Evolución de las herramientas toxicológicas para la evaluación de nanopartículas

18|01|2013
Blanca Suárez PhD

Nacimos para innovar y **NUESTRA VISIÓN** es ser referente, con proyección internacional, y proveedores de conocimiento tecnológico.

Nuestras claves

- Un proyecto con el compromiso de **todas las personas**
- Una **apuesta decidida** por la innovación tecnológica
- Una **integración** de tecnologías internas y externas
- Una estrategia centrada en nuestros **grupos de interés**
- Una **gestión** basada en la **mejora continua**

Productos nano

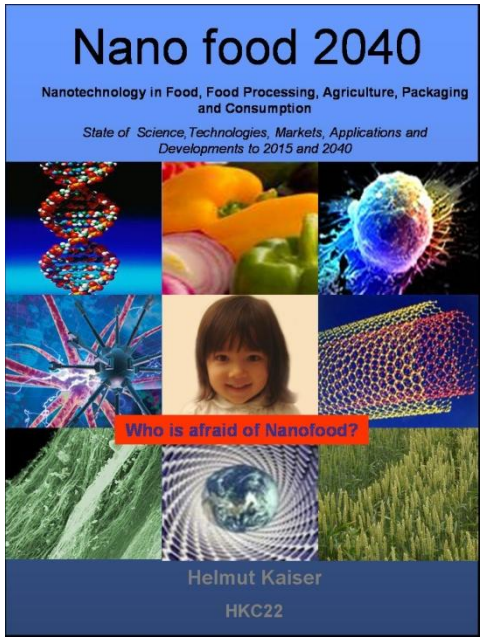
VS

EU votes for labels on nano, cloned and GM food

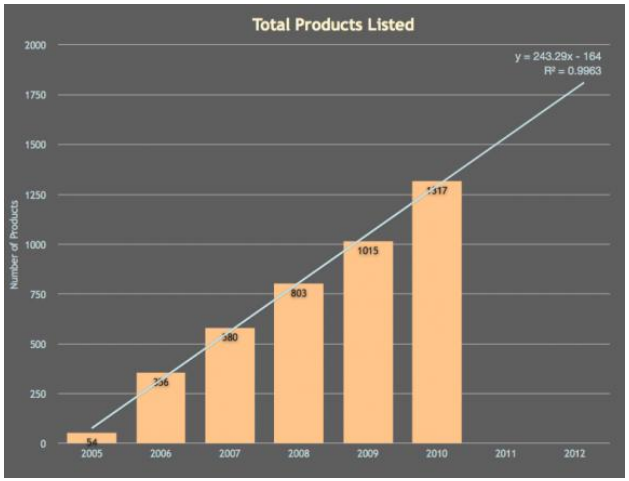
Ecologist
5th May, 2010

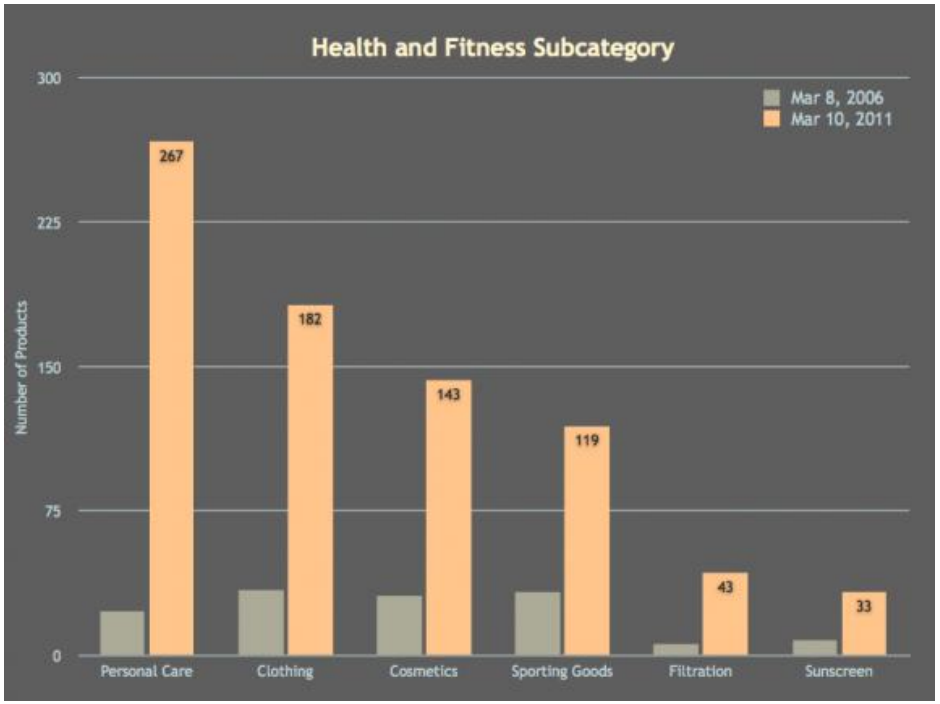
UK and other member states expected to fight proposals to bring in compulsory labelling for consumers on novel foods

