Danish Ministry of the Environment
Environmental Protection Agency

Dermal Absorption of Nanomaterials

Part of the ”Better control of nano” initiative 2012-2015

Environmental Project No. 1504, 2013

Title:

Dermal Absorption of Nanomaterials

Part of the ”Better control of nano” initiative 2012-2015

Authors:

Poland, C.A. - Institute of Occupational Medicine (IOM)
Read, S.A.K. - IOM
Varet, J. - IOM
Carse, G. - IOM
Christensen, F.M. - COWI A/S
Hankin, S.M - IOM

Published by:

The Danish Environmental Protection Agency
Strandgade 29
1401 Copenhagen K
Denmark
www.mst.dk/english

Year:

2013

ISBN No.

978-87-93026-50-6

Contents

Preface

The project “Dermal absorption of nanomaterials” was carried out during the period January to May 2013.

This report and the accompanying database (see appendix 4) are intended to provide a comprehensive evaluation of the knowledge base regarding the dermal absorption/penetration of nanomaterials based on the currently available scientific literature.

These results regarding skin as an exposure route for nanomaterials are part of the “Better control of nano” initiative conducted by the Danish EPA with the aim of further clarifying possible risks to consumers and the environment.

The project was carried out by the Institute of Occupational Medicine (IOM) with COWI A/S as subcontractor.

The project group consisted of the following members:

Craig A. Poland, B.Sc.(Hons.), M.Sc., Ph.D., IOM
Sheona A.K. Read, M.Chem.(Hons.), Pg.Cert., IOM
Julia Varet, M.Sc., Ph.D., IOM
Gillian Carse, B.Sc.(Hons.)., IOM
Steven M. Hankin, B.Sc., Ph.D., Dip.Med.Tox., Pg.D, IOM
Frans M. Christensen, M.Sc.(Eng.)., COWI A/S
Christina Ihlemann, Cand. Scient., Danish Environmental Protection Agency
Gregory Moore, Cand. Scient., Ph.D., Danish Environmental Protection Agency
Anne Mette Boisen, Cand. Scient., Ph.D., Danish Environmental Protection Agency

Main authors:

Poland, C.A., Read, S.A.K., Varet, J., Carse, G., Christensen, F.M., Hankin, S.M.

Reference group:

Dr. Maxine McCall, Commonwealth Scientific and Industrial Research Organisation (Australia)
Prof. Jesper Bo Nielsen, Southern University of Denmark.

The role of the reference group was to provide independent expert advice and input to the project authors on evaluated literature, current research gaps and experimental models.

The project was financed by the National Budget Agreement 2012 on Better Control of Nanomaterials and their Safety (“Bedre styr på nano”).

Danish EPA, May 2013

Abbreviations List

Abbreviation	Definition
AES	Auger Electron Spectroscopy
AOT	Sodium bis(2-ethylhexyl) sulfosuccinate
APS	(3-amino-propyl)-trimethoxysilane
BSI	British Standards Institution
C_{60}	Fullerene
CDC	US Centre For Disease Control
CEN	European Committee For Standardization
CFA	Coal Fly Ash
CLSM	Confocal Laser Scanning Microscopy
CNT	Carbon nanotube
CSIRO	Commonwealth Scientific And Industrial Research Organisation
CTAB	Cetyltrimethyl ammonium bromide
Danish EPA	Danish Environmental Protection Agency
DCMS	Decylmethyl sulfoxide
dH_2O	Distilled water
ddH_2O	Double-distilled water
DHLA	Dihydrolipoic acid
DLS	Dynamic Light Scattering
DMEM	Dulbecco's Modified Eagle Medium
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
EC	Epidermis cells
ECVAM	European Centre For Validation Of Alternative Methods
EDX	Energy Dispersive X-Ray Spectroscopy
EELS	Electron Energy Loss Spectroscopy
EFSA	European Food Safety Authority
ETAAS	Electro Thermal Atomic Absorption Spectroscopy
FDC	Franz-type Diffusion Cell
FITC	Fluorescein 5-isothiocyanate
FMA	Fluorescein methacrylate
FP	Framework Programme
GN	Gold nanorods
H	Human
HEK	Human epidermal keratinocytes
HSE	Health and Safety Executive
ICCR	International Cooperation On Cosmetics Regulation
ICP	Inductively Coupled Plasma
IHCP	Institute For Health And Consumer Protection
IPPSF	Isolated perfused porcine skin flap
ISO	International Organization For Standardization
JRC	Joint Research Centre
LC	Langerhans cells

Abbreviation	Definition
MAIL	Multi-Photon-Absorption-Induced Luminescence
MC	Multi collector
MPM	Multi-photon microscopy
MS	Mass Spectroscopy
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MWCNT	Multi-walled carbon nanotubes
NIOSH	National Institute Of Occupational Safety And Health
NIR	Near Infrared
NP	Nanoparticle
NR	Nile Red
OECD	Organisation For Economic Cooperation And Development
OES	Optical Emission Spectrophotometry
P	Porcine
PAA	Poly(acrylic acid)
PAH	Polyaromatic hydrocarbons
PBS	Phosphate buffered saline
PDADMAC	Poly(diallyldimethylammonium chloride)
PEG	Polyethylene glycol
PIXE	Particle-induced X-Ray Emission
PMMA	poly(methyl methacrylate)
PS	Polystyrene
PSS	Poly(sodium-4-styrenesulfonate)
QD	Quantum dots
QSAR	Quantitative Structure Activity Relationship
R	Rodent
RBS	Rutherford Back Scattering
RIP-oN	REACH Implementation Project on Nanomaterials
RITC	Rhodamine B isothiocyanate
RIVM	National Institute For Public Health And The Environment
ROS	Reactive oxygen species
SC	*Stratum corneum*
SCCS	Scientific Committee on Consumer Safety
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCID	Severe combine immune-deficient
SEM	Scanning Electron Microscope
SG	*Stratum granulosum*
SGV	*Stratum germinativum*
SIMS	Secondary-Ion Mass Spectrometry
SLS	Sodium lauryl sulphate
SPM	Scanning Probe Microscopy
SR	*Stratum reticulare*
SS	*Stratum spinosum*
STIM	Scanning Transmission Ion Microscopy
SWCNT	Single-walled carbon nanotubes
TCSCP	Time-Correlated Single Photon Counting
TEER	Transepidermal Electrical Resistance
TEM	Transmission Electron Microscopy
TG	Test Guideline
TIMS	Thermal Ionisation Mass Spectrometry
TMAOH	Trimethylammonium hydroxide

Abbreviation	Definition
US EPA	United Stated Environmental Protection Agency
UV	Ultraviolet
WPMN	Working Party On Manufactured Nanomaterials
XPS	X-Ray Photoelectron Spectroscopy

Sammenfatning og konklusioner

Denne rapport med tilhørende litteratur-database indgår i rækken af projekter under indsatsen ”Bedre styr på nano”, som administreres af Miljøstyrelsen. Projektet indsamler og vurderer eksisterende litteratur i relation til viden om systemisk optag/absorption af nanomaterialer via hudkontakt med det mål at beskrive state-of-the-art, herunder at identificere manglende viden i relation til forbrugereksponering.

Nanomaterialers specifikke egenskaber har gjort mange nye industrielle anvendelser mulige og et stigende antal forbrugerprodukter indeholder nanomaterialer, som kan medføre bevidst eller utilsigtet hudeksponering. Dette skal ses i lyset af den begrænsede viden om nanomaterialers optag over huden og mulige sundhedsmæssige effekter.

Metoden til indsamling og analyse af den eksisterende viden omfattede udvikling af en systematisk søgestrategi baseret på søgetermer og –sætninger med relevans for hudabsorption af nanomaterialer. Søgningen og den efterfølgende trinvise gennemgang af de identificerede studier var inspireret af vejledningerne i ” the Cochrane Handbook for Systematic Reviews of Interventions”, såvel som hvad der p.t. anses for god praksis i relation til gennemgang af litteratur vedrørende nanotoksikologi. Vurderingen af hvert af de identificerede studier er dokumenteret i en MS Access database, som ledsager denne rapport. Denne viden blev efterfølgende anvendt til at evaluere hvilken rolle forskellige fysisk-kemiske egenskaber spiller for hudpenetration/hudabsorption af nanomaterialer. Dette er sammenfattet under følgende generelle fysisk-kemiske egenskaber:

- Størrelse
- Sammensætning
- Overfladekemi
- Form

En af de væsentligste udfordringer ved at vurdere litteraturen om fysisk-kemiske egenskabers indflydelse på hudpenetration/hudabsorption er, at det er svært at drage konklusioner fordi: i) rapporteringen af de fysisk-kemiske egenskaber er mangelfuld, og/eller ii) de eksperimentelle forsøgsparametre varierer på en ikke systematisk måde. Manglen på information om nanopartiklers fysisk-kemiske egenskaber er udbredt, mens ændringen af de eksperimentelle parametre er mest udfordrende, da flere karakteristika såsom form, ladning, overfladebehandling og størrelse kan variere samtidigt. Dette forårsager, at det er meget svært at sammenligne resultater indenfor og ikke mindst mellem studier.

Trods disse udfordringer kan der drages nogle væsentlige konklusioner. Selvom der er mange modsatrettede resultater, synes litteraturen at indikere, at absorption gennem huden af partikler i nanostørrelse er mulig, omend i meget begrænset omfang og at graden af hudpenetration, afhængig af partiklernes kemiske sammensætning og de eksperimentalle forhold, kan være større end for større partikler. Størrelsen af partiklerne er således en væsentlig parameter for hudabsorption, men ser ikke ud til i sig selv at være bestemmende for absorptionen, da andre egenskaber også markant influerer på muligheden for hudabsorption. Derudover er størrelse ikke en konstant parameter da agglomerering af partikler kan forekomme over tid og er afhængig af de eksperimentelle forhold (f.eks. afhængig af forekomsten af overfladeaktive stoffer i den formulering, som nanomaterialet testes i). Selvom agglomerering menes at være en væsentlig parameter (og en væsentlig eksperimentel variabel), er omfanget af agglomerering ofte ikke rapporteret i den videnskabelige litteratur.

Selvom det er vist, at selve partikel-sammensætningen har lille effekt på hudpenetration/absorption skal sammensætningen (herunder urenheder) tages i betragtning i relation til dermal toksicitet. En anden udfordring relateret til at vurdere hudabsorption af nanomaterialer er at bestemme, hvorvidt en påvist absorption forekommer som en fast partikel eller som optag af opløst materiale (f.eks. som metal ioner). Dette kan have væsentlig indflydelse på systemisk tilgængelighed, distribution, såvel som metabolisme og udskillelse.

Nanomaterialers overfladekemi udgør kontakten mellem nanopartikler og de biologiske omgivelser, herunder kontakt med huden. En række studier indikerer således at fysisk-kemiske egenskaber relateret til overfladekemi er væsentlige for nanopartiklers evne til at penetrere huden. Overfladeladning, som påvirkes af overfladebehandling (coating og funktionalisering) er den mest studerede egenskab. Det er dog svært at uddrage klare konklusioner om overfladekemiens indflydelse på hudpenetration/absorption, da karakteriseringen af overfladekemien i litteraturen generelt er mangelfuld og i mange tilfælde kun består af kvalitative indikationer. Derudover varierer flere overfladeegenskaber indenfor det samme eksperiment, således at det ikke er muligt at forudsige, hvilken effekt individuelle egenskaber har på hudabsorption. Trods disse komplekse resultater kan der spores en svag tendens til større hudabsorption for positivt ladedepartikler, selvom der også findes modstridende studier.

Formen af nanomaterialer anses for en væsentlig egenskab i relation til eksponering og toksicitet via inhalation, men er endnu ikke velundersøgt i relation til hudabsorption. Ikke-sfæriske partikler har været undersøgt i en række studier, men der er ikke identificeret studier som kritisk og systematisk evaluerer formens/længdens rolle for hudabsorption. Ingen af de studerede ikke-sfæriske partikler har opfyldt kriterierne for hvad der typisk anses for fibre (dvs. længde over 5 µm) og der mangler også studier af andre former, såsom plader (f.eks. grafen).

Udover at vurdere indflydelsen af fysisk-kemiske nøgleparametre på hudabsorption har rapporten også evalueret de testmetoder (*in vitro*, *in vivo* og *in silico*), som anvendes til at studerede hudabsorption. Mange forhold, såsom klargøring af testmateriale, anvendte hjælpestoffer og dyreart, såvel som bestemmelse og kvantificering af partikler er fælles for såvel *in vitro* som *in vivo* testmetoder. Disse forhold er diskuteret i rapporten og bidrager til usikkerheden forbundet med sammenligning af resultater fra forskellige studier pga. manglende harmonisering, som til en vis

grad bunder i eksisterende test guidelines. Anvendelse af *in silico* metoder, såsom "Quantitative Structure Activity Relationships" (QSAR) til at forudsige hudabsorption for konventionelle kemikalier er stadig i sin vorden for nanomaterialer. Udviklingen af *in silico* metoder forekommer dog yderst vigtig givet diversiteten af nanomaterialer nu og i fremtiden.

Rapportens beskrivelser af manglende viden i relation til fysisk-kemiske egenskaber, testmetoder og detektionsgrænser for hudabsorption leder til en række anbefalinger af metoder og endpoints til at vurdere hudabsorption af nanomaterialer. Anbefalingerne fokuserer på behovet for mere robuste og harmoniserede test metoder/guidelines til at bestemme hudabsorption og behovet for en systematisk tilgang til variationen af fysisk-kemiske karakteristika for at forstå deres relative indflydelse på hudabsorption. Dette inkluderer anbefalinger relateret til klargøring af testmateriale, hudmodeller, hjælpestoffer, dosis, varighed, samt bestemmelse og kvantificering af partikler. Rapporten afsluttes med anbefalinger relateret til prioriterede nanomaterialer til testning, herunder hvordan dette måske nærmere bør relatere til prioriterede fysisk-kemiske egenskaber frem for enkelte eller grupper af nanomaterialer.

Executive Summary

This report and accompanying literature database forms part of a series of projects regarding nanomaterials in Denmark ("Better control of nano") commissioned by the Danish Environmental Protection Agency (EPA). The report constitutes a literature study to gather and evaluate the existing knowledge on the systemic absorption of nanomaterials *via* dermal exposure with the intended aim of describing the current state of the art as well as gaps in knowledge on dermal absorption of nanomaterials in relation to consumer exposure.

There exists concerns that whilst the properties of nano-scale materials have enabled numerous industrial applications and the consumer market already offers increasing numbers of products containing nanomaterials, these may result in skin exposure (intentionally or unintentionally) and knowledge concerning passage of nanoparticles through the skin and subsequent effects is limited. The approach to collating and analysing the current state of knowledge involved the development of a systematic search strategy based on key search terms and phrases of relevance to dermal absorption of nanomaterials. The search and subsequent stepwise review of identified studies took into account guidance provided in the Cochrane Handbook for Systematic Reviews of Interventions as well as currently considered best practice in reviewing nanotoxicological literature. The identified studies and their appraisal culminated in the formation of an MS Access Database which accompanies this report. This knowledge base has been used to evaluate the role of various physicochemical properties on nanoparticle absorption into the skin as summarised under the following broad physicochemical properties, concerning the role of:

- Size
- Composition
- Surface Chemistry
- Shape

One of the key challenges in assessing the literature on the physicochemical properties influencing dermal penetration/absorption of nanomaterials is that it is difficult to draw conclusions due to either: i) limitations in the reporting of physicochemical data, and/or, ii) the alteration of multiple experimental parameters in a non-systematic way. The issue of a lack of information on nanoparticle physicochemical properties is common, yet the most challenging aspect is the alteration of multiple experimental parameters whereby multiple characteristics such as shape, charge, coating, size can all be changed. This means that little meaningful comparison of results can be made within a single experimental study, let alone between studies.

Despite such challenges, some key conclusions can be drawn. Whilst there are many conflicting results, on balance the literature seems to suggest that absorption of particles in the nano-range through the skin is possible although occurs to a very low degree and that the level of penetration, depending on chemistry and experimental conditions, may be greater than for larger particles. The

role of size is considered a critical component of dermal absorption but this in itself does not seem to guarantee absorption or lack of as other properties can also influence dermal absorption markedly. In addition, particle size is not necessarily a constant parameter as agglomeration of particles can occur over time and also in relation to experimental conditions (e.g. presence of surfactants within particle vehicle formulation). However, whilst this issue of agglomeration has been suggested as being important (as well as an important experimental variable), agglomeration state is often not reported within studies.

Although particle composition has been shown to have little effect on dermal penetration/absorption of nanoparticles, composition (either in terms of the bulk or as a contaminant such as iron) should be considered in relation to dermal toxicity. Another challenging issue noted in relation to nanoparticles is determining whether absorption (when detected) occurs as a solid particle or as soluble fraction (e.g. metal ions). This may have important connotations in terms of systemic availability, distribution as well as metabolism and excretion.

Surface chemistry, due to its prominence in forming the interface between a nanoparticle and the biological environment, is seen as a key group of physicochemical properties dictating dermal interaction and has been indicated by several studies to be an important factor influencing the ability of nanoparticles to penetrate into the skin, with surface charge (through the modification of surface coating/functionalisation) being the most investigated aspect. However, elucidating a clear relationship between the many aspects of surface chemistry and dermal absorption/penetration is difficult as the level of characterisation of the surface chemistry of the nanoparticles studied is often poor and in many cases only a qualitative indication is provided. In addition, such issues are further confounded by the multiple alterations of surface properties within the same experiment, meaning that it is not possible to predict the effect of one single property on dermal absorption. However whilst the results are complex and inconclusive, there does appear to be a slight tendency towards greater uptake of positively charged particles although there are conflicting studies in relation to this.

Shape has been seen as a key physicochemical property influencing toxicity in relation to inhalation exposure to nanomaterials, yet knowledge of its role in dermal absorption is limited. Whilst there are examples of non-spherical nanoparticles being evaluated within the literature, there is no study which critically evaluates in a systematic way the role of particle shape/length on absorption. Indeed those non-spherical particles which have been evaluated do not meet the criteria of what is conventionally considered to be a fibre (i.e. length greater than 5 μm) and there is also an absence of other shaped particles such as platelets (e.g. graphene) being evaluated.

In addition to appraising the role of key physicochemical properties on dermal absorption of nanomaterials, this report also considered the test methods (*in vitro*, *in vivo* and *in silico*) used in the evaluation of absorption. There exist many cross-cutting issues such as sample preparation, vehicle and species employed, as well as particle detection and quantification that are common to both *in vitro* and *in vivo* test methods. These issues are discussed in the report, and add to difficulties in comparing results of differing studies due to non-harmonised approaches which, to a certain extent, may be enhanced by current test guidelines. The use of *in silico* approaches such as quantitative structure activity relationships (QSAR) to predict dermal absorption is apparent for

conventional chemicals yet is still very much within its infancy for nanomaterials. However, due to the challenges that the vast array and diversity of nanomaterials brings, this is considered to be an important area for further development.

Other key gaps are described in relation to the physiochemical properties, test methods and detection methods related to dermal absorption leading to report recommendations on methods and endpoints to assess dermal absorption for nanoparticles. Such recommendations focus around the need for more robust and harmonised testing approaches (guidelines) for determining dermal absorption of nanomaterials and the need for a systematic approach to alteration of key physicochemical characteristics to understand their relative role in dermal absorption. This also involves recommendations surrounding sample preparation, dermal models, nanoparticle vehicles, dose, duration and particle detection and quantification. The report concludes on recommendations for relevant and priority candidate nanomaterials and how this may relate more to priority candidate physicochemical properties rather than a single or group of nanomaterials.

1. Introduction

1.1 Danish initiative for "Better control of nano"

The Danish government and the Red-Green Alliance (a.k.a. Enhedslisten) have signed an agreement called "Bedre styr på nano" ("Better control of nano") for four years (2012-2015) that focuses on the use of nanomaterials in products on the Danish market and their consequences on consumers and the environment. The Danish Environmental Protection Agency (EPA) has initiated a series of projects with the aim of further clarifying possible risks to consumers and the environment. The current project and accompanying literature database is part of this series.

1.2 Project outline

As part of this series of projects on key issues regarding nanomaterials in Denmark, such as occurrence, extent of consumer and environmental exposure and assessment of potential risk, the Danish EPA commissioned the present literary study on the systemic absorption of nanomaterials *via* dermal exposure. The overall aim of the project was to gather and evaluate the existing knowledge in the area and assess the need to generate new knowledge, as well as to develop recommendations for the most suitable skin models, measurement methods and relevant candidate nanomaterials for experimental testing in the future.

This study provides a comprehensive evaluation of the knowledge base regarding the dermal absorption of nanomaterials, based on a review of the currently available scientific literature. The evaluation includes:

- assessment of the extent of dermal absorption of nanomaterials;
- identification of nano-specific characteristics that may influence dermal absorption of nanomaterials;
- evaluation of which test method(s) would most closely simulate the transport of nanomaterials through the skin; and
- assessment of the specific research areas that require more knowledge.

1.3 Dermal absorption of nanomaterials

Nanomaterials are defined in different ways by different organisations, typically focusing on materials having at least one dimension in the range of 1 to 100 nanometres. Some definitions, require that the nano-scale confers them specific properties,[1] whereas other consider the size distribution of the particles in a material.[2] This review does not address a specific definition as it is typically not clear what definition is considered in a journal article and the focus is rather on more broadly collecting evidence about materials with nano-scale dimensions. The identified literature related to dermal absorption or penetration of nanomaterials mainly addresses nanoparticles (i.e.

objects with nanoscale in all three dimensions) and, to some extent, fibres (two dimensions). Furthermore, some studies with larger sized particles, which may be used to draw conclusions in a weight of evidence approach, have also been included. Nanomaterials can be natural (e.g. from volcanic activities), unintentionally produced/generated (e.g. from combustion), or engineered (i.e. nanomaterials intentionally made for industrial and/or research purposes). Due to the interesting properties that nanomaterials display, their applications are increasing and their use is growing fast. Nanomaterials are used in many industrial processes but also incorporated in many goods destined to the consumers. As a consequence, consumers are increasingly exposed to nanomaterials yet to date, the risks in terms of health and safety represented by their use are still not fully known. Risk is defined as the product of exposure and hazard. Hazard assessment is the assessment of the potential for a chemical/material to cause adverse effects to an individual entering in contact with it. It involves hazard identification and dose-response studies. Exposure is generally used as a proxy for the dose and exposure is defined as "*the contact between an agent and a target*".[3]

Depending on the product the consumer is using, exposure to chemicals/particles can occur along several routes: the respiratory tree, digestive tract and skin being the main routes of exposure. The skin represents a total surface of around 1-2 m^2 and[4,5], as a consequence, a large area with potential exposure to chemicals, particles and other materials, in the form of cosmetics, clothing, handling materials etc. It is worth noting that while most industrial and environmental skin exposures are unintended, infrequent, and covering a limited area, the exposure to cosmetic products is very much different as it is clearly intended, often repeated, in periods very frequent, (e.g. sun blockers), and often covering a significant area of the body.

1.3.1 The skin

The structure of the skin presents some alterations according to the region of the body as physiological adaptations. The skin is structurally divided into three layers: the outside layer or epidermis, the dermis and the inside layer, the hypodermis, as schematically represented in Figure 1.

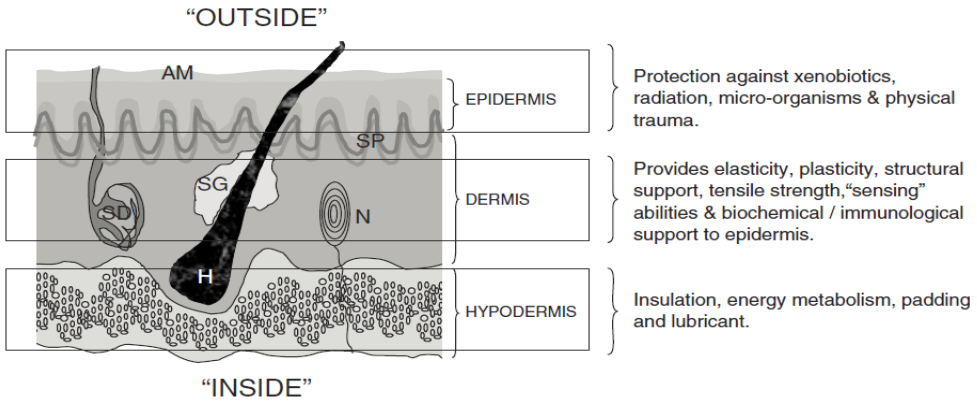

FIGURE 1: SCHEMATIC REPRESENTATION OF SKIN STRUCTURE AND ASSOCIATED FUNCTIONS. Reproduced from [6]. Note that the relative thickness of each layer is not to scale. Several adnexal structures are shown (SP = Superficial Plexus, SG = Sebaceous Gland, SD = Sweat Duct, N = Pacinian corpuscle, H = Hair). In humans the skin is covered with a thin layer of lipids known as the acid mantle (AM), which comprises sebum, cell debris and sweat residua.

The main cell type found in the epidermis is the keratinocyte, which are known to produce a fibrous structural protein called keratin. Keratinocytes cells are structurally and functionally organised into

several layers, with gradually increasing keratin content and rich-lipid extracellular matrix along the epidermis, termed (from the inside to the outside):

- *stratum basale* (SB), composed of dividing cells that proliferate and push the cells toward the outside of the skin where they gradually morphologically and functionally change and flatten and finally die in the outer layer (*stratum corneum*);
- *stratum spinosum* (SS);
- *stratum granulosum* (SG);
- *stratum lucidum* (SL), a thin clear layer between stratum granulosum and *stratum corneum*;
- *stratum corneum* (SC), constituted of flat, dead cells, called corneocytes, that desquamate.

The epidermis is schematically depicted in Figure 2.

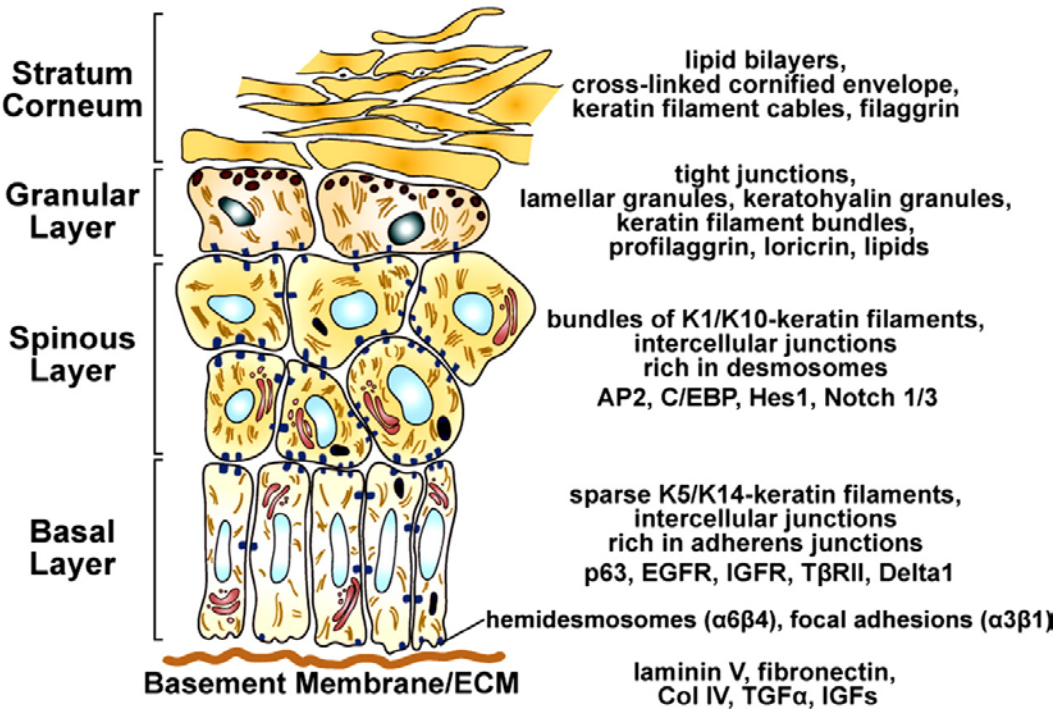

FIGURE 2: SCHEMATIC REPRESENTATION OF THE EPIDERMIS. Reproduced from [7]. The program of epidermal differentiation is shown in this schematic, illustrating the basement membrane at the base, the proliferative basal layer, and the three differentiation stages: spinous layer, granular layer, and outermost stratum corneum. To the right, key molecular markers are shown.

The epidermis is also composed of many other cells types, such as cells from the immune system (e.g. Langerhans cells and melanocytes which produce the pigment melanin under exposure to ultra-violet (UV) light). The epidermis also contains hair follicles, which are invaginations of the sub-SC that penetrate deep into the epidermis and reach the dermis.

The dermis is a layer of the skin mainly composed of extra-cellular matrix such as collagen and elastic fibres produced by dermal fibroblasts. The dermis also contains various structures such as

sebaceous gland, blood vessels and nerves. The hypodermis is similar to the dermis but contains larger blood vessels and nerves as well as fat structure.

1.3.2 Skin patho-physiology

The skin is an organ composed of many different cell types and structures with specialised functions. The main role of the skin is to act as a protective barrier between the inside and outside of the body. The skin also ensures physiological functions such as hydro-regulation, thermo-regulation, sensory information, protection against UV radiation and immune defence. Therefore, its integrity and functionality are very important for the health of an individual. Exposure of the skin to chemicals or other materials may result in a variety of pathological effects ranging from surface effects to deeper topical effects or even systemic effects if penetration through the skin occurs.[6] Interaction with the surface of the skin can induce damages such as irritation, which is reversible, or corrosion, which is irreversible by definition although, whilst severe corrosive effects may remain visible as scars, milder corrosive effects may fade and not be seen after some time.

Penetration of a material though the skin barrier can trigger also numerous toxic effects. Compounds that reach the *stratum granulosum* (SG), for example, can interact with the viable keratinocytes, and trigger an inflammatory reaction. Compounds that reach the *stratum spinosum* (SS) can interact with Langerhans cells (from the immune system) and initiate an allergic reaction responsible for phenomenon such as contact dermatitis. Carcinogenic processes may also be induced in the skin following dermal exposure depending on the chemical and its ability to penetrate the skin and reach the viable layers, where it can potentially induce skin cancer. All of these effects can be grouped as dermal toxicity. However, when a compound manages to cross the epidermis, it becomes accessible for the dermis and potentially accessible to the systemic circulatory and lymphatic systems. As a result such compounds can cause effects in other distal organs within the body, by translocation through the circulatory system or by triggering systemic reactions.[1] These can potentially lead to a wide range of toxicological effects and disease such as systemic inflammation, organ toxicity and cancer.

1.3.3 Engineered nanomaterials in products and potential for skin exposure

The properties of nano-scale materials have enabled countless industrial applications. In terms of consumer exposure, the market already offers many products containing nanomaterials and for most of them the skin is likely to be a potential route of exposure. Although powder-based materials raise concerns in terms of respiratory exposure, for the skin as a route of entry nanomaterials in suspensions such as suncreams are likely to be of higher concern due to their direct application.[1] Nonetheless, the risks associated with the use of both types of nanomaterials, dry materials and suspensions, and the skin as a route of entry should be considered.

Recently, a review published in 2010 provided an overview of the most common nanomaterials found in consumer products.[8] Sourcing from the Project on Emerging Nanotechnologies inventory (http://www.nanotechproject.org), the authors counted more than 400 products containing metal nanomaterials and destined to topical applications for consumers.[8] Cosmetics in the form of moisturisers, make-up etc. are one of the major applications of nanomaterials. The most commonly used nanomaterials are TiO_2 and ZnO in sunscreens because these materials provide effective filters

against UV radiation. Liposomes, dendrimers and carbon-based materials such as fullerenes are also being used in the cosmetics industry to improve the delivery of cosmetic agents.[9] Silver is also commonly used in anti-bacterial and burn dressings and as such, is in direct contact with the skin. It is important to note that such products containing silver are generally applied to damaged skin, which has risk implications, an issue discussed later in this report. Other common examples are quantum dots which are mainly used for diagnostic in medicine. However, consumers can be exposed to various other products not designed for skin application but which use may result in exposure of skin to nanomaterials. For example, silver and other metals are being added into textiles due to properties such as anti-bacterial, water repellent, UV absorption, tear and wear resistance etc. as shown in Table 1. Nanomaterials are also introduced in many other products such as motor oil, fuel catalyst, cleaning products etc. whose use introduce potential exposure to the skin for consumers.[8]

TABLE 1: COMMON NANOMATERIALS AND PROPERTIES USED FOR TOPICAL CONSUMER PRODUCTS AND OTHER APPLICATIONS RELEVANT IN TERMS OF POTENTIAL SKIN EXPOSURE [8,10,11]

Nanomaterials	Properties	Examples of consumer applications with potential dermal exposure
Carbon nanofibres, nanotubes, fullerenes	• Increased tensile strength • High chemical resistance • Anti-oxidant • Delivery system	• Sporting goods • Textiles • Cosmetics • Therapeutics • Diagnostics
Carbon black nanoparticles	• Improved abrasion resistance and roughness and high chemical resistance	• Sporting goods, • Composites, • Textiles • Cosmetics • Pigment
Clay nanoparticles	• Electrical, heat and chemical resistance • Block UV light • Flame retardant • Anticorrosive • Adsorbing properties	• Composites • Sunscreens • Textiles
Metal nanoparticles (Ag, Au, Cu)	• Antimicrobial • Anti-odour • Self-sterilisation • Others	• Textiles, • Surface coating (handles, pan surfaces etc.), • Burn dressing • Cosmetics • Dietary supplement • Therapeutics
Metal oxide nanoparticles (TiO_2, Al_2O_3, ZnO, MgO, CeO_2)	• UV absorption • Photo-catalytic ability • Photo-oxidising activity against chemicals and biological species • Antimicrobial/self-sterilization	• Sunscreen • Cosmetics • Textiles, • Self-cleaning coating (paints etc.) • Cleaning products • Sporting goods • Motor oil • Diesel catalyst

In conclusion, there is growing number of products containing nanomaterials that are likely to reach the general consumer and cause intended or unintended exposure of the skin. Importantly, the range of products that the consumer face with likelihood of exposure to nanomaterials *via* the skin is broader than cosmetics and also now includes a wide range of nanotextiles, medicines and various other consumer merchandise.

1.3.4 Skin as a route of exposure

As mentioned previously, the skin represents a total surface of around 2 m^2 and as a consequence, forms a large area over which there is a potential for exposure to chemicals, particles and other materials, in the form of cosmetics, clothing, handling materials etc. Several parameters are known to govern the penetration of chemicals through the skin, such as the physicochemical properties of the skin and of the compound, but also the conditions of exposure. Following many years of stricter regulations on occupational inhalation exposures, the dermal route has also in many occupational settings become a very significant route of exposure.

Due to its structure and hydrophobic nature, the *stratum corneum* is one of the most important barriers against external compounds reaching the skin. Skin appendages such as hair follicles, sebaceous glands and sweat glands on the contrary have been highlighted as possible alternative routes of entry of the skin and have even been targeted for this effect for purposes such as vaccination and drug delivery.[12-16] Anatomical, age and species variations in the skin structure are responsible for significant modifications in the efficiency of skin as a barrier against the environment.[6]

For chemicals, several physicochemical characteristics have been highlighted as critical in terms of absorption through the skin. It is usually suggested that molecules with a molecular weight higher than 500 Da do not generally penetrate the skin, although this is a rough cut-off since exceptions have been found.[17] The solubility of a molecule measured in terms of partition between an aqueous and a lipophilic solvent is also a parameter used to critically estimate the absorption of a chemical *via* the skin, since the SC is very hydrophobic by nature. In addition, absorption of molecules that are charged is also impaired by the presence of charges negative and positive on the main protein found in the epidermis or keratin.

1.3.5 Dermal absorption

Dermal absorption is defined as the transport of a compound from the outer surface of the skin into the skin and into the body and for those chemicals that are absorbed, the vast majority is by passive diffusion following Ficks Law. Therefore skin absorption implies that the compound becomes systemically available. Skin absorption should not be confused with skin permeation, which is the diffusion of a compound into a certain skin layer, nor with skin penetration which is the diffusion into deeper layers, as represented in Figure 3.

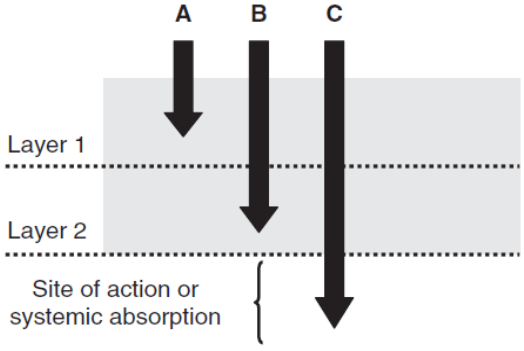

FIGURE 3: REPRESENTATION OF SKIN PERMEATION (A), SKIN PENETRATION (B) AND SKIN ABSORPTION (C). Permeation is diffusion of a penetrant into a certain skin layer. Subsequent diffusion through that layer represents penetration (in this example, the substance has "penetrated" layer 1). Penetration through layers of skin to either the site of action or systemic circulation represents absorption.[6]

Skin absorption/ penetration is governed by many parameters relative to structure of the skin, the physicochemical characteristics of the compound and finally the conditions of exposure.[6] The anatomical region of the skin, in terms of structure and appendages, the age and species of origin, the donor and its physio-pathological status will greatly influence the dermal absorption of a compound. In terms of compounds, physicochemical characteristics such as charge, hydrophobicity/hydrophilicity and size will be critical in determining its absorption *via* the skin. Exposure conditions such as choice of vehicle, area of contact, volatility, occlusion, and skin treatments such as clipping, shaving, epilation etc., will also influence the resulting dermal absorption.[6] In addition, the conditions in which exposure occurs can also influence the level of absorption of a chemical through the skin. The nature of the formulation is also critical in terms of absorption of a compound through the skin, and sometimes even minor changes in the formulation can critically change the level of absorption of a compound. Thus, the content of solvents or detergents and the acidity of a formulation, for example, may alter the uptake. The volatility of a compound in a formulation will also influence its level of absorption through the skin. Similarly, the conditions in which the exposure to the skin occurs such as covered or un-occluded will alter the absorption. Finally, another factor critical in terms of absorption through the skin is the integrity and functionality of the skin on which contact with a compound happens. Indeed, the level of hydration of the skin, the integrity of all its layers, the presence of cuts or any skin disease is likely to influence the level of absorption of a compound through the skin.

As absorption through the skin considers full penetration of the skin layers so that the penetrant can become systemically available, it is important to consider that many studies may only consider penetration as they may not consider systemic availability (and thereby not be able to show dermal absorption). An example of this would be the evaluation of penetration depth of particulate into the skin using microscopy to detect particle interaction with cell layers. Such a study may show penetration to lower layers of the skin such as the dermis yet not show absorption as systemic availability is not demonstrated. This is not to say such results are of lesser importance as they may still demonstrate particle penetration and interaction with sensitive cell layers thereby potentially causing toxicological effects. In addition, whilst absorption may not have been demonstrated, this does not necessarily mean that it will not occur, especially where penetration to deep cell layers is evident as while penetration may not result in absorption, absorption must result from penetration (i.e. there can be no dermal absorption without dermal penetration but there can be penetration without absorption).

This may be further complicated where studies discuss or state absorption, yet actually show penetration as the terms are often used interchangeably. For example, the 1996 study by Tan *et al.* states that:

> *"The results from this pilot study showed that levels of titanium in the epidermis and dermis of subjects who applied microfine titanium dioxide to their skin were higher than the levels of titanium found in controls. Studies with larger cohorts are necessary to establish if this absorption is statistically significant."*[18]

The phrase “if this absorption” is interesting as the result itself states that levels in the epidermis and dermis were higher which therefore suggests increased penetration, not absorption *per se*. Therefore, where the degree of penetration or absorption is unclear or the results are equally relevant to both terminologies, the phrase absorption/ penetration will be used.

2. Methodology

2.1 Literature search strategy

The peer-reviewed literature provides the most substantial resource of information for this review. In order to collate relevant references from the literature, a systematic literature search strategy was developed based on the main concepts being examined in the review. The approach taken to development of the search strategy and identification of relevant studies took into account guidance provided in the Cochrane Handbook for Systematic Reviews of Interventions,[19] whilst recognising and accounting for the inherent differences between the current nanotechnology knowledge base and the medical literature.

As a first stage in the development of the literature search strategy, key search terms and phrases of relevance to the project scope were derived from recognised standard terminology and nomenclature (e.g. current documents from the British Standards Institution (BSI), the International Organization for Standardization (ISO) and the European Committee for Standardization (CEN)). Following consultation with and further input from members of the Danish EPA and the project's Reference Group, the final set of search terms/phrases was agreed as follows:

- absorption
- assay
- bioavailability
- biodistribution
- characterisation
- characterization
- "consumer products"
- corrosion
- cosmetics
- derma-abrasion
- dermal
- disease
- distribution
- exposure
- fullerene
- "hazard assessment"
- "hazard identification"
- "*in silico*"
- "*in vitro*"
- "*in vivo*"
- irritation
- modeling
- modelling
- models
- nanocomposites
- nanofiber
- nanofibre
- nanomaterial
- nano-object
- nanoparticle
- nanoparticulate
- nanoplate
- nanorod
- nanotube
- nanowire
- penetration
- permeation
- "personal care"
- physicochemical
- physico-chemical
- QSAR
- "quantum dot"
- sensitisation
- sensitization
- skin
- sun block
- sun lotion
- sunscreen
- susceptibility
- systemic
- "systemic absorption"
- "test guidelines"
- "test methods"
- "testing strategy"
- "toxicity assessment"
- "toxicology assessment"
- translocation
- ultrafine
- uptake

A systematic matrix-based search strategy was then developed by using Boolean logic operators (AND, OR and NOT) to combine the key search terms into defined search strings. The draft search strategy was trialled in the United States National Library of Medicine's PubMed database, and further refined to provide a search strategy that was robust and fit-for-purpose for the project.

The final search strategy, agreed with the Danish EPA, was as follows:

> (Ultrafine OR nano-object OR nanoparticle OR nanoparticulate OR nanomaterial OR nanotube OR nanofiber OR nanofibre OR nanowire OR nanocomposite OR nanoplate OR nanorod OR fullerene OR "quantum dot") AND (dermal OR skin) AND (absorption OR penetration OR uptake) AND *(Search Term)*

where the following words were applied sequentially as the *(Search term):*

assay	"*in silico*"	susceptibility
bioavailability	"*in vitro*"	sun block
biodistribution	"*in vivo*"	sun lotion
characterisation	irritation	sunscreen
characterization	modelling	systemic
"consumer products"	modeling	"systemic
corrosion	models	absorption"
cosmetics	permeation	"test guidelines"
derma-abrasion	"personal care"	"test methods"
disease	physicochemical	"testing strategy"
distribution	physico-chemical	"toxicity assessment"
exposure	QSAR	"toxicology
"hazard assessment"	sensitisation	assessment"
"hazard identification"	sensitization	translocation

2.2 Identification of studies

In order to gain a comprehensive summary of the available evidence, in Task 1.2 the final search strategy was undertaken in the following databases:

- The United States National Library of Medicine
  - PubMed
  - TOXLINE
- Thomson Reuters (formerly ISI) Web of Knowledge

The references obtained from each individual search were then collated in reference manager software (RefWorks). An inherent consequence of performing multiple searches across several databases is the inclusion of duplicate references in the collated dataset. RefWorks was therefore used to remove duplicates in the collated set of references to form a final “computational dataset”. The number of references obtained from each search in the three databases is presented in Table 2 overleaf. Overall, following the removal of duplicates, the computational dataset consisted of 714 unique references.

TABLE 2: NUMBER OF REFERENCES OBTAINED FOR THE FINAL SEARCH STRATEGY IN PUB MED, WEB OF KNOWLEDGE AND TOXLINE DATABASES

	No. of references obtained for the final search strategy: (Ultrafine OR nano-object OR nanoparticle OR nanoparticulate OR nanomaterial OR nanotube OR nanofiber OR nanofibre OR nanowire OR nanocomposite OR nanoplate OR nanorod OR fullerene OR "quantum dot") AND (dermal OR skin) AND (absorption OR penetration OR uptake) AND ***(Search term)***		
Search term	**PubMed**	**Web of Knowledge**	**TOXLINE**
assay	152	27	27
bioavailability	24	9	14
biodistribution	13	9	7
characterisation	8	41	4
characterization	53	41	35
"consumer products"	13	11	17
corrosion	2	0	1
cosmetics	101	33	49
derma-abrasion	0	0	0
disease	26	25	20
distribution	90	62	57
exposure	93	84	87
"hazard assessment"	1	1	1
"hazard identification"	1	1	1
"*in silico*"	0	0	0
"*in vitro*"	268	146	185
"*in vivo*"	137	94	97
irritation	27	10	16
modelling	1	2	1
modeling	1	3	2
models	53	21	113
permeation	148	59	95
"personal care"	6	6	5
physicochemical	43	30	24
physico-chemical	1	2	1
QSAR	0	0	1
sensitisation	1	1	1
sensitization	0	2	0
susceptibility	3	5	3
sun block	0	0	-
sun lotion	0	1	0
sunscreen	71	32	42

TABLE 2 (CONT.)

	Number of references obtained for the final search strategy: (Ultrafine OR nano-object OR nanoparticle OR nanoparticulate OR nanomaterial OR nanotube OR nanofiber OR nanofibre OR nanowire OR nanocomposite OR nanoplate OR nanorod OR fullerene OR "quantum dot") AND (dermal OR skin) AND (absorption OR penetration OR uptake) AND ***(Search term)***		
Search term	**PubMed**	**Web of Knowledge**	**TOXLINE**
systemic	54	33	41
"systemic absorption"	7	4	6
"test guidelines"	0	0	0
"test methods"	0	0	8
"testing strategy"	1	1	1
"toxicity assessment"	1	1	1
"toxicology assessment"	0	0	0
translocation	13	10	6
Total references (including duplicates)	**1413**	**807**	**969**
Total references (excluding duplicates)	**536**	**308**	**376**
Total references collated in the computational dataset (excluding duplicates)	**714**		
Manual screening	**- 509** **= 205**		
Additional searches	**+ 28**		
Total number of references for further appraisal	**= 233**		

Results of a systematic matrix-based search strategy will inherently contain references which are not relevant to the scope of the project. The titles and, where required due to non-specific titles, abstracts of the references in the computational dataset were therefore manually screened by an expert reviewer to identify any which were considered not to be of relevance to the aims of the project. Those determined to be of potential relevance for the project in terms of scope were selected by the reviewer and included in a "screened dataset" for taking forward into the further appraisal. The determination of whether a publication was considered not relevant was based upon the topic of the article. For example, whilst the computational approach captured an article by Zhang *et al.*, the topic of the article was the detection of silver nanoparticles in fruit (pears)[20] and therefore not relevant to the dermal penetration of nanomaterials in humans. As the focus of the project is on the dermal penetration of solid nanoparticles relevant to consumers, it was decided that publications on the medicinal uses of certain forms of nanoparticles such as liposomes, nano-emulsions and lipid nanocapsules and other lipid based nanostructures would not be taken forward. An example of such a study is that of Mamot *et al.* addressing the "Tolerability, safety,

pharmacokinetics, and efficacy of doxorubicin-loaded anti-EGFR immunoliposomes in advanced solid tumours: a phase 1 dose-escalation study".[21] However, where studies appeared potentially relevant to aims of understanding what characteristics of particles could influence their penetration through the skin, these were included irrespectively. In total, 205 references from the peer-reviewed literature were included in the screened dataset for further appraisal.

In addition to searching the peer-reviewed literature in the above databases, the project team also searched for published information and reports from relevant organisations such as the Scientific Committee on Consumer Safety (SCCS), Commonwealth Scientific and Industrial Research Organisation (CSIRO), Organisation for Economic Co-operation and Development Working Party on Manufactured Nanomaterial (OECD WPMN), Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), as well as standards organisations such as ISO and CEN, EU Framework Programme (FP) 6/7 projects, and other international organisations such as the National Institute for Occupational Safety and Health (NIOSH). In addition to searching specific websites for publications, a general search was carried out using Google to ensure coverage of additional relevant literature and in addition, the Reference Group was consulted for recommendations. Based on the results of this searching, a further 28 references were included in the screened dataset. Thus, a total of 233 information sources were taken forward for further appraisal.

2.3 Assessment of the relevance & reliability of the identified studies and appraisal of relevant studies

A standardised process was developed and used to appraise each information source in the screened dataset in terms of its relevance and reliability for use in the project. This process was facilitated in an MS Access Database, which was provided to the Danish EPA in addition to this report.

For each information source, an entry in the database was created providing a full reference and an indication of the source type (i.e. abstract, journal publication or report). Based on a more comprehensive assessment of the information available in the abstract, an assessment of the relevance (high, low) and specificity (high, low) of the information source was made by the reviewer. The criteria for relevance was based on whether or not the publication in whole or in part dealt with the issue of dermal absorption and/or toxicity of solid nanoparticles applied to the surface of the skin. Where publications were not deemed relevant, they were discounted from further appraisal (although remain in the database) and therefore this represents the last screening juncture before comprehensive review. In terms of specificity, this impacted to a much lesser degree on the full appraisal of the publication because those studies of high relevance but low specificity were still appraised. The criterion for specificity was based on the question of if the article specifically dealt with dermal penetration/toxicity or if this was only part of the considerations of the article. For example, the article by Campbell *et al.* was highly specific as it addressed only nanoparticle deposition in mammalian skin after topical exposure,[22] whilst a review by Borm *et al.* was considered non-specific as dermal penetration was not the central theme of the article;[23] the article considered dermal effects at a cursory level and also dealt with other exposure routes (e.g. inhalation) and effects in greater detail.

Of the 233 publications included in the screened dataset, approximately 56 publications were determined to be not relevant based on the abstract and a reason for this was recorded in the Access database comments box of the citation. An example is the study by Aillon *et al.*, entitled "Effects of nanomaterial physicochemical properties on *in vivo* toxicity".[24] Whilst the title appeared sufficiently of interest to warrant its inclusion in the MS Access Database, upon review of the abstract it became clear that the study deals only with systemic distribution after injection. The reference made to the term "dermal" in this paper related to a brief discussion of how routes of exposure such as dermal, oral and inhalation are most commonly studied, yet the focus of this study was on parenteral administration. Therefore, the study was discounted from further appraisal. Thus, 177 publications were identified for full appraisal. The IOM has been able to gain access to approximately 146 of them with 26 not accessible by the IOM within the resources of the project and 5 not available in English (Japanese). A more comprehensive appraisal of these 146 publications was performed according to a standardised appraisal framework.

The standardised appraisal framework, detailed in Table 3 overleaf, is intended to help rank the studies when considering their impact and contribution to the objectives of the project and provide a basis for the consistent assessment of the evidence base identified. The framework draws principally on two published methodologies for literature assessment:

- Klimisch *et al.*, which describes a standard base set of criteria and corresponding codes (Klimisch codes) for assessing the quality and potentially the validity of toxicological and eco-toxicological scientific studies and data sets[25];

- Card and Magnuson[26], which describes a two-step process where application of Klimisch codes to assess study reliability comprises the first step. The second step focuses on assessing the level of nanomaterial characterisation which was done by comparing the information presented within a study against ten parameters of nanomaterial characterisation. In this approach, the greater the number of nanomaterial characteristics described within a study, the greater the score.

The developed framework consists of 7 criteria against which the information source is assessed. Each criterion comprised of a set of scored categories. These individual scores are then summed to provide an overall score for the study in terms of its relevance and reliability allowing the identification of studies considered to be of highest quality and impact. Whilst those studies obtaining a high score would be considered to be of the highest quality and impact, those obtaining a low score will not be discounted but instead considered in light of any caveats which has impacted on its score.

TABLE 3: APPRAISAL FRAMEWORK USED TO ASSESS THE IDENTIFIED STUDIES

Criteria	Scored categories
1. Study's level of output	6 Systematic review 5 Review 4 Journal publication 3 Final report 2 Interim report 1 Abstract
2a. Test material/analyte characterisation undertaken	4 Determined & reported, with *in situ* characterisation 3 Determined & reported - characterised as supplied 2 Reported - as per label 1 Inferred from data presented 0 None
2b. Experimental design	3 Internationally accepted standard method e.g. OECD TG 2 Non-standard, documented method 1 Undocumented method 0 No experimental detail provided
2c. Cumulative property score (as per Card & Magnuson, 2010)	A score of 1 is accrued per key physicochemical property reported
3. *Experimental* method chosen for simulating dermal absorption of nanomaterials in humans under realistic conditions	5 Human 4 *In vivo* - Direct application 3 *Ex vivo* – Skin absorption models 2 *In vitro* – Skin absorption models (multi-cellular e.g. EPiSkin) 1 *In vitro* – Skin absorption models (single cell e.g. keratinocytes)
4. *Computational* method chosen for simulating dermal absorption of nanomaterials in humans under realistic conditions	3 PBPK model (multi-compartment) 2 PK/TK model (multi-compartment) 1 PK/TK model (single-compartment)
5. Methods used for labelling nanomaterials	3 Validated with post exposure confirmation 2 Validated without post exposure confirmation, or non-validated with post exposure confirmation 1 Non-validated without post exposure confirmation 0 None
6. Methods used for measuring the retrieval of nanomaterials post exposure	5 Validated method for *in situ* detection 4 Non-validated method for *in situ* detection 3 Validated method for *ex situ* detection e.g. mass balance study 2 Semi-quantitative detection e.g. local accumulation only 1 Qualitative detection e.g. histopathology with microscopy 0 None
7. Reliability	4 Reliable, without restriction 3 Reliable, but with restriction 2 Not reliable 1 Not assignable

Completed appraisals of the 146 accessible information sources are provided in the appendix to this report. A summary diagram indicating the tasks and the sequential approach to refining the dataset with results is shown below in Figure 4.

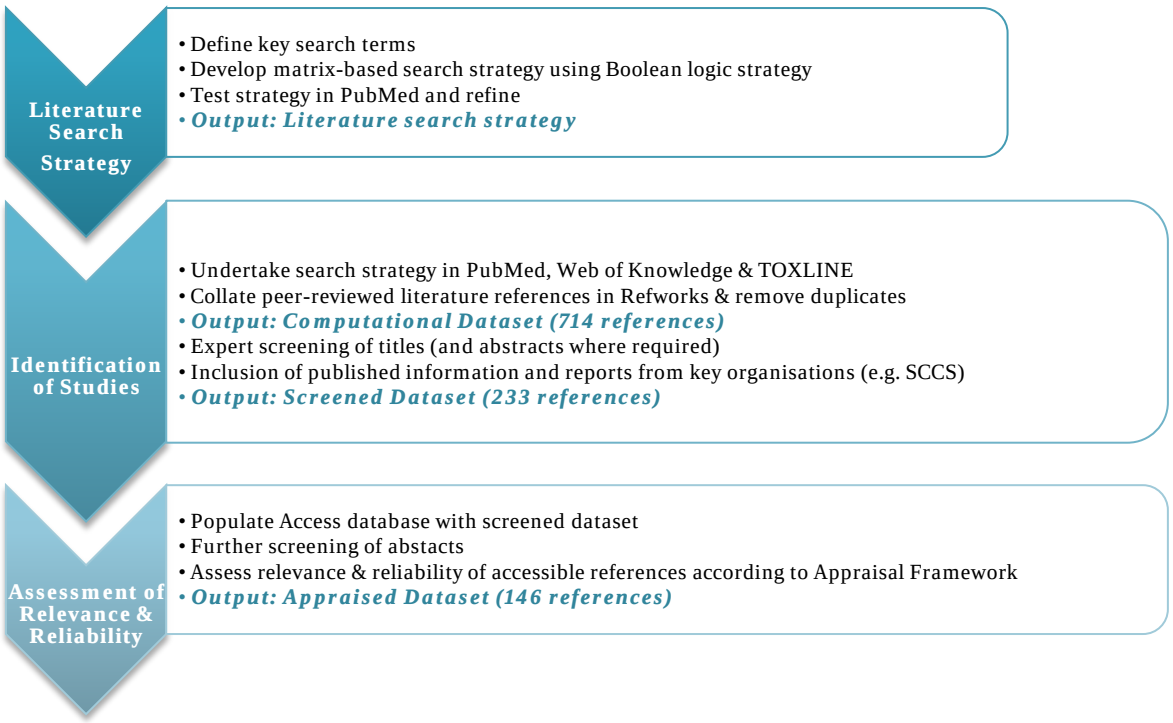

FIGURE 4: SUMMARY OF PROJECT TASKS AND APPROACH

2.4 Identification of key issues related to dermal penetration of nanomaterials, identification of research gaps and recommendations

Based on the detailed review and appraisals of the 146 references, physicochemical factors influencing dermal penetration of nanomaterials and experimental setups for investigating dermal penetration of nanomaterials were analysed, as summarised in Chapter 3. Research gaps identified are discussed in Chapter 4, followed by recommendations for methodologies/experimental setup for investigating dermal penetration of nanomaterials in Chapter 5 and recommendations related to choice of candidate nanomaterials for further research in Chapter 6.

3. Dermal Penetration and Absorption of Nanomaterials

3.1 Introduction

The potential for penetration of nanomaterials through the epidermis into the viable dermis and the ability to become systemically available within the body has raised considerable concern. Indeed, such concerns have been raised as early as 1996 with the publication of a pilot study by Tan *et al.* in which they raised the hypothesis that 10-50 nm microfine (or nano-sized) titanium dioxide "*particles may have a greater potential to be percutaneously absorbed than commercial grade titanium dioxide*".[18] Such concerns have been reiterated within numerous reports and studies since[27-32] and there is still a great deal of confusion. As described in several studies, it is still debated whether nanoparticles will penetrate the skin and have any toxicological impact.[33] This is likely to be in part due to the complexity in nanotoxicology and wide variety of nanoparticles available for testing. In addition, establishing dermal penetration/ absorption in terms of particle detection, relevance of models is in itself highly challenging and should not be underestimated. This is particularly the case for nanomaterials which present various challenges in terms in their detection and penetration analysis.

It is well established that the properties, behaviour and biological effects of engineered nanomaterials can be influenced by a range of physicochemical parameters, including size, shape and surface area. For example, nanoparticles in the lower nanometre range may penetrate biological membrane barriers that normally prevent the entry of (larger) particulate materials.[34,35] It is therefore possible that, if internalised in the form of nanoparticles, some insoluble or partially-soluble materials may be able to reach certain parts of the body that could not be reached by larger particles. It is now widely acknowledged that adequate characterisation of a nanomaterial is necessary to accompany any toxicity study and forms an integral part of the risk assessment. Zuin *et al.* amongst others, [36] highlighted that toxicity studies on carbon nanotubes (CNT), fullerenes, metal oxides (e.g. titanium dioxide, zinc oxide), silica and quantum dots (QD), require adequately characterised nanomaterials to interpret potential causes of biological effects. They suggested that, ideally, each toxicological assay should be accompanied by a detailed characterisation of all the physicochemical properties of the investigated material that could have biological relevance. In support of this, Boverhof & David recommended that good characterisation data using a minimal characterisation dataset should accompany and be required for all studies on nanomaterials.[37] As outlined in Chapter 2 characterisation, based on the Card and Magnussen methodology, has been an integral part of the literature appraisal in this project.

As highlighted by the EU's Scientific Committee on Consumer Safety (SCCS)[38], the selection of key physicochemical parameters that can adequately describe a nanomaterial, and the selection of characterisation methods that can be used to measure them, will depend on the composition, properties, and intended use(s) of the nanomaterial. It remains challenging to identify a short list of priority parameters for the characterisation of nanomaterials due to gaps in current knowledge regarding the relationship between their physicochemical properties and adverse health effects. The postulation and debate over which parameters to characterise for nanomaterials has been on-going for some years, with numerous reports by international committees and working groups making suggestion of the most important properties and endpoints to characterise, including: the expert working group convened by the International Life Sciences Institute Research Foundation/Risk Science Institute[39]; the European Centre for Ecotoxicology and Toxicology of Chemicals[30]; the EU's Scientific Committee on Emerging and Newly Identified Health Risks[40,41]; the German Chemical Industry Association[42]; the OECD Working Party on Manufactured Nanomaterials[43,44]; the Dutch National Institute for Public Health and the Environment (RIVM)[45]; the European Food Safety Authority (EFSA)[46]; the REACH Implementation Project on Nanomaterials (RIP-oN 2)[47]; and the SCCS[38], the latter being specifically in relation to nanomaterials intended for use in cosmetic products.

Within the peer-reviewed literature, exhaustive lists of physicochemical properties to characterise are often suggested and it has been acknowledged by Warheit that recommendations of ideal physicochemical properties to characterise often become "laundry lists" without adequate prioritisation.[48] In many instances the suggestions are generic (e.g. surface chemistry) and are made in the absence of any detailed understanding of the characteristic, the relevance and applicability of any interpretable data on the characteristic should it be available, or the availability of a technique to gather such data.

Most recently, the International Committee on Standardisation (ISO) has published a guidance document outlining a list of physicochemical properties for detailed description of manufactured nano-objects subject to toxicological testing.[49] These properties, as well as proposed methods for their characterisation, are outlined in Table 4.

TABLE 4: OVERVIEW OF PHYSICOCHEMICAL PROPERTIES REQUIRED FOR DETAILED DESCRIPTION OF MANUFACTURED NANO-OBJECTS SUBJECTED TO TOXICOLOGICAL TESTING, USING SELECTED STANDARD TERMINOLOGY FROM THE ISO CONCEPT DATABASE.[50]

Property	Description	Example measurement methods
Equivalent diameter	Diameter of a sphere that produces a response by a given particle-sizing instrument, that is equivalent to the response produced by the particle being measured	Dynamic light scattering; nanoparticle tracking analysis; small angle X-ray Scattering; size exclusion chromatography; analysis of SEM, TEM or SPM images; differential mobility analysis.
Aggregation/ agglomeration state	Aggregate: particle comprising strongly bonded or fused particles where the resulting external surface area may besignificantly smaller than the sum of calculated surface areas of the individual components; Agglomerate: collection of weakly or loosely bound particles or aggregates or mixtures of the two in which the resulting external specific surface area is similar to the sum of the specific surface areas of the individual components.	Analysis of SEM or TEM images; small angle X-ray scattering; X-ray diffraction; small angle neutron scattering; rheology methods; centrifugal liquid sedimentation; nanoparticle tracking analysis.
Shape	External geometric form of a powder particle.	Analysis of SEM, TEM or SPM images.
Surface area	Extent of available surface area as determined by given method under stated conditions	Methods based on gas or liquid adsorption isotherms; liquid porosimetry; analysis of SEM, TEM or SPM images.
Surface chemical composition	Material composition within a few atomic layers of the surface	Auger electron spectroscopy (AES); X-Ray photoelectron spectroscopy (XPS); Secondary ion mass spectrometry (SIMS); Energy Dispersive X-Ray Analysis; Electron Energy Loss Spectroscopy (EELS); Raman and other molecular spectroscopies.
Surface charge and surface charge density	Type of charge (positive or negative) and the amount that can be bound to the surface of a material.	Isoelectric point; electrophoretic light scattering; electrophoresis; electrosmosis; electric sonic amplitude; colloidal vibration current
Solubility	Maximum mass [of a substance] that is soluble in a given volume of a particular solvent under specified conditions.	There are no specific methods for the assessment of the solubility of nano-objects, however, consider reporting equilibrium dialysis, inductively coupled plasma mass spectrometry (ICP-MS) or inductively coupled plasma optical emission spectroscopy (ICP-OES) as possible measurement methods.
Dispersibility	Degree to which particles can be broken down to some minimum size.	Methods to assess the dispersibility are based on equivalent diameter measurements stated above.

A range of techniques have been adapted or developed for the characterisation of nanomaterials, including microscopic, spectroscopic, spectrometric and chromatographic techniques. It is important to note that different techniques will suit different sample forms (e.g. aerosol, suspensions etc.) and, in many cases, no individual technique can satisfy a meaningful characterisation of nanomaterials.[51-53] Multiple techniques might therefore be used where possible in order to formulate an appropriate understanding of the nanomaterial's properties, and the optimum set of required techniques should be selected based on the specific nanomaterial type and form under investigation. The need for multi-method characterisation and material-specific

selection of techniques applies across a range of nanomaterial properties and would facilitate the gathering of data on multiple metrics.[47]

In addition to determining which properties are relevant to characterise, another challenge is when and where the properties of nanomaterials should be characterised. Whilst characterisation of nanomaterials as-produced or as-supplied is the most direct and currently realistic approach to obtaining physicochemical information about the material being studied, the data may not appropriately represent the properties of the material when in contact with the environment in which it is being observed, for example in air or physiological environments of *in vivo* or *in vitro* assays. Exclusive reliance on pre-determined (often estimated) characterisation parameters will limit the comparability of studies and confidence in the interpretation of results.

In its guidance on the safety assessment on nanomaterials in cosmetics,[38] SCCS highlighted that characterisation of a nanomaterial in a cosmetic formulation is more difficult compared to characterisation in a raw material. Depending on the concentration of nanomaterial contained in a cosmetic formulation/matrix, and the nature of the formulation/matrix, SCCS suggested that a suitable characterisation scheme may be needed that includes isolation, purification and concentration steps (if necessary) before analysis of the nanomaterial. Characterisation in a cosmetic product should also provide information on any changes in the nanomaterial characteristics during formulation, e.g. in terms of primary/secondary particle sizes, chemical composition, surface characteristics, etc. Similar care is needed during toxicological evaluations.

Sayes and Warheit suggest that adequate particle characterisation should be performed in three distinct phases, primary, secondary, and tertiary,[54] where: primary characterisation is performed on particles as-synthesised or as-received in its dry native state; secondary characterisation is performed on particles in the wet phase as a solution or suspension in aqueous media (which could be ultrapure water, vehicle solution or cell culture media); and tertiary characterisation are performed on particles following interactions with cells under *in vivo* or *in vitro* condition. This could include particle characterisation in blood, lung fluids, urine, as well as interactions with proteins, fats, and specific cell types. The authors state that, although characterisation in the tertiary phase is complex and nontrivial, it is the most relevant toxicological characterisation, depending upon the questions that are being addressed.

Characterisation after administration is particularly advantageous where the possibility of physicochemical changes in the material before and after administration exists. Examples of potential changes include aggregation, physisorption or chemisorption of biomolecules and changes in surface chemistry. While characterisation after administration is considered a goal to work towards, it is recognised that in many cases, improved characterisation at the point of administration will be the more realistic and feasible option in the shorter term especially as characterisation at the point of administration is the most relevant in terms of inherent properties of causing penetration/ absorption or harm. It is recognised that in many cases characterisation at the point of administration will remain to be essential for the comparison of studies. Understanding particle characteristics after administration is likely to be interesting in relation to understanding why harm is caused. For example if we compare to chemical toxicity and understanding the route to

such toxicity, it is important to understand the metabolism of the chemical and how this may change it.

Sample preparation is widely recognised as one of the most critical steps towards successful characterisation and subsequent toxicological testing of nanomaterials, in which there are many variables to consider when designing a method for preparation. Common issues regarding sample preparation include storage and stability of the test material; the chemical composition of the test media; characterisation of stock dispersions, and; characterisation of samples (prepared from stock dispersions) prior to administration/testing. Guidance on sample preparation for the physicochemical characterisation of nanomaterials, covering properties including particle size distribution, shape, specific surface area, octanol-water partition coefficients, degree of agglomeration and dispersion behaviour, has been published by the OECD.[55]

An important component of sample preparation is the need to have "reliable" sampling, such that the test aliquot used for measurement represents the physical and chemical characteristics of the entire sample. The characterisation of particle properties like size, form and specific surface area requires very careful sampling and sample splitting practices to be followed. Also in relation to sample preparation, it is known that the observations and interpretation of toxicity as a result of exposure to agglomerates may or may not be associated with the primary particle's characteristics. As such, it is necessary to be aware that aggregates and agglomerates of nanomaterials can form in solution, powder and aerosol forms, and their presence is influenced by a number of factors including the method of synthesis, storage, handling and environmental conditions. The state of agglomeration or aggregation is recognised as an important parameter influencing the interpretation of characterisation and testing of nanomaterials ("as received", "as used", "as dosed/as exposed") and should therefore be considered during sample preparation.

In addition to aggregation and agglomeration, the behaviour of particles in solution presents some additional important aspects and challenges to recognise. In particular, it can be difficult to distinguish between when a nanomaterial is dispersed and when it is dissolved due to its small particle size. Dispersion stability is an important parameter to assess in the context of sample preparation. The dispersion of particles is determined by intermolecular forces involving particle-particle interactions as well as those between the particles and their environment. Due to attractive forces (e.g. Van der Waals interactions) particles tend to agglomerate unless stabilised by surface charge or steric effects. As a result, the state of dispersion is dynamic and determined primarily by the environment of the nanoparticles. In solution, slight modifications in pH, ionic strength, and concentrations of molecular constituents can significantly alter the dispersion of particles.

If a nanomaterial is *soluble* in biological or environmental media, then it is likely to be presented to the test system in its *molecular* or *ionic* form and can therefore be expected to elicit the same response as bulk (non-nanoscale) *solubilised* substances. If, however, the nanomaterial under investigation is *insoluble* or *sparingly soluble* in biological or environmental media, then it will likely be presented to the test system in a *particle* form. In addition, nanoparticles may interact with the liquid phase components, partially or totally yielding soluble or dispersed transformation products (as well as some solubilised nanomaterial itself).[55] All of these factors may influence the overall toxicity and fate processes of nanomaterials in toxicological assessments, which complicates

the ability to compare one study to the next and draw overall conclusions regarding the influence of physicochemical properties on observed effects.

When it comes to an eventual risk assessment, one should carefully consider whether the nanomaterial as prepared for testing is (dis)similar to the nanomaterial to which humans are exposed. For example, exposure often takes place to agglomerated/aggregated nanomaterials, where during toxicity testing, it is often attempted to keep the nanomaterial dispersed as primary particles. It should therefore be considered if such attempts at improved dispersal represent a true reflection of real use or if this is a "worst-case scenario".

Physicochemical properties are known to have a decisive influence on the absorption of bulk compounds through the skin.[56] Information regarding the influence of nanoparticle physicochemical characteristics on dermal penetration/ absorption is emerging within the scientific literature. This chapter outlines the current state of knowledge in this area, with a view to identifying gaps and future research needs.

3.2 Nanoparticle physicochemical characteristics influencing dermal penetration/ absorption

This section discusses physicochemical properties of (nano)particulates that may or may not affect the ability of the particles to penetrate the skin and potentially become systemically available. In order to attempt to understand the role each property may play in dermal penetration/ absorption of nanomaterials, each property is considered in isolation starting with size as the defining criteria of a nanoparticle and followed by composition, shape and surface chemistry.

3.2.1 Role of size

Particle size (distribution) is the defining criteria for nanoparticles[1] but the relevance of this to concerns over health has its genesis in the observation of generally greater toxicity with nanomaterials at the same mass basis when compared with larger particles of the same composition and this has long been known[57]. The concern that nanoparticles due to their small size may show enhanced penetration over larger, micron-sized particles was raised by Tan *et al.* in 1996[18] and has received particular attention in the intervening years with a range of studies investigating dermal penetration of nanomaterial of diverse compositions, surface properties and sizes. This focus towards the nanoscale has led to increased interest in the importance of understanding the physical dimensions of a particle and, for collections of particles, the size distribution used within studies with in-depth analysis of size distribution becoming an increasing prerequisite for publication.

Looking to other areas of nanotoxicology than dermal exposure/toxicity, we often see comparative studies looking at the effect of size and surface area by making very broad comparisons between materials differing markedly in size (e.g. 260.2 nm versus 14.3 nm carbon black[58]). However, whilst taking a more polarised analysis of size does show significant differences between nano-sized and larger materials, there are also spectrums of toxicity within the nano-size range and this has been observed for numerous materials[59,60] as well as modes of toxicity (e.g. oxidative stress[61]). An interesting example of this was shown in the 2007 study by Pan *et al.* where the size dependent

cytotoxicity of 0.8 to 15 nm gold nanoparticles was investigated using a range of cell lines including connective tissue fibroblasts and melanoma cells.[62] Whilst all cell types internalised the gold nanoparticles and showed signs of stress, the smaller particles (< 1.4 nm) were more toxic than their larger equivalents, so that the 15 nm nanoparticles were relatively non-toxic even at doses 60 fold higher than those resulting in toxicity with 1.4 nm particles. This is interesting as despite all the particles investigated possessing nano-dimensions, they were not equally toxic to cells. This article is also important as it demonstrates that even very small differences in size can influence particle behaviour and activity. By comparing 1.2 and 1.4 nm gold particles, Pan and colleagues showed that the size of particles was able to influence the mode of cell death with the 1.2 nm particles causing apoptosis whilst the 1.4 nm particle caused necrotic cell death.[62] Whilst the results of Pan have not yet (to the best of our knowledge) been confirmed in other studies and therefore should be considered with caution, they do suggest the potential for even small changes in size to alter particle behaviour and that this should be considered.

In relation to investigating the role of particle size on dermal penetration, there are actually relatively few studies which take a broad size comparison as noted above in other branches of nanotoxicology and similarly there are few which look at very small differences in size, such as Kimura *et al.* who analysed 2.8. 6.6, 9, 12, and 41.6 nm fluorescein isothiocyantae-dextrans.[63] In addition, the issue of particle size, as with most physicochemical properties, is not straight forward. This is because a large proportion of the studies identified focus on a single particle size meaning that robust comparisons of the role of particle size in determining dermal penetration depth is challenging. This is due to numerous confounding factors such as differing experimental models, species, doses, duration, but perhaps most challenging, particles of differing physicochemical characteristics outside that of simply size. For example, if we consider the same material such as quantum dots but look for differing sizes used within studies, we also see differences in coating such polyethylene glycol (PEG)[64] versus dihydrolipoic acid (DHLA),[65] as well as differences in application vehicle (phosphate buffered saline (PBS) with 10% isopropyl myrisate versus glycerol). Even taking two studies looking at PEG-coated quantum dots with the same size,[64,66] we see differences in species, vehicle and charge. This means that in a side-by-side comparison, no definitive conclusions can be drawn as penetration depth may have been affected by any number of differing experimental parameters or a combination of them and not just differences in size.

The results of dermal penetration based on particle size are highly conflicting and together with the issues of multiple variables within the same experiment described above, these issues hinder the drawing of solid conclusions. To aid in the evaluation of the role of size in terms of an overview of the weight of evidence rather than comparing single studies, Table 5 shows the differing particle size ranges and the minimal/maximal penetration depth observed with supporting references to show the weight of evidence. It should be noted that this table is an amalgamation of *all* studies identified and does not differentiate between composition, models (although it does exclude single cell culture models), species or other physicochemical properties. For further information and detail about the study parameters, see Appendix 2 for a summary table of particle and study characteristics or the accompanying MS Access Database.

TABLE 5: PARTICLE SIZE RANGES IN RELATION TO MINIMAL AND MAXIMAL DERMAL PENETRATION DEPTH

Size Range (nm)[#]	Minimal Penetration Depth	Reference	Maximal Penetration Depth	Reference
0-5	SC	63,67,68	SB	69
5-10			SC	63,70-74
10-20	SC	22,63,65,66,72,75-79	Dermis	80,81
20-40	SC	63,66,73,82-87	Dermis*	64,84,88,89
40-60	SC	63,73,76	SB	90
60-80	SC	76	Dermis**	64
80-100			SC	22,82
100-250			SC	22,79,82,90,91
250-500			SC[§]	15,82,84,85,90,92
500-1000+			SC[§]	64,89,92

N.B. The table does not record penetration into single cell culture nor the presence of a positive result in the receptor fluid in the case of diffusion cell models unless penetration depth in cell layers is provided. No penetration is recorded as SC for maximal/minimal penetration to show no penetration into viable cell layers. Given the concerns raised by Jonaitis *et al.*[93] and limited data presented, the findings of Wu *et al.*[94] have not been recorded in the table.

[#] Size recorded as primary particle size where a range is given; *Perifollicular dermis in the case of reference [89]; **The study by Nabeshi[64] states only that 70 nm silica and 35 nm quantum dots penetrated the SC and were systemically available although no further detail was given. As such, dermis was selected on a precautionary basis; [§]A very small fraction of the applied dose was noted in lower levels of the skin layers including the dermis but penetration was considered as minimal.[84,92]

Taking a broad comparison of particles less than 100 nm compared those over 100 nm, it appears from the studies identified that those > 100 nm do not penetrate thorough the SC with any great efficiency. Taking for example the study of Sadrieh *et al.*, the authors noted that after 22 days of administration of submicron (300-500 nm) or nano-sized (30-50 nm) TiO_2 to mini-pigs there was no detectable levels of TiO_2 within the sentinel organs (liver, spleen) for either size range. Whilst they did see very low levels of TiO_2 in cell layers below the SC, including the dermis by TEM, these were very small. A representative section overlain with the TiO_2 particle distribution frequency is shown in Figure 5. This led the authors to conclude that minimal penetration occurs through the viable epidermis, with TiO_2 particles primarily found in the SC and upper follicular lumens, and highly aggregated between the layers of keratin in the SC.[84] Interestingly, in this comparative study the authors noted that there was no observable trend with particle size and both sub-micron and nano-sized particles were observed in similarly low levels representing a small fraction of the applied dose.

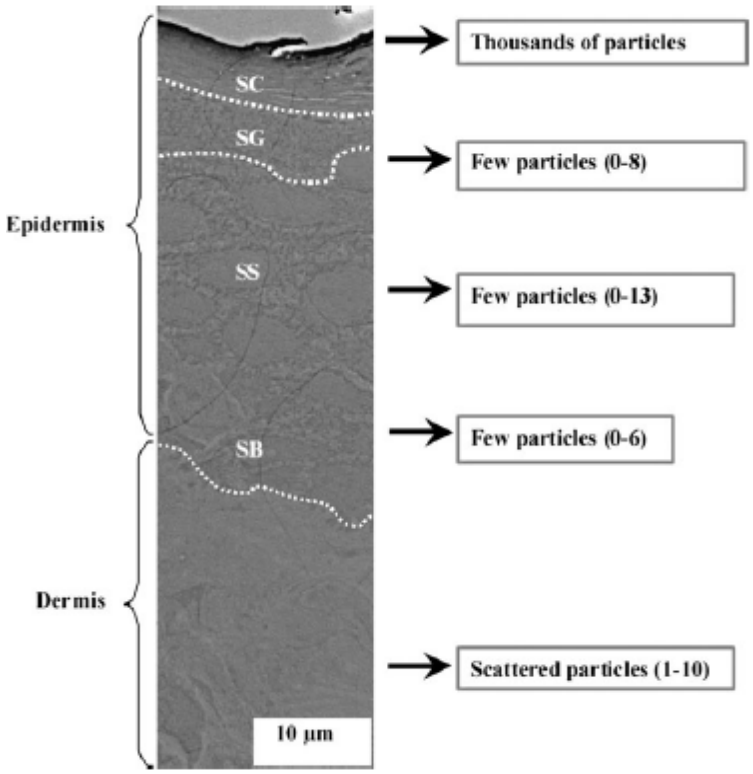

FIGURE 5: REPRESENTATION OF SUBMICRON TIO_2 PARTICLE DISTRIBUTION FREQUENCY IN DIFFERENT LAYERS OF MINIPIG ABDOMINAL SKIN AFTER 22 DAYS APPLICATION OF SUB-MICRON TIO_2. Reproduced from Sadrieh *et al.*[84] Numbers in parentheses are estimates of the numbers of TiO2 particles observed in each layer. Figure SC: stratum corneum; SS: stratum spinosum; SG: stratum granulosum; SB: stratum basale.

Such results of low penetration at the sub-micron and micron size range was also demonstrated by Tinkle *et al.* using fluorosphere beads of 500 nm, 1 µm, 2 µm or 4 µm. Using a flexion model to mimic the movement of the human wrist, the authors applied the beads and assessed penetration at different time points. They found that the 500 nm and 1-µm beads penetrated into the epidermis in 2 of 11 skin samples (18%) flexed for 15 min, in 5 of 12 samples (41%) flexed for 30 min, and in 9 of 16 samples (56%) flexed for 60 min showing increased penetration with time.[92] They also noted penetration into the dermis in two samples after flexing for 60 min. In agreement with the findings of Sadrieh *et al.*, the fluorospheres that penetrated through the SC represented only a very small percentage of the applied beads, and the pattern of penetration was random suggesting a lack of definitive penetration. Another point of importance when considering the variability in results and difficulty in comparing studies is that no penetration was observed in non-flexed tissues at any time point.[92]

Whilst these results provide us with a general impression of very low penetration of sub-micron and micron sized particles, it is those studies which perform a comparison between nano-sized and larger particles in the same study that are of most interest due lower potential for inter-study variability. In addition to the study by Sadrieh *et al.* already described which showed no relationship between size and penetration into the skin, there are nine further studies identified, which compared dermal penetration of different sizes. Senzui *et al.* looked at the penetration of rutile TiO_2 at 35 nm and 250 nm through Yucatan micropig skin that had been left intact, the SC

stripped using tape (as a model of skin injury), or had the hair removed using tweezers. The skin was fitted to a Franz diffusion cell and 2 µL of a 10% suspension of TiO_2 in silicone applied to the upper portion and left for 24 h.[85] After the incubation time, the surface of the skin was removed by cyanoacrylate stripping and the remaining epidermis and dermis separated using heat and assessed for titanium content using ICP-MS. The results showed no permeation of titanium into the receptor fluid of the Franz diffusion cell nor did the results show a significant increase in the separated epidermis or dermis as well as no difference between the 35 nm and 250 nm particles. In addition, irrespective of skin condition (intact, stripped or plucked) there were no increases in uptake. It could perhaps be argued that the mode of analysis was insensitive and relatively non-specific as the surface 'residue' of particles was removed after application by cyanoacrylate stripping and the epidermis and dermis separated with TEM undertaken. However even if insensitive, it does show that if penetration did occur, it did so in very small quantities that were below the detection limit of the ICP-MS analysis (detection limit not recorded) with no appreciable difference between nano-sized and non-nano-sized particles.

This lack of difference between 35 nm and 250 nm particles is in contrast to the study of Sonoavane *et al.* who noted size dependent skin permeation though a Franz diffusion cell mounted with rat skin. The authors noted greater permeation for 15 nm gold nanoparticles as compared to 102 nm and 198 nm gold particles with a decreasing permeability coefficient through rat skin with increasing size.[81] A similar result was observed by Vogt *et al.* who applied 40 nm as well as 750 nm and 1500 nm fluorescent beads to excised human skin (*in vitro* without use of a diffusion cell) for a period of 15-16 h. They found that by using fluorescence and laser scanning microscopies, only 40 nm particles deeply penetrated into vellus hair openings and through the follicular epithelium suggesting size dependent penetration *via* hair follicles which they suggested as a route to cutaneous antigen presenting cells as a potential method of transdermal vaccine delivery.[89] Using similar fluorescent polystyrene nanoparticles (diameters 20 and 200 nm), another study also noted that such polystyrene nanoparticles accumulated preferentially in the follicular openings in porcine skin, and that the follicular localisation was favoured by the smaller particle size as detected using surface confocal laser scanning microscopy (CLSM). However in cross section, they also observed that non-follicular structures/areas do not offer an alternative penetration pathway for the particles to penetrate "*whose transport was clearly impeded by the stratum corneum*".[79]

This result was supported in a later study, again using 20 nm and 200 nm fluorescent polystyrene nanoparticles to assess dermal penetration in porcine skin suspended in a Franz diffusion cell. This study by Campbell *et al.* found that applied particles were only able to penetrate into the skin layers in the final stages of desquamation (stratum disjunctum) and that this uptake was in itself minimal and independent of contact time (up to 16 h) and of nanoparticle size tested. Using a compromised skin model (removal of 4 tape-strips immediately prior to mounting in the Franz cell) they noted that the particles still could not penetrate beyond the most superficial layers, corresponding to a depth of 2–3 µm, of the SC (the outermost, 15–20 µm skin layer).[22] These results, in part, conflict with the results of Rancan *et al.*[90] who did note penetration of 42 nm silica particles into explant human skin but, in correlation with the previous study, the authors did state that particles mainly accumulated in skin furrows, hair follicles and hair shafts and that even skin with a mild barrier disruption could efficiently block penetration of particles of 75 nm or greater. This is interesting as it raises the issue of follicular versus non-follicular penetration and if follicular penetration should

be seen as more or less of concern than non-follicular penetration. Indeed, numerous publications have suggested hair follicles as route of dermal delivery of nanoparticles [12-14,16,95,96] and the express intention of the Vogt *et al.* study was to investigate hair follicles as a route of dermal vaccination due to the locality of dendritic cells near the hair follicle. However, there are concerns about the role of hair shafts in potentially overestimating permeation of nanoparticles as penetration of the hair shaft may not necessarily represent penetration and distribution of the lower dermal layers. In addition concerns were raised by Senzui *et al.* who stated that the use of split skin as recommended by OECD Test Guideline (TG) 428 may over estimate permeation of nanoparticles as the hair follicle is cut and therefore may provide for a route into the receptor phase.[85]

This suggestion that the skin can effectively block penetration of particles 75 nm or greater is contradicted or becomes ambiguous by the 2004 study by Kohli *et al.* who, using porcine skin in a modified diffusion cell as a model looked at the penetration of 50, 100, 200 and 500 nm latex particles.[82] They found that 50 nm nanoparticles could penetrate the skin and that 100 nm and 200 nm particles could not (in line with the results of Rancan *et al.*) but they also noted that 500 nm particles *could* also penetrate the skin in contradiction to the 75 nm cut-off. The penetration of the 50 nm and 500 nm was said by the authors to be due to the negative surface charge, who speculate that the permeation seen may be a result of repulsive forces between negatively charged lipids within the skin and particles at the surface and that "*these forces may result in the temporary initiation of channels within the skin allowing for particle permeation*".[82] Whilst an interesting conclusion, it should be noted that particles used in the study by Rancan *et al.* were also negatively charged at neutral pH.[90] Both of these studies suffer from a lack of a physiological *in vivo* model and it is possible that the differences noted result from the different species used (human versus porcine). However, it may also be the case that the Rancan *et al.* simply drew too strong a conclusion on the presence of a size cut-off without further extending the particle range as Kohli did.

Within the peer reviewed literature, one of the most relevant studies in terms of comparing nano- and larger (non-nano) sized particles is that of Gulson *et al.* This study consisted of two parts. An indoor trial involving 3 human subjects[106] established the conditions for a larger trial of 20 human volunteers performed at an Australian beach.[90] The larger trial is of high relevance due to the use of a realistic *in vivo* approach whereby sunscreens were applied to humans undergoing normal activities at a beach, and the comparison of two different sizes of ZnO particles enriched for tracing with the stable isotope ^{68}Zn. The two sizes of particles, with average diameters of ~20 nm and > 100 nm, provide a large degree of difference in opacity of the sunscreens applied to skin, as shown in Figure 6. Within the study, two groups of 10 volunteers ranging in age, skin classifications and ethnicity had sunscreen containing nano or bulk particles of ZnO enriched with ^{68}Zn applied to their backs twice a day for 5 consecutive days by a single person (not a subject of the study) handling the sunscreen tubes and applying sunscreens to the backs of subjects to reduce application variability and potential cross contamination.[97] Blood and urine were collected a week before the trial started, twice daily during the sunscreen application phase, and 6 days after the final sunscreen application.

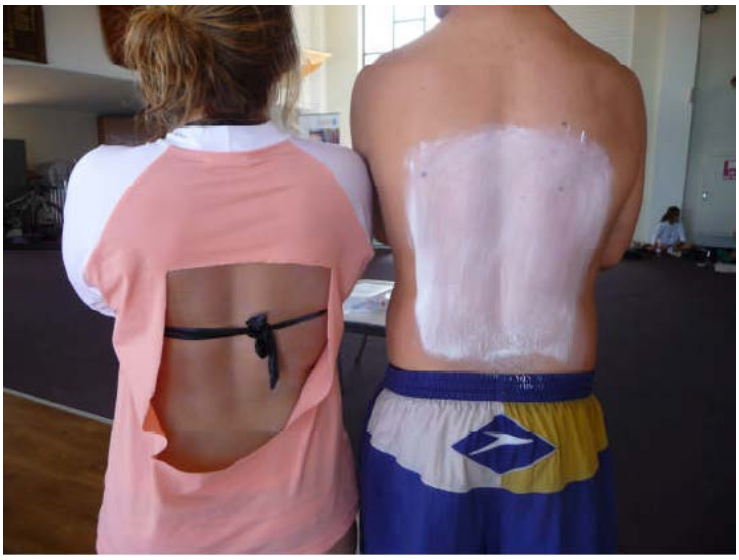

FIGURE 6: APPEARANCE ON THE SKIN OF TEST FORMULATIONS CONTAINING NANOPARTICLES (LEFT) OR BULK PARTICLES (RIGHT). Reproduced from [98].

In addition and in order to reduce contamination, the sun-protecting clothing (shown in Figure 6) was put on before the sunscreen application in the morning, and taken off after sunscreen removal at the end of each day by a person assigned to washing the clothing each day (this was a different person from the sunscreen applicator, and who also was not a subject of the study).[97] Towels were also collected and washed daily by this person. Prior to eating, subjects washed hands well, and ate with utensils. Subjects were monitored by observers at all times, and by each other, to check their hands were not put in their mouths to reduce risk of hand-to-mouth contamination.[97] However, it was acknowledged that external contamination of urine was a potential problem, especially for females, possibly due to sweat running down the back (taking a little sunscreen with it) and collecting in swimming costumes and on the skin which may then have externally contaminated the urine when collected.[97] The smaller trial alerted the study directors to this possibility, and so it was rigorously implemented that hands etc. were well washed before and after giving urine samples, but they acknowledged that this couldn't eliminate the problem entirely.[97] For this reason, results focused on data from blood samples, which did not suffer from this contamination.

The study showed that "*the overwhelming majority of applied ^{68}Zn was not absorbed, although blood and urine samples from all subjects exhibited small increases in levels of tracer ^{68}Zn*".[99] A strength of this study was that the actual penetration dose was placed in context; the amount of tracer detected in blood after the 5-day application period was ~1/1000th that of total Zn in the blood compartment. Of interest, this study noted that levels of ^{68}Zn in blood from females receiving the nano sunscreen were significantly higher than males receiving the same treatment and higher than all subjects receiving the bulk sunscreen.[99] This gender difference was hypothesised to be due, in part, to underlying skin thickness as females on average have thinner skin than males.[98] Similarly to the smaller study, Gulson *et al.* stated clearly that it was not known whether the tracer was absorbed as ZnO particles or soluble Zn or both.

Overall, *on balance* the results seem to suggest that penetration of particles in the nano-range into the skin is possible although occurs to a low degree and that this is, depending on chemistry and

experimental conditions, may be greater than for larger particles. In terms of dermal absorption, information is severely limited with very few studies showing systemic availability. One of the only studies showing a more robust demonstration of absorption (rather than simply penetration) was that of Nabeshi *et al.* who demonstrated that a 28-day application of fluorescent 70nm silica nanoparticles to the ears of mice (250 µg/ear/day) resulted in particles being detected in the skin, the regional lymph nodes, parenchymal hepatocytes present in liver, cerebral cortex and the hippocampus. [64] Such results were also noted for 35nm quantum dots. Whilst the results do suggest absorption, only the 70nm and 35nm particles were examined and the full spectrum of 70, 300 and 1000 nm silica nanoparticles used within the study [64] were not investigated. It should also be noted that detection of the particles was be TEM alone, with elemental profiling to prove that the electron dense region identified as being a silica or quantum dot nanoparticle was in fact that. The detection of such particles was all the more surprising given the sampling methodology of the tissue. Indeed, TEM analysis allows the evaluation of only minute areas of tissue and therefore to understand the frequency of such "nanoparticles" in the tissue, the sampling methodology (numbers of pieces of tissue analysed, area of analysis etc.) needs to be detailed which it is not.

However beyond such targeted evalautions, and even when only comparing bulk and nano-forms there is considerable variability in results and experimental setups in the relatively few studies published, limiting the ability to draw any firm conclusions. One potential source of variability outside that of differing composition, coatings, models, shapes etc. that is of significance in relation to particle size is the effect of changes in particle agglomeration/aggregation state. Indeed, as noted by Labouta *et al.* "*aggregation of applied particles over time limits the availability of the individual particles that would have a higher probability of penetrating the resilient barrier*".[100] Therefore, understanding the particle characteristics such as aggregation/agglomeration state at the start of, during and at the end of experimental applications could be useful in understanding the reasons behind conflicting results. This degree of agglomeration may also be affected by the nature of the vehicle as well as the nature of application (e.g. spreading versus massage).

3.2.2 Role of composition

In relation to nanoparticle composition, this can be considered in two ways, the primary bulk composition of the particle (e.g. for a carbon nanotube this would be carbon) or, if the nanoparticle contains a minor constituent or contaminant that exerts a biological effect. This first point is considered by splitting the bulk composition of particles into relevant groups so that penetration of, for example metal oxides such as TiO_2 can be considered in isolation with other such studies investigating this material. As can be seen in Table 6, there are a wide variety of nanoparticles compositions that have been considered in relation to dermal penetration. However it must be borne in mind that within each compositional group, there can and is considerable variability in terms of particle characteristics such as size, crystallinity, charge, coating etc. Therefore, whilst a single bulk composition may have been studied to a greater extent than another, the variability in physicochemical properties within this group may be so significant that there is in fact little truly comparative literature.

TABLE 6: VARIETY OF NANOPARTICLE COMPOSITIONS STUDIED FOR DERMAL PENETRATION. For each type, example references are shown and are reflective of the numbers of studies

Composition	Reference
Cobalt	101
Copper Oxide	87
Dextran Beads	63,92
Fullerenes	102,103
Gold	72,73,80,81,104
Iron*	70,105
Polymer/Polystyrene	15,22,63,79,82,89,106
Quantum dots	64-66,68,71,75,88,107-110
Silica	64,90,111
Silver	76,83,90,112
Titanium Dioxide	63,78,84-86,91,94,113,114
Zinc Oxide	63,77,91,99,115-118

*including Cobalt-Ferric magnetic nanoparticles.

In looking across the peer reviewed literature for dermal penetration of nanomaterials, the studied particle compositions may reflect the industrial relevance of these materials in terms of their use (current or future) in consumer products or in the case of quantum dots and fluorescent polymer/polystyrene nanoparticles, their ease of use in terms of tracking and manipulation for studying penetration. Overall, the frequency of study for the particles outlined above could be considered as follows:

Titanium Dioxide > Zinc Oxide > Polymer/Polystyrene > Silica > Silver > Iron > Fullerenes > Dextran Beads > Copper > Cobalt

When looking at bulk composition and the level of dermal penetration noted in studies using a specific material type, there appears to be very little pattern between bulk composