UNIVERSITÀ DEGLI STUDI DI MILANO
School of Pharmacy – Laboratory of Toxicology

Nanotecnologie nel settore cosmetico: prospettive e problemi per la valutazione di sicurezza

Marina Marinovich

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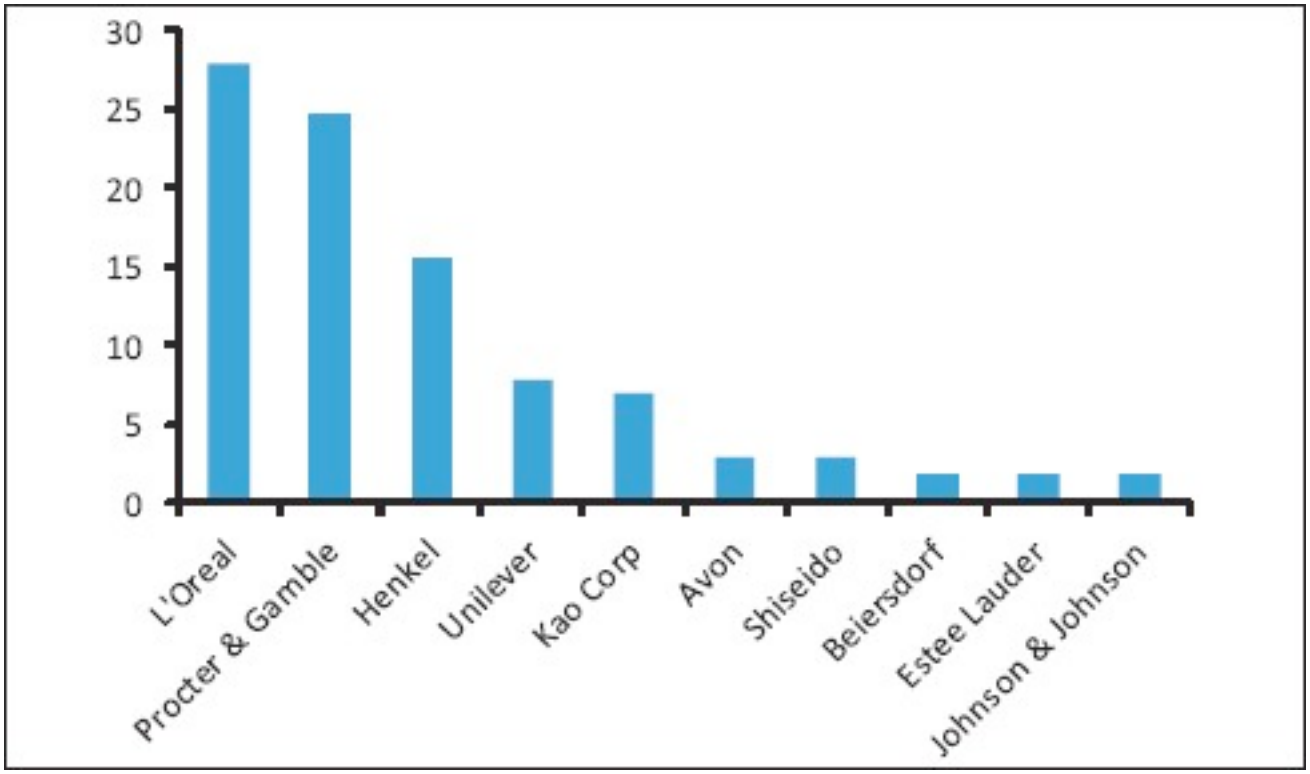
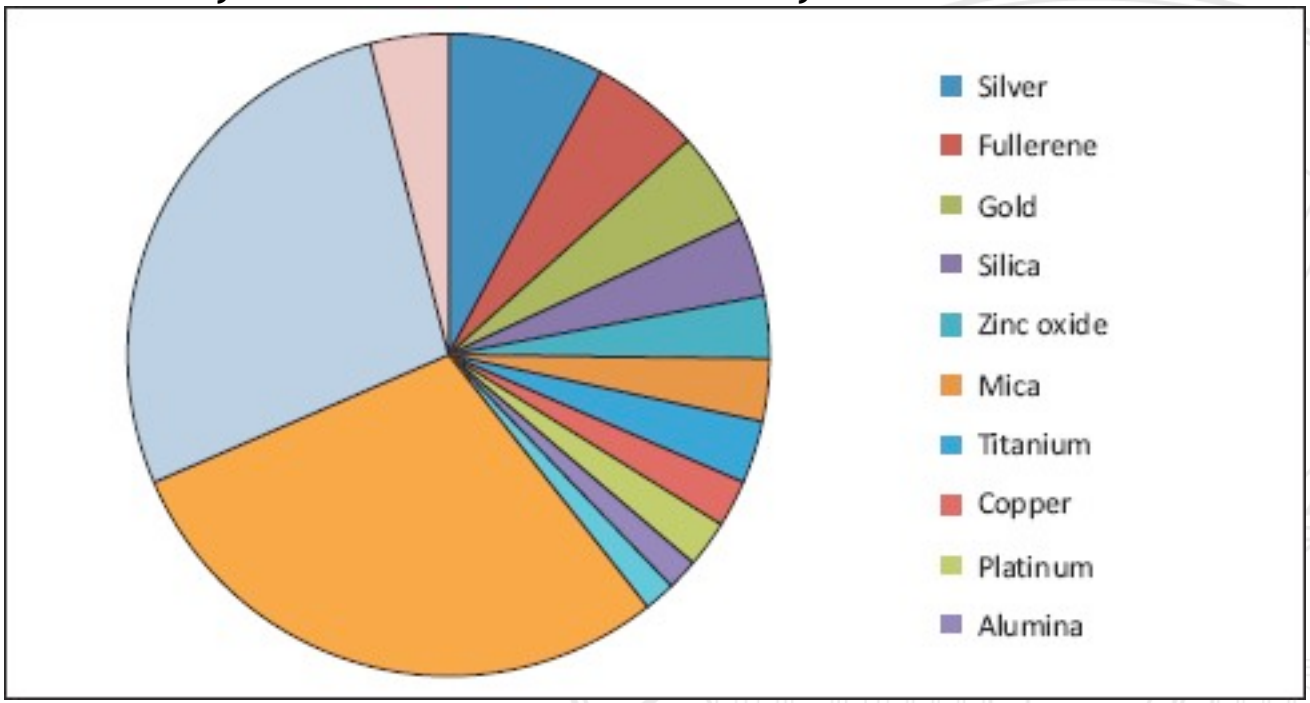
Nanomaterial and cosmetic industries



