects in humans after dermal application.

This does not apply to other applications that might lead to inhalation exposure to ZnO nanoparticles (such as sprayable products).

Rationale for conclusions on ZnO nanomaterials

Based on provided information and open literature it was concluded

- No evidence for absorption of ZnO nanoparticles through the skin
- Minor contribution to blood Zn pool (µg vs mg) was noted after dermal application of ZnO nanoparticles in a sunscreen formulation
- Amount of solubilized zinc in ZnO-containing cosmetic formulations is likely to be small
- No risk to consumers in absence of systemic exposure
- Different particle sizes, surface modifications and crystalline structures do not significantly alter uptake, bioavailability and overall safety profile

In view of the lung inflammation induced by ZnO particles after inhalation exposure, the use of ZnO in cosmetic products which may result in inhalation is of concern.

Example:
SCCS ZnO evaluation

ZnO with following characteristics is considered not to pose a risk for humans after dermal application.

- *ZnO nanoparticles of purity ≥96%, with wurtzite crystalline structure and physical appearance as clusters that are rod-like, star-like and/or isometric shapes, with impurities consisting only of carbon dioxide and water, whilst any other impurities are less than 1% in total.*
- ZnO nanoparticles with a median diameter (D50: 50% of the number below this diameter) of the particle number size distribution above 30 nm, and the D1 (1% below this size) above 20 nm.
- ZnO nanoparticles that are either uncoated or coated with triethoxycaprylylsilane, dimethicone, dimethoxydiphenylsilanetriethoxy-caprylylsilane cross-polymer, or octyltriethoxy silane. *Other cosmetic ingredients can be used as coatings as long as they are demonstrated to the SCCS to be safe and do not affect the particle properties related to behaviour and/or effects, compared to the nanomaterials covered in the current opinion.*
- ZnO nanoparticles that have a comparable solubility to that reported in the dossier, i.e. below 50 mg/L (approximately the maximum solubility of the ZnO nanomaterials for which data are provided in the dossier).

SCCS Addendum to Opinion on ZnO, 2013

Experience sofar

- Within one nanomaterial (TiO_2, ZnO) grouping was found to be possible
  - Extensive data was provided (phys-chem)
  - Exposure data (skin penetration) was available
  - Toxicity data available both submitted and in open literature
- Application focus on local effects (lack of skin penetration)
- Domain and limitations based on extensive information provided

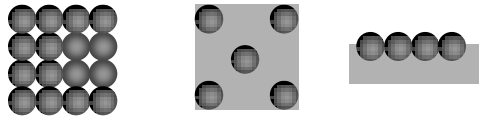
Conclusion:

when a sufficient amount of data is available interpolation within one type of nanomaterials seems to be possible and a description of a group is possible.

Source-to-adverse outcome pathway

Nanomaterial

Powder
Embedded in matrix
or on surfaces

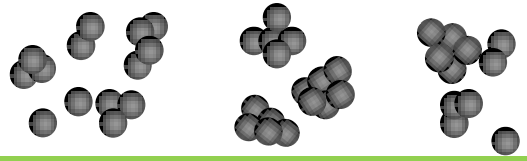


Life-cycle

Changes of phys.-chem. properties

Dispersion

Aerosol
Suspension



Uptake in the body

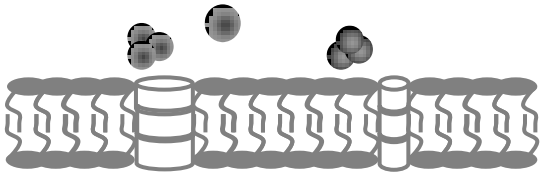
Deposition in the lung,
Alveolar, intestinal, dermal absorption

Modification in the body

Surface coating changes
Agglomeration, desagglomeration

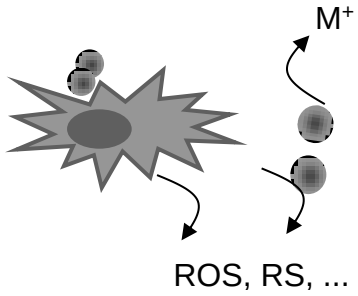
Distribution in the body

crossing of biological barriers
Tissue distribution,
Intracellular distribution



Primary Effect

Inflammation
Catalysing formation of reactive compounds
Ion release
Direct interaction with cellular structures



Toxic Effect

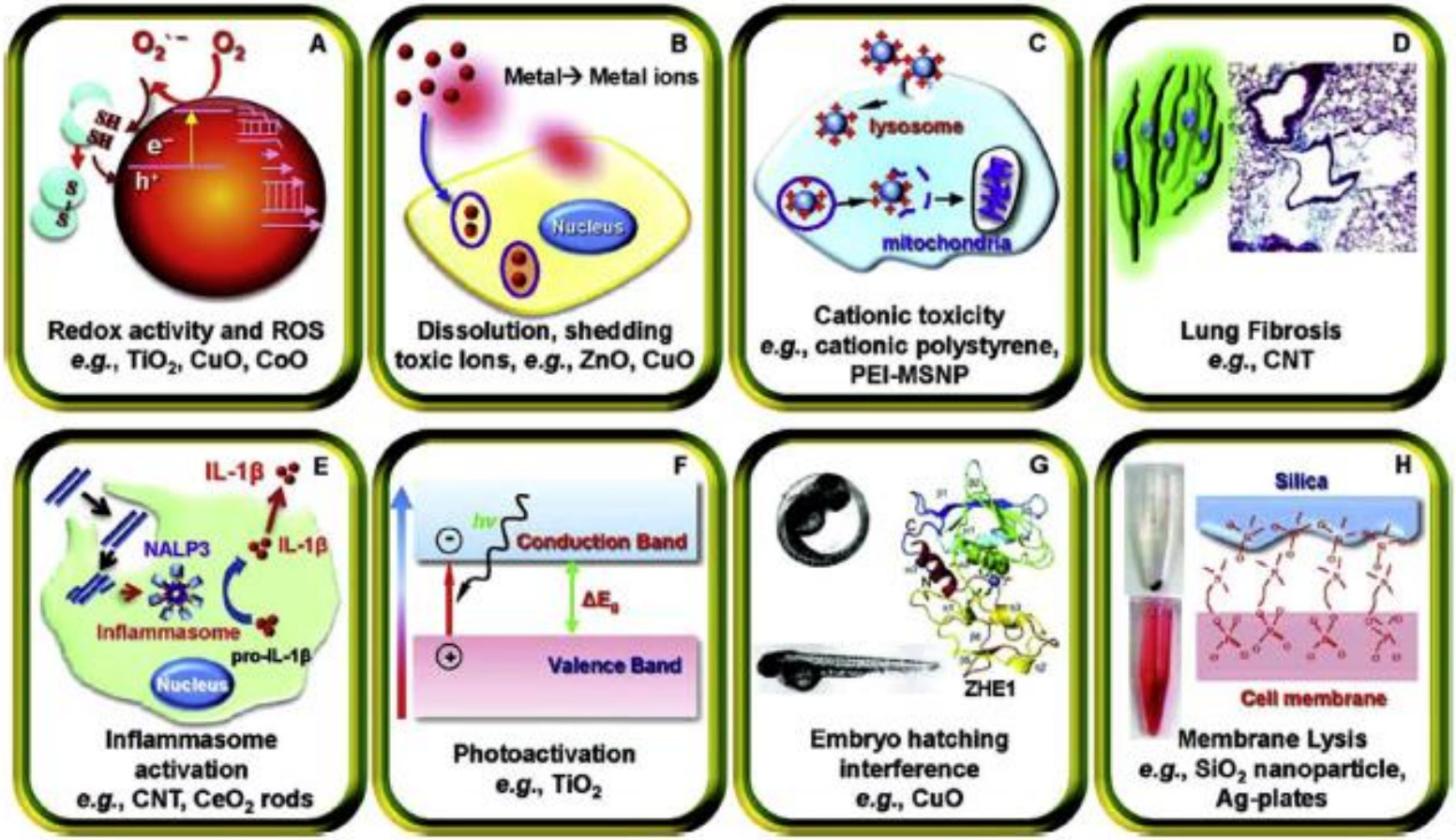
Organ toxicity

Source to Adverse Outcome Pathway

Material properties
Use
Releas e
Uptak e
Distri-bution
early event
Apical Effect

Oomen et al., 2014

Mechanisms for toxicity of nanomaterials



Nel et al., 2013

What do we know in relation to toxicity?

- Size/surface chemistry/coating
  - Important for toxicokinetics
- Chemical composition
  - Possible intrinsic chemical toxicity
- Solubility
  - Release of toxic ions
- Morphology (nanofibers)
  - Fiber length, rigidity, persistence (fiber paradigm)

What do we want/need to know?

Physicochemical characteristic that is associated with an adverse effect (toxicity)

Main principles / modes of action of nanomaterials

(i) the release of toxic chemical constituents from NMs (e.g. Cd from quantum dots, ionic silver from Ag NPs)— i.e. ***NM dissolution***;

(ii) direct effects from physical contact with NMs, influenced by their size, shape and surface properties, and which produce interferences with important biological functions for example by altering conformation of biomolecules — i.e. ***NM surface effects***;

(iii) the inherent properties of the material, such as photochemical and redox properties resulting from bandgap or crystalline form — i.e. ***NM structure effects***; and

(iv) the capacity of NMs to act as vectors for the transportof other toxic chemicals to sensitive tissues — i.e. ***NM Trojan horse effects***.

Lynch et al., 2014

Screening for adverse outcomes can be done in *in vitro* assays

- High-throughput screening

  - Identify hazard potential for
    - Cytotoxicity
    - Inflammation
    - Genotoxicity
  - Comparison of various nanomaterials to select the least toxic (prioritization high risk group)