MEPs have voted almost unanimously in



Aplicaciones seguras
Opinión pública positiva





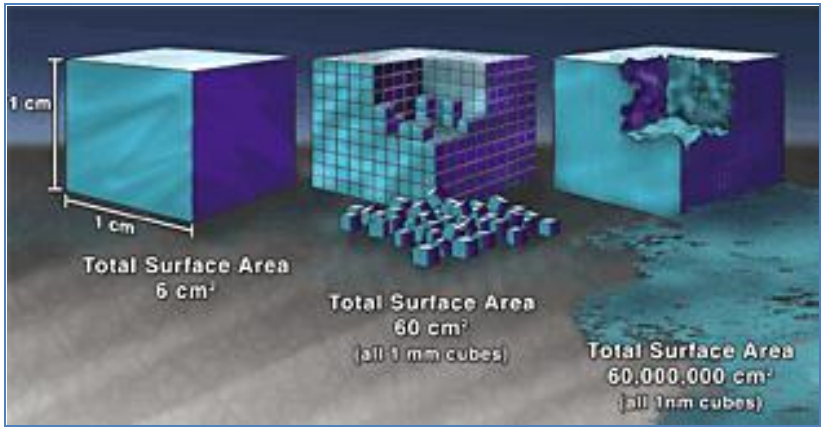
EEUU > Europa > Asia

Fuente: Nanotechnology consumer product inventory

La legislación actual no es capaz de evaluar la seguridad de los nanomateriales:

- Pérdida de competitividad
- Pérdida de confianza del consumidor

Definición –son materiales (origen natural, accidental o manufacturado) cuyos principales constituyentes tiene una dimension entre 1-100 nm. En el caso de aglomerados o agregados 50% de estos materiales poseen una o mas dimensiones en la nanoescala.

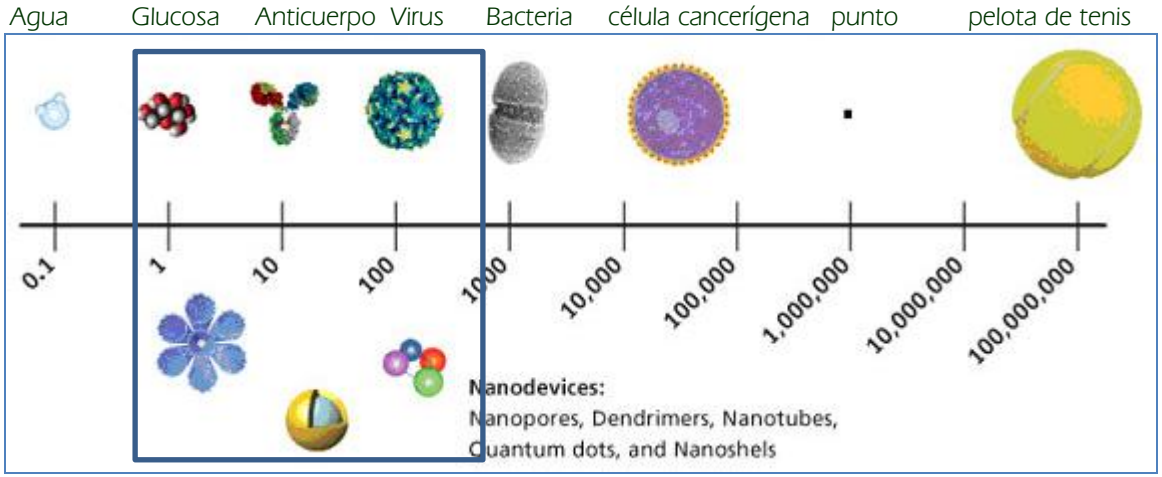


Tamaño	Número	Area de superficie
1 cm	1	6 cm2
1 mm	1000	60 cm2
1 um	10^12	6000 cm2
1 nm	10^21	60.000.000 cm2

Mayor área de superficie > reactividad > contacto con células y tejidos

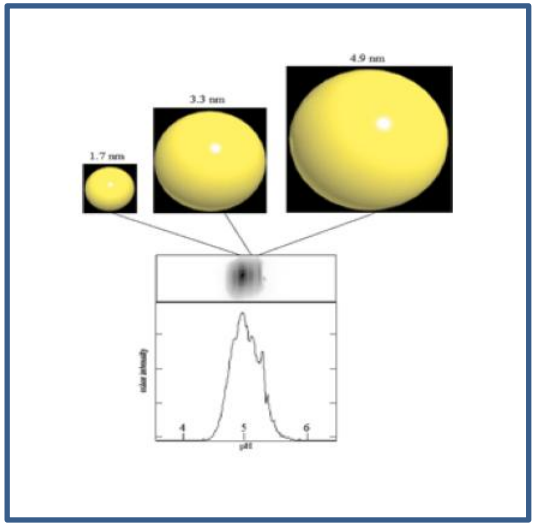
¿Por qué preocuparse?