J Pharm Bioall Sci 2012;4:186-93

TYPE AND FORM OF COSMETIC NANOMATERIAL

Liposomes
Nanoemulsions
Nanocapsules
Solid lipid nanoparticles
Nanocrystals
Nanosilver and Nanogold
Dendrimers
Cubosomes
Hydrogels
Buckyballs



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Why is the risk assessment of nanomaterial so difficult?

Examples of genoxicity of NMs compared to their bulk counterparts

	Nano (<100 nm)	Bulk (> 5µm)
CuO	++	+
TiO_2	-	++
Fe_2O_3	--	--
MnO_2	+	-
Al_2O_3	+	-

HAZARD ASSESSMENT

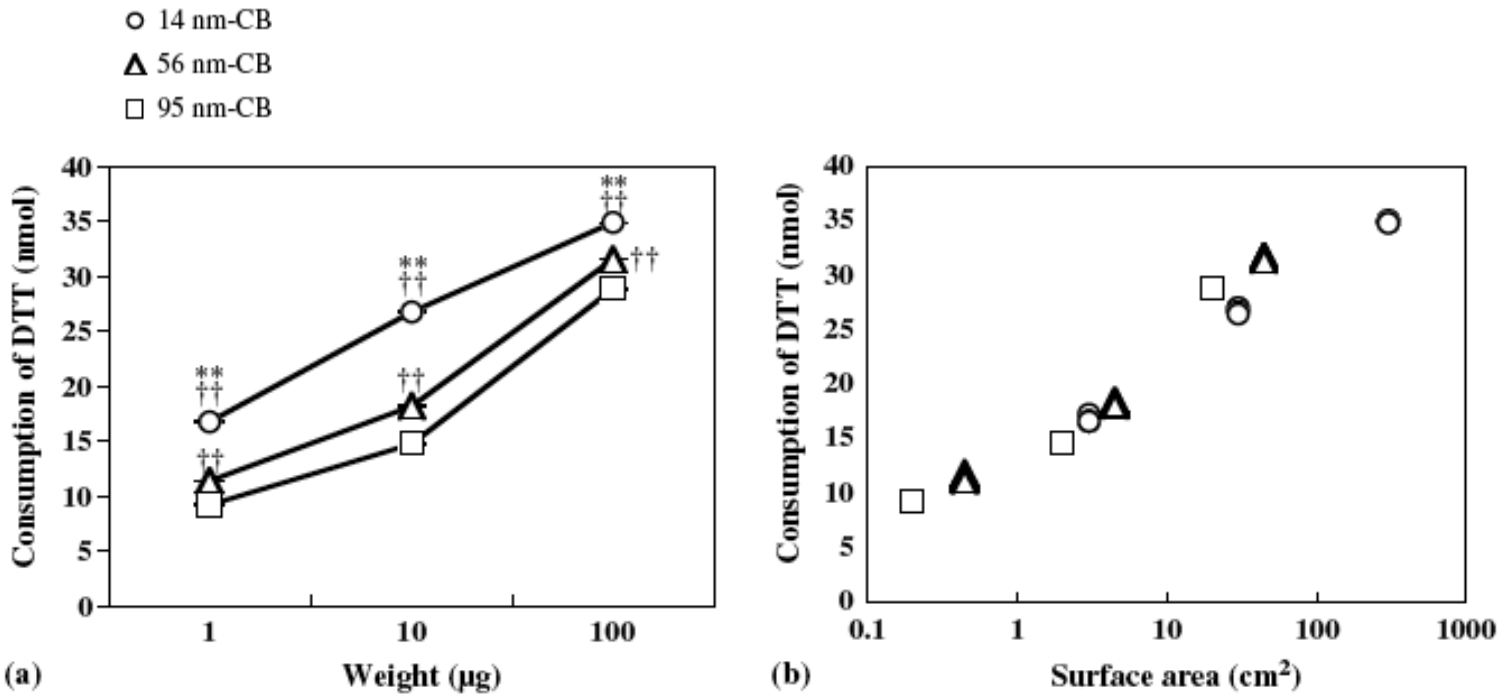
Toxicology paradigm:

the health effects are correlated to the **mass** of the agent to which the individual is exposed, resulting in an accumulated mass as internal or organ dose/exposure

For nanoparticles the **concentration number** and the resulting **total surface area** determine the interactions with biological systems.

Therefore the surface area and number concentration appear to be more reasonable parameters for doses in terms of exposure.

Innate oxidative capacity of CB nanoparticles



CB nanoparticles (1–100 μg/ml) having particle sizes of 14 nm (circle), 56 nm (triangle) and 95 nm (square) were incubated with 100 μM DTT in a 250 mM Tris–HCl buffer (pH 8.9) for 30 min at 37 C in a water bath. The data of (a) are presented as the mean ± SEM of six samples ; **p < 0.01 compared with 56 nm;†† p < 0.01 compared with 95 nm).

Chemosphere, 2006

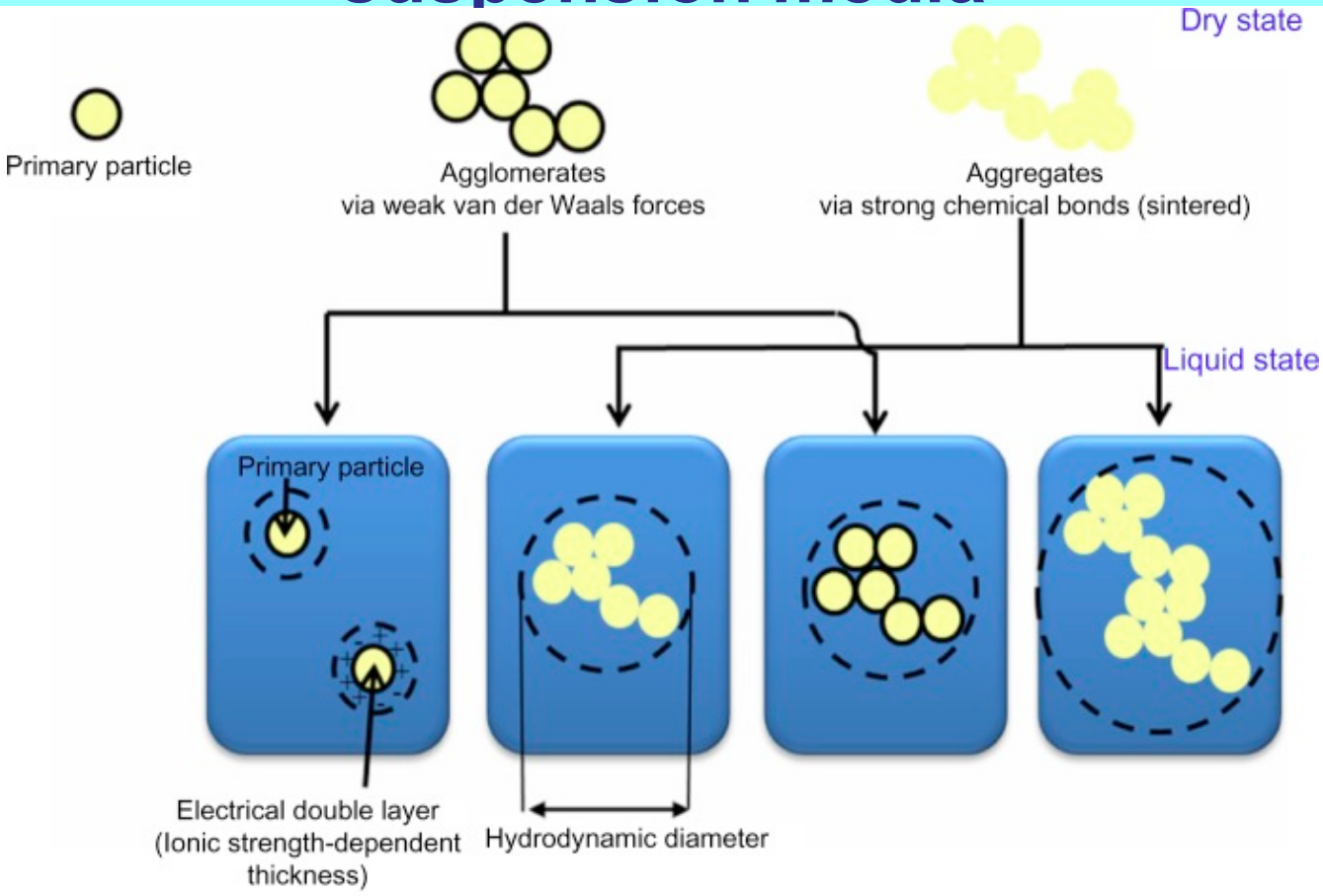
HAZARD ASSESSMENT

No identificazione della sostanza = no valutazione del pericolo

- Per “nanomateriale» s’intende un materiale naturale, derivato o fabbricato contenente particelle allo stato libero, aggregato o agglomerato, e in cui, per almeno il 50 % delle particelle nella distribuzione dimensionale numerica, una o più dimensioni esterne siano comprese fra 1 nm e 100 nm.

- In casi specifici, e laddove le preoccupazioni per l’ambiente, la salute, la sicurezza e la competitività lo giustifichino, la soglia del 50 % della distribuzione dimensionale numerica può essere sostituita da una soglia compresa fra l’1 % e il 50 % (2011/696/UE).

Various states of nanoparticles in different forms of dry powder and liquid in suspension media

- 

Dominant repulsive forces:
– High surface charge
– Thicker double layer
– Steric forces

Weak repulsive forces – agglomeration:
– Low surface charge
– Thinner double layer
– No steric forces

Kwon et al., 2014

Material characterisation for hydroxyapatite forms

Material	Morphology	Average particle size [nm]	Specific surface area [m2/g]
HPC (non-nano)	irregular shaped	1200 x 2100	67
HA-NP	mainly nano-sized plates	3 x 20 x 45	154
HA-NR	mainly nano-sized rods	5 x 90	166
HA-NN	needles	3 x 20 x 100	106
HA-FN	intermediate morphology between rods and needles	95 x 740	27

Albrecht et al., 2009

SCCS recommendation

- Where a nanomaterial **loses its nanostructure**, e.g. by solubilisation in a formulation, test medium, or biological environment, **it will no longer be expected to behave any differently from its non-nano (chemical) equivalent**

- It may still pose a toxicological hazard at the **local level** because of its chemical constituents, or at **systemic level** if before disintegration the nanostructure delivered the chemical constituents to a biological site where such a concentration of the conventional form would have not reached

Nanomaterial characterisation

EFSA, 2011 (food)

The characterisation should ideally be determined in five stages i.e.

- as manufactured (pristine state),
- as delivered for use in food products,
- as present in the food matrices,
- as used in toxicity testing, and
- as present in biological fluids and tissues during testing.

SCCS, 2012 (cosmetics)

The characterisation of nanomaterials for use in a cosmetic product should include description of the pristine nanoparticles

- in the raw material form as manufactured
- after addition to a final cosmetic formulation
- during toxicological investigations

Open points

- *…The characterised materials do not necessarily correspond to those materials which have been used for toxicity testing….*
- …The test material was insufficiently characterised and it is not clear whether the doses used correspond to test material as 5% aqueous solution or to the active ingredient…..
- *…The study did not adhere to an OECD or EU test guideline and it is not clear whether it was performed according to GLP principles. No conclusion can be drawn from this study…*

Advantages and disadvantages for different techniques to measure NP in the 1–100 nm size range

	SEM	TEM	FFF	CPS	PTA	AUC	DLS	SP-ICP-MS
Minimum size	++	+++	+++	+	+	++	+++	+
D								
A								
S								
In								
Ease of use	–	–	+	++	+	+	++	+
Cost	–	–	++	++	++	+	+++	+

It is important to note that currently there is no single method that can be regarded a 'gold' standard for characterisation of the different physicochemical parameters of nanomaterial as such, nor is there one suited to fully assess a nanomaterial in a cosmetic product.

AUC, analytical ultracentrifugation; CPS, centrifugal particle sedimentation; DLS, dynamic light-scattering; FFF, field flow fractionation; PTA, particle tracking analysis; SEM, scanning electron microscopy; SP-ICP-MS, single particle inductively coupled plasma; TEM, transmission electron microscopy.

Calzolai et al 2012

Problemi analitici

- Modifiche durante la preparazione del campione
- Contaminazione da parte del sistema analitico (per es. sonde per la sonicazione di campioni liquidi o utilizzo di celle al quarzo)
- Pochi metodi arrivano a 1 nm
- Interferenza di nanoparticelle di background
- Il campione è rappresentativo dell'intera formulazione?
- Mancanza di standard di riferimento (basati teoricamente su matrici)
- Mancanza di procedure standard per la preparazione del campione ➡ mancanza di riproducibilità
- Mancanza di un'unica tecnica per le diverse misure che si possa applicare in maniera routinaria

HAZARD IDENTIFICATION AND DOSE-RESPONSE CHARACTERISATION

- A limited number of *in vitro* test methods developed and validated for conventional chemicals
- **None of the methods has yet been validated for nanomaterials**.
- In silico modelling approaches: **not yet available** / reliable for nanomaterials
- dispersion/ aggregation, adsorption, stability and distribution into the tissue to be taken into consideration.

Skin corrosivity and skin irritation

Conventional chemicals

5 validated *in vitro* alternatives are available [OECD 430-1]

:

1. TER test (rat skin transcutaneous electrical resistance test)
2. EpiSkinTM
3. EpiDermTM
4. SkinEthicTM
5. EST-1000 (epidermal skin test-1000)

Nanomaterials

- The tests based on colorimetric assays (such as sulforhodamine B dye, MTT assay) may not be suitable because of possible interaction between reagents and the nanomaterials (disperse/ absorb light)
- The measurement of cytokines and chemokines in the test system may provide additional information (e.g. IL-1 , tumor necrosis factor (TNF-a) IL-8, interferon). However, they may bind/ adsorb on nanomaterial surfaces, and this may lead to false negative results.

Mutagenicity/genotoxicity

Conventional chemicals

- Tests for gene mutation:
  i) Bacterial reverse mutation test [OECD 471]
  ii) *In vitro* Mammalian cell gene mutation test [OECD 476]

- Tests for clastogenicity and aneugenicity
  i) *In vitro* Micronucleus test [OECD 487] or
  ii) *In vitro* Mammalian chromosome aberration test [OECD 473]

Nanomaterials

- there are doubts if the Ames test is an accurate representative test for genotoxicity.
- bacterial cells lack uptake of nanomaterials through endocytosis, and also that some nanomaterials have bactericidal activity.
- the use of metabolic activation system for nano-substances is questionable
- proteins in the S9 may interfere with the nanomaterial alter bioavailability of the nanomaterial, and thus reduce sensitivity of the assay.

Cytotoxicity assay	Detection principle	NP interference	Altered readout	Particle
Cell viability MTT	Colorimetric detection of mitochondrial activity	Adsorption of substrate	⇩indication of cell viability	Carbon NP
LDH	Colorimetric detection of LDH release	Inhibition of LDH	⇩indication of necrosis	Trace metal-containing NP
Annexin V/ propidium iodide	Fluorimetric detection of PLserine exposure (apoptosis marker) Propidium iodide-staining of DNA (necrosis marker)	Ca2+-depletion Dye adsorption	⇩indication of apoptosis ⇩indication of necrosis	Chitosan NP Carbon NP
Neutral red	Colorimetric detection of intact lysosomes	Dye adsorption	⇩indication of cell viability	Carbon NP
Caspase	Fluorimetric detection of Caspase-3 activity	Inhibition of Caspase-3	⇩indication of apoptosis	Trace metal-containing NP
Stress response DCF	Fluorimetric detection of ROS production	Fluorescence quenching	⇩indication of oxidative stress	Carbon nanoparticles
Inflammatory response ELISA	Colorimetric detection of cytokine secretion	Cytokine adsorption	⇩indication of cytokine concentration	Carbon NP Metal oxide NP

European Journal of Pharmaceutics and Biopharmaceutics 72 (2009) 370–377

Exposure assessment

No indication that the use of consumer/cosmetic products that contain nanomaterials is likely to be any different from the use of other products that contain conventional ingredients.

but

The rule >500 Da and log Pow <-1 or >4, = 10% dermal absorption is not likely to be relevant for most nanomaterials and therefore the 10% default absorption will not be applicable. In view of this, **dermal absorption** of nanomaterials will need to be determined **experimentally**.

Exposure challenges

- The tested nanomaterials are in **exact form/ composition** as intended for use in a cosmetic formulation, and as the formulation is delivered to the end-user?
- **The concentration** of a nanomaterial may decrease during a test due to sedimentation, binding with other moieties in the test medium, or adhesion to glass/plastic ware.
- To ascertain the **stability and uniformity of the nanomaterial** in a test medium to ensure that the applied concentration/ dose is maintained for the intended period during the test.
- To determine the **possible interaction** of the nanomaterial with other component of a test medium/ formulation.

IN VITRO *vs.* IN VIVO exposure

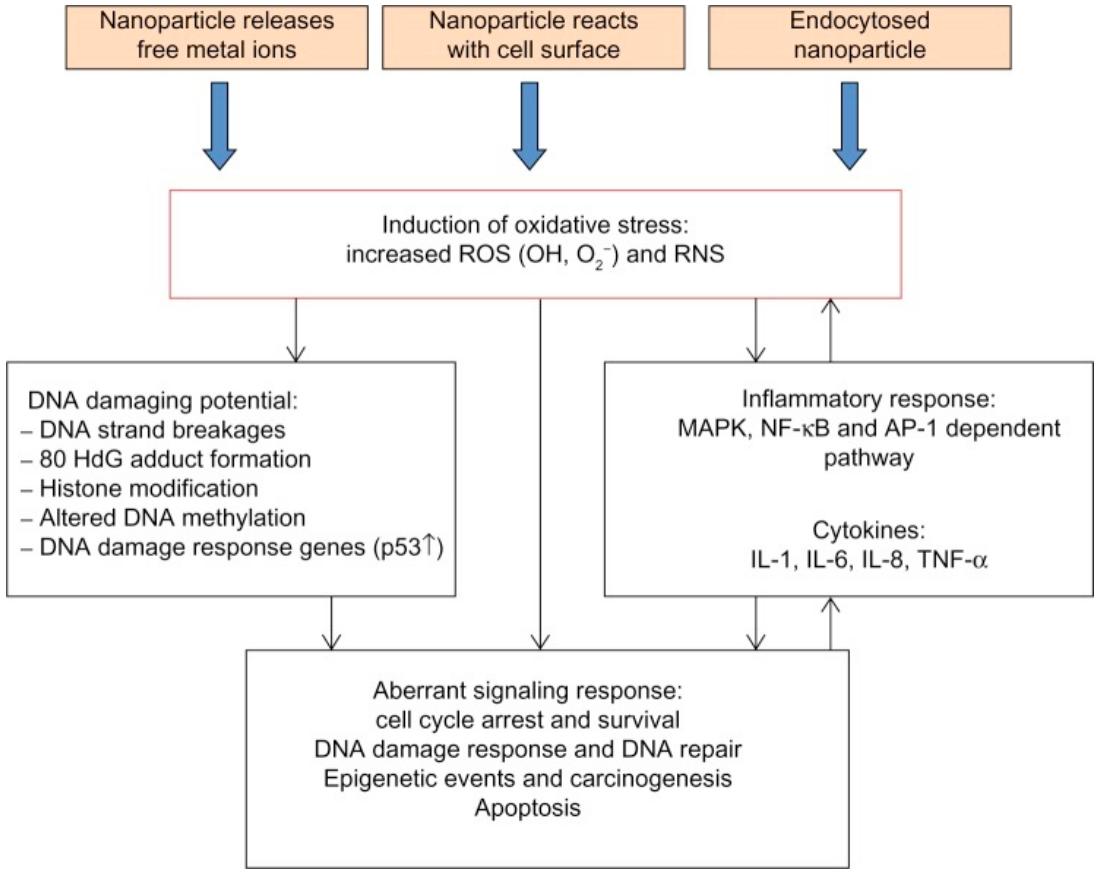
- Rough estimates indicate that in most of these studies the nanoparticles to cell ratio was far beyond 1000:1, which largely exceeds any realistic dose in vivo.

- Generally, **2 x 10^5 nanoparticles per cell**, or 2 x 10^{10} nanoparticles per 10^5 cells, are applied

- In vivo,for breathing of ambient aerosols, on average **6 nanoparticles** will be daily deposited **per cell** in the alveolar region.

- Maximally, but not realistic, at the highest possible nanoparticles aerosol, an alveolar surface **cell** will receive on average **120 nanoparticles per hour.**

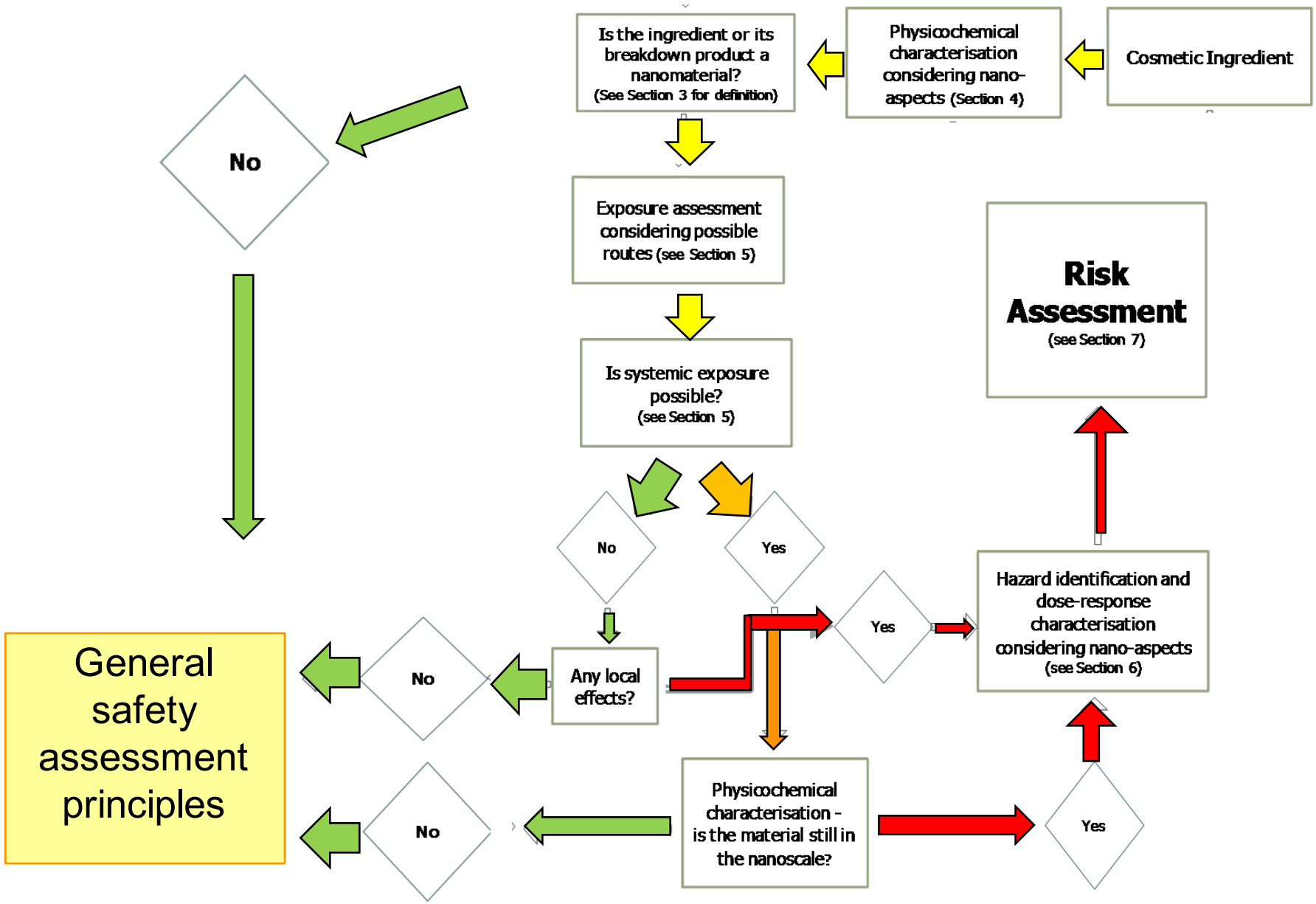
Putative mechanisms underlying the effects of zinc oxide and silica NP



Kwon et al., 2014

RELEVANCE, ADEQUACY AND QUALITY OF THE DATA IN SAFETY DOSSIERS ON NM

- If nanomaterial loses its nano-structure, it will no longer be expected to behave any differently from its non-nano (chemical) equivalent.
- the safety of a nanomaterial must not be assumed or argued simply on the basis of its chemical composition alone.
- safety of a nanomaterial cannot be assumed on the argument that the bulk form of the materials is safe (and vice versa)
- a safety dossier on nanomaterial(s) contains sufficient data (physicochemical properties, exposure, toxicological effects, and safety evaluation) and supporting information to enable adequate risk assessment. It should include data from the open literature.



Adapted from SCCS/1484/12