  - Identify mechanism of toxicity ("omics" pathways)

In general NOT for safety evaluation/risk assessment

Recent developments: Principal component analysis (PCA)

Sayes et al., Int J Nanomedicine 2013:8, 45-56.

Principle characteristics, being engineered size, concentration, agglomerated size in water, zeta potential as measure of surface charge, pH, and age of suspension. Measured in absence of biological (medium, cells, serum) components to avoid added variables.

Lynch et al., Nano Today 9, 266-270, 2014

Three principle components being intrinsic properties (e.g. shape, porosity, structural configuration, bandgap), extrinsic properties (e.g. surface interactions/transformations, biomolecules), composition aspects (e.g. inherent molecular toxicity, charge, hydrophobicity, coating).

However,…..

Primary physicochemical descriptors of nanomaterials may not be the most appropriate to predict their toxicological behaviour, in part as many of these are "context dependent", i.e. are affected by the surrounding matrix (pH,ionic strength, biomolecules or macromolecules etc.), the route of exposure, etc.

Many nanomaterial properties are interdependent such that changing one property may inadvertently result in change to several others, e.g. changing nanomaterial shape/length may cause surface defects or change the surface chemistry.

Nanomaterials age and are transformed throughout their lifecycle.

Lynch et al., 2014

Conclusions

Aim is to provide information for safety evaluation and risk estimation of nanomaterials

We need to identify a set of characteristics that can be linked to an adverse (toxic) outcome.

It is therefore important that in the Principle Component Analysis (PCA) somehow toxicity outcomes and mechanisms of action need to be included.

In addition, also the (toxico)kinetics need somehow to be considered as unexpected target sites may be reached by nanomaterials due to their surface characteristics (coatings).

Some grouping is possible within one type of nanomaterial as demonstrated by the SCCS outcomes.

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  - NanoMILE project (WP 9 data intergration/QPAR……)