- Entidades nuevas desde un punto de vista toxicológico
- Utilización de masa como medida puede no ser adecuado
- ¿Son adecuados los procedimientos habituales?
- Diversidad de nanomateriales (inorgánico, orgánico)
- Solubilidad, aglomeración
- Calidad del preparado, pureza
- Detección y caracterización del nanomaterial

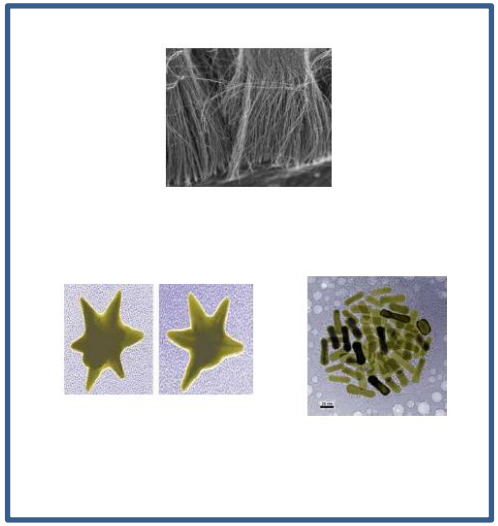


Las nanopartículas tienen tamaños comparables a estructuras subcelulares y en teoría pueden interaccionar con éstas

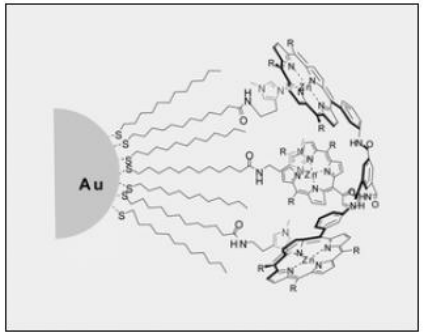
Tamaño

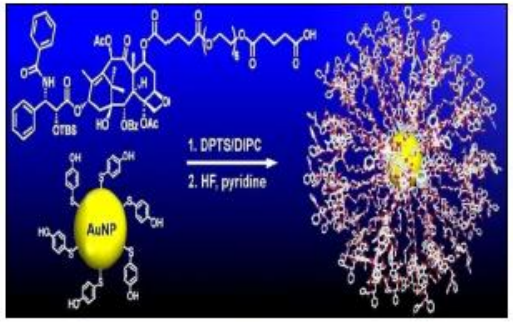


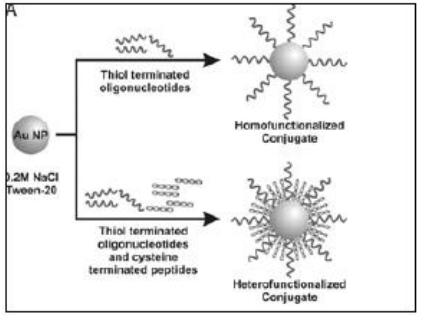
Forma



Funcionalización

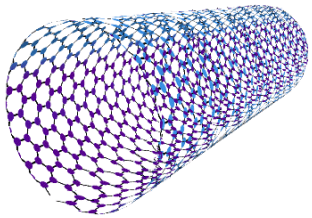






Premisas

•La única característica común entre nanopartículas es el nombre!



Nanotubos de carbono

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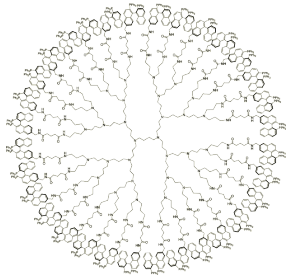

Dendrimeros

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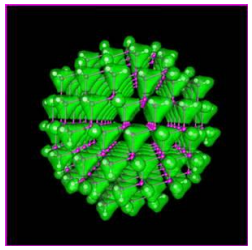

Quantum dots

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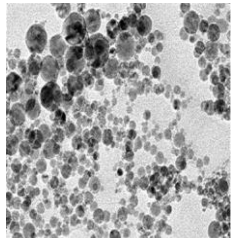
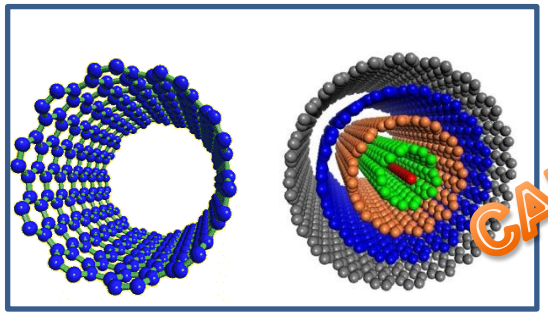


Dioxido de titanio

Nanotubos de carbono – 0.5-2µm x1-2 nm



Costes en EEUU:
>249 millones de $
34-53 años

Evaluación toxicológica

Tamaño?
Longitud?
Presencia/ausencia de catalizadores?
Estado puro? (pristine nanomaterials)
Funcionalizados?

Identificar características/
Tests que sirvan para predecir toxicidad

Diseño seguro/Safe by design

TOXICOLOGICAL HIGHLIGHT

How Meaningful are the Results of Nanotoxicity Studies in the Absence of Adequate Material Characterization?

David B. Warheit[1]

DuPont Haskell Global Centers for Health and Environmental Sciences, Newark, Delaware

Received November 6, 2007; accepted November 6, 2007

For the very few people who may not have an understanding of nanotechnology, here is a quick overview. Nanotechnology is an emerging multidisciplinary technology that involves the synthesis of molecules in the nanoscale (i.e., 10^{-9} m) size range. The origin of the term "nanotechnology" is derived from the Greek word "nano," meaning "dwarf." From a chemistry and material science perspective, the development of new products using nanomaterials is exciting because, for a given particle-type, as one moves down the nanoscale (i.e., as the particle size is decreased within the nanoscale range), fundamental physical and chemical properties appear to change—often yielding completely new and different physical/chemical properties.

For example, titanium dioxide particle-types, lose their white color and become colorless at decreasing size ranges < 50 nm. Other particle-types, known for electrical insulating properties, may become conductive at the nanoscale; or insoluble substances can become more soluble below 100 nm. Accordingly, these alterations in physical properties have generated great interest in this new technology (Colvin, 2003).

Given the excitement associated with all of the nanotechnology applications, evaluating the potential hazards related to exposures to nanoscale materials and its products has become an emerging area in toxicology and health risk assessment. The development of a safety database for nanoscale particles is evolving as new particles, materials, and exposure methodologies are being developed (i.e., implications research). Nanoparticle-types (often defined as < 100 nm in one dimension) may have different health impacts when compared to fine-sized (bulk) particle-types of similar chemical composition. In this regard, data from some pulmonary toxicity studies in rats demonstrate that exposures to ultrafine/nanoparticles produce enhanced toxicity responses when compared with larger-sized particles of similar chemical composition (Donaldson *et al.*, 2001; Oberdorster, 2000). Particle surface area and particle number determinations have been postulated to play significant roles in the development of nanoparticle-related lung toxicity. In particular, some reports indicate that inhaled ultrafine/nanoparticles, following deposition in the alveolar regions of the lung, largely escape alveolar macrophage surveillance and transmigrate to the pulmonary interstitium or the systemic circulation following deposition in the alveolar regions of the lung (Donaldson *et al.*, 2001; Oberdorster, 2000). Alternatively, other recent studies indicate that the toxicity of some nanoparticulates may be related, in large part, to the surface reactivity of the particles, indicating that the particle surface–cellular interactions may take precedence over the core particle or particle size/surface area *per se* in influencing the development of inflammatory and cytotoxic responses in the lung (Warheit *et al.*, 2007a,b).

Particle surface and interfaces are important components of nanoscale materials. As the particle size is reduced, the proportion of atoms found at the surface is enhanced relative to the proportion inside its volume. This results in nanoscale particles, which are likely to be more reactive, thus generating more effective catalysts from an applications standpoint. However, from a health implications perspective, reactive groups on a particle surface are likely to modify the biological (potentially toxicological) effects. Therefore, changes in surface chemistry forming the "shell" on a (core) nanoparticle-type may be important and relevant for health effects. In addition, surface coatings can be utilized to alter surface properties of nanoparticles to prevent aggregation or agglomeration with different particle-types, and/or can serve to "passivate" the particle-type to mitigate the effects of ultraviolet radiation induced reactive oxidants. It is interesting to note that surface coatings, functioning to reduce aggregation and to facilitate particle dispersion, enhance the efficacy of the particle-type in its *designed application*, but may also accelerate translocation of the nanoparticle from the respiratory tract to the systemic circulation and thereby significantly increase nanoparticle distribution throughout the body (Borm *et al.*, 2006; Oberdorster *et al.*, 2005). To capture this concept of the importance of nanoparticle core-shell dynamics, it

[1] For correspondence via E-mail: david.b.warheit@usa.dupont.com.

Particle and Fibre Toxicology

BioMed Central

Review

Open Access

The limits of testing particle-mediated oxidative stress in vitro in predicting diverse pathologies; relevance for testing of nanoparticles

Ken Donaldson*[1], Paul JA Borm[2], Vincent Castranova[3] and Mary Gulumian[4]

Address: [1]MRC/University of Edinburgh Centre for Inflammation Research, ELEGI Colt Laboratory, Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh, EH16 4TJ, UK, [2]Zuyd University, Nieuw Eyckholt 300, Heerlen, Limburg, 6400 AN, The Netherlands, [3]National Institute for Occupational Safety and Health, 1095 Willowdale Road, Morgantown, West Virginia, USA and [4]National Institute for Occupational Health, P O Box 4788, Johannesburg 2000, University of the Witwatersrand, Witwatersrand, South Africa

Email: Ken Donaldson* - ken.donaldson@ed.ac.uk; Paul JA Borm - p.borm@hszuyd.nl; Vincent Castranova - vic1@cdc.gov; Mary Gulumian - Mary.Gulumian@nioh.nhls.ac.za

* Corresponding author

NANOTOXICOLOGY

Signs of stress

What is the best way to find out if nanoparticles are toxic? New results suggest that measuring oxidative stress could eventually allow us to screen the thousands of new nanoparticles made every year.

Vicki Stone[1] and Ken Donaldson[2]

are at [1]Centre for Health and Environment, School of Life Sciences, Napier University, Edinburgh EH10 5DT, UK; [2]MRC/University of Edinburgh Centre for Inflammation Research, Queen's Medical Research Institute, Edinburgh EH16 4TJ, UK.

e-mail: v.stone@napier.ac.uk; ken.donaldson@ed.ac.uk

The rapid expansion of nanotechnology has resulted in a vast array of nanoparticles that vary in size, shape, charge, chemistry, coating and solubility. Take carbon nanotubes, for example, which have been intensively studied because they have new and unusual mechanical, electronic and other properties. The potential toxicity of these materials has attracted attention because of their apparent similarities to asbestos and other carcinogenic fibres (Fig. 1). Carbon nanotubes are long, thin (just nanometres in diameter) and insoluble — all factors that contribute to fibre toxicity in the nanomaterials cause most oxidative injury within cells[2].

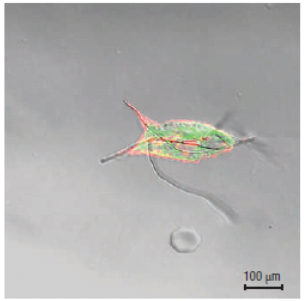


Figure 1 A rat lung cell attempting to ingest carbon nanotubes that are longer than the distance that the cell can stretch, which means that the rat cannot remove such nanotubes from the body. This optical microscopy image is superimposed with confocal images of the protein cytoskeleton

However, when testing the toxicity induced by carbon nanotubes, should we consider single- or multiwalled tubes? Long or short nanotubes? How long or short? Should we remove metal catalysts? Do we use functionalized or non-functionalized particles? Should we use pristine tubes or should they be tested in the form in which they might actually be used? The list of variations is endless and poses a real problem for toxicologists.

However, the challenge for toxicologists is not to test every variation of a new nanoparticle generated but, instead, to identify key factors or tests that can be used to predict toxicity, permit targeted screening and allow materials scientists to generate new, safer nanoparticles with this structure-toxicity information in mind.

Nel and co-workers have now taken a major step in this direction by systematically

Registro Europeo de Productos Químicos (REACH)

En Europa existe el Registro Europeo de Productos Químicos (REACH). El REACH (registro, evaluación, autorización y restricción) es un sistema integrado único europeo y nace con el fin de:

a. Proteger la salud humana y del medio ambiente
b. Mantenimiento y promoción de la competitividad de la industria europea
a. Transparencia
b. Integración con esfuerzos similares
c. Disminución de las pruebas con animales

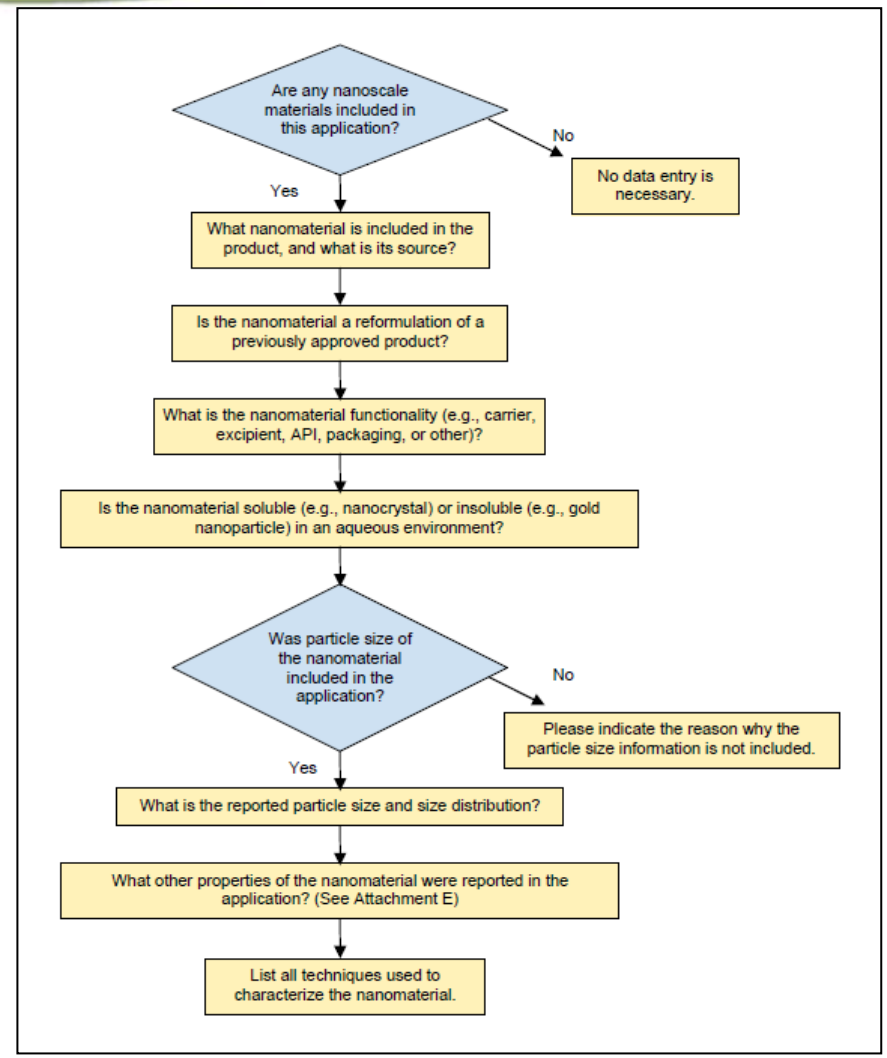
Los estudios toxicológicos que exige REACH no están adaptados para estudios a nanopartículas. Desde Europa se está reformando la normativa para que se permita evaluar la seguridad de los productos nano.

- Kit de irritación Episkin
- Corrosividad en piel Episkin, Epiderm y TER
- Phototoxicidad 3T3 NRPT
- Absorción en piel (humano/cerdo) con Franz cells
- Genotoxicidad (AMES, micronucleous test, mamífero)
- Embriotoxicidad (células madre embrionarias, ensayo micromasa y embrión total)

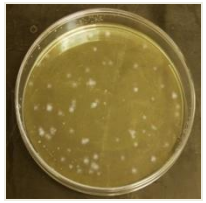
Ninguno ha sido validado para nanoformulaciones

Proyectos del FP6 y FP7 están enfocados en el desarrollo de nuevas estrategias *in vitro*: Nanoderm, Nanother, Skintreat…

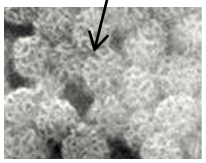
Ambito regulatorio

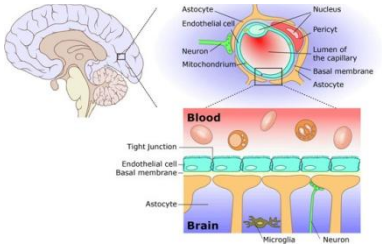


Source: Nanomaterial product review flowchart (Office of Pharmaceutical Science 2010)

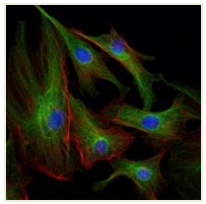


Genotoxicidad

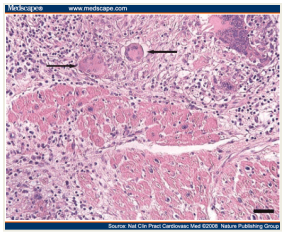




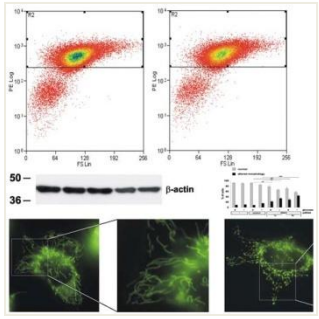
Barreras fisiológicas



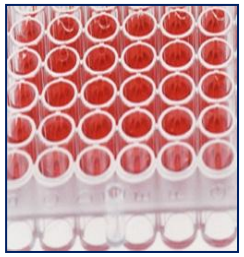
Uptake celular



Inflamación



Stress celular

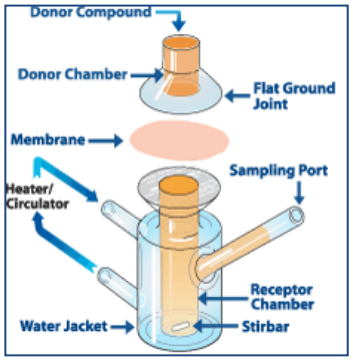


Citotoxicidad

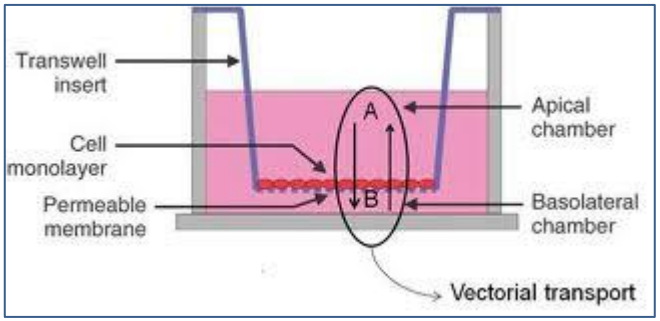
Se ha observado una correlación entre el área de superficie, capacidad de generación de stress celular y efectos proinflamatorios

Permeabilidad

Absorción percutánea



Absorción intestinal



Problemática:

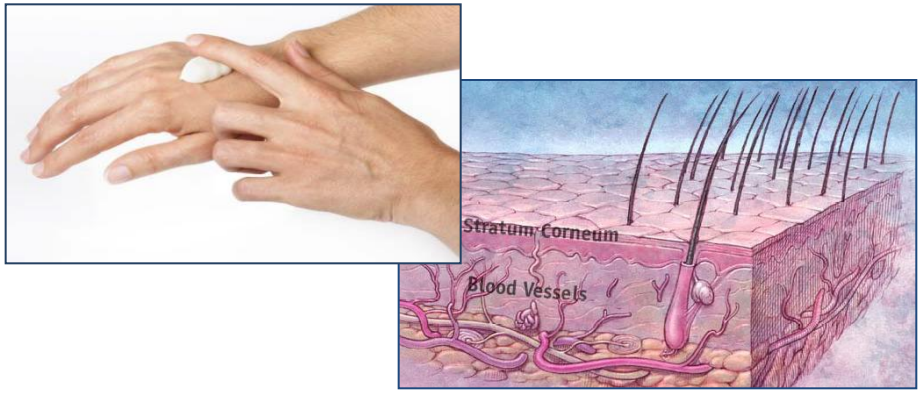
- Detección
- Cuantificación
- Standards

PIEL

•Evidencia de que existe penetración (principalmente stratum spinosum y quizás dermis para partículas muy pequeñas (<10 nm)

•No hay evidencia de que partículas > 20 nm penetren
La piel

•Es posible que áreas de la piel tenga absorción diferente (flexing)

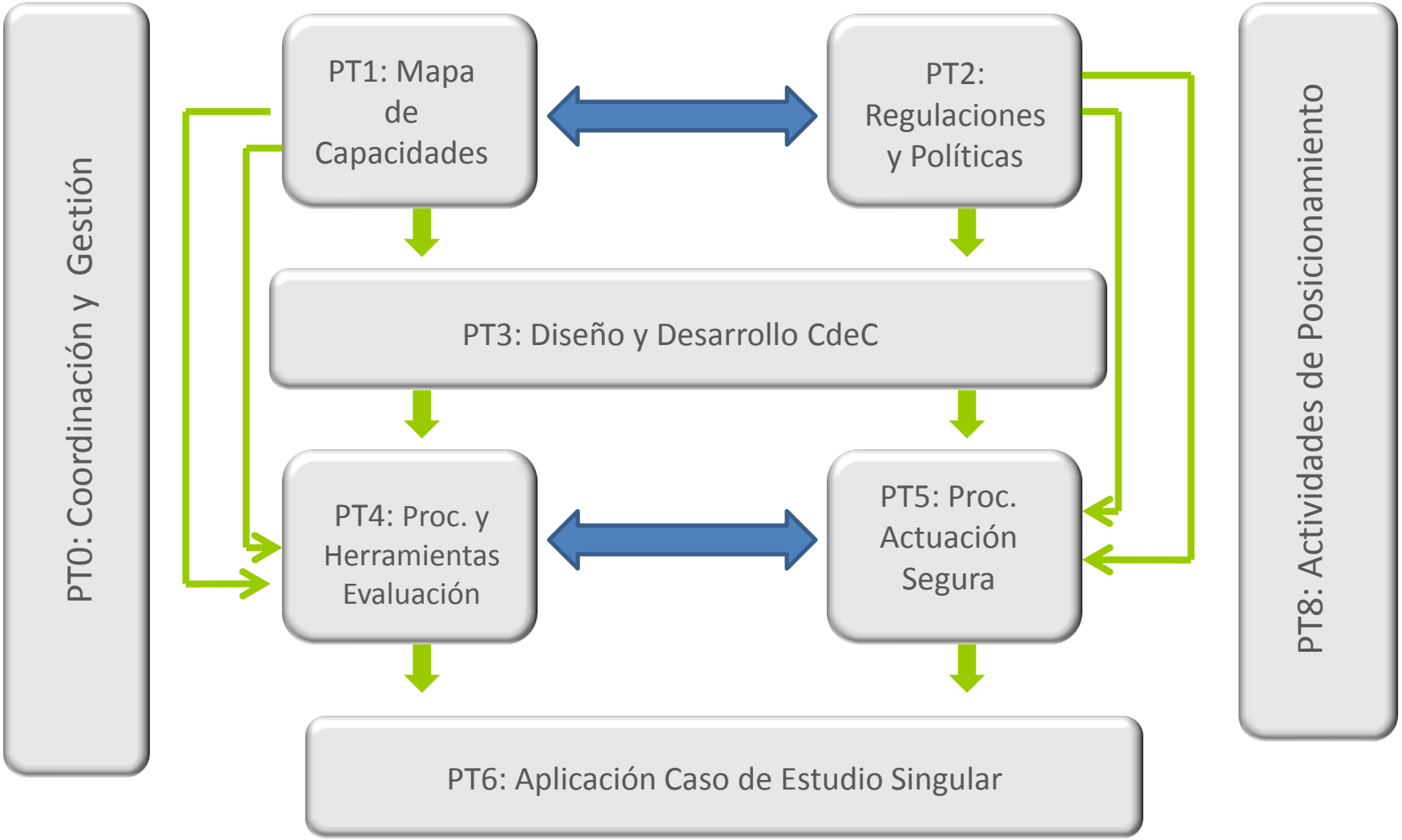


Rutas de exposición-Piel

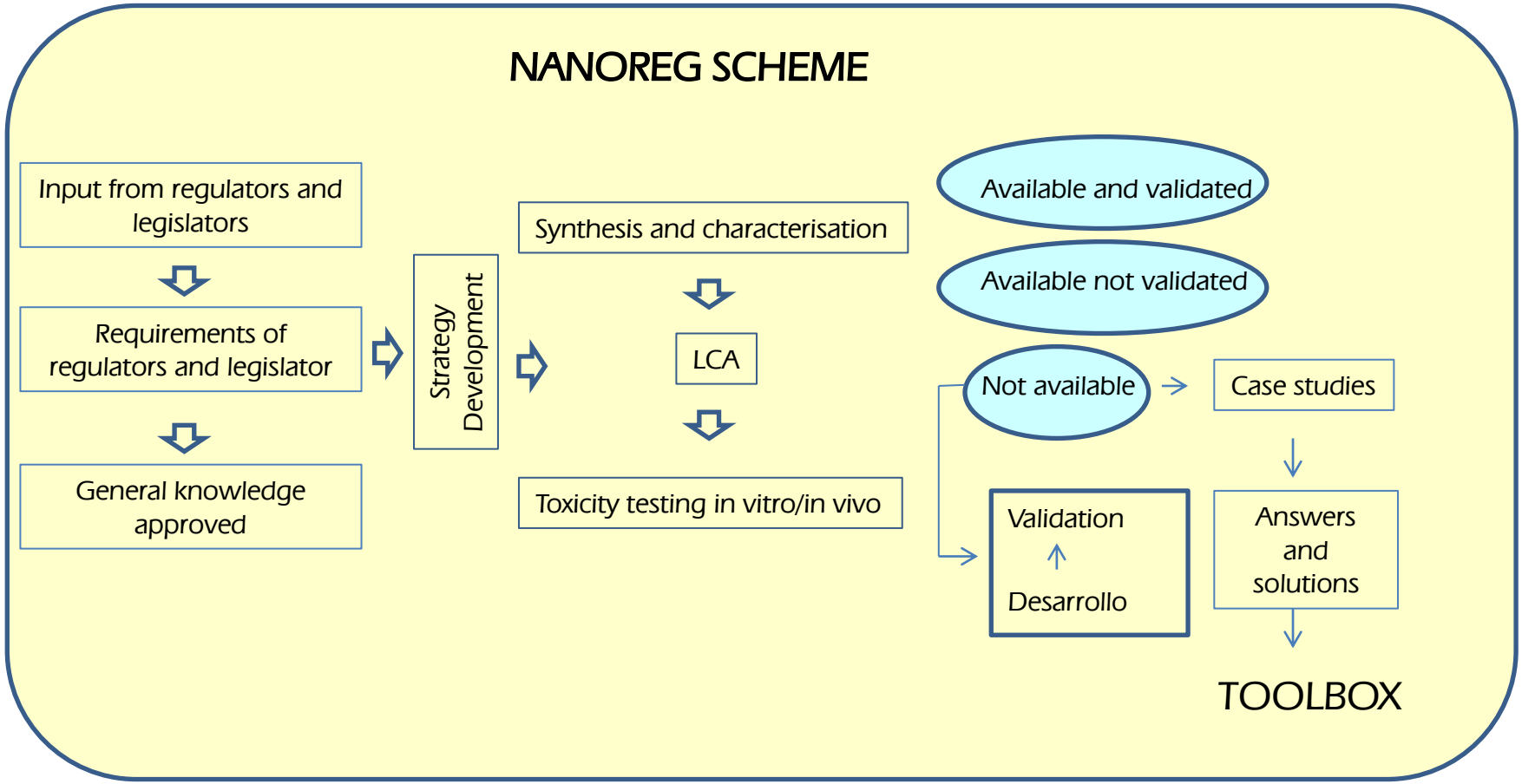

- A pesar de que estos productos se deben utilizar en piel sana, un 2% de la población europea padece eczema y una gran proporción es atópica

- Se desconoce el comportamiento de estas formulaciones en piel envejecida

Proyecto EHS-Advance



NANoREG – A common European approach to the regulatory testing of nanomaterials



Parque Tecnológico de Bizkaia, Edificio 202
48170 Zamudio | Bizkaia | Spain
T. 0034 94 6002323
suarezb@gaiker.es | www.gaiker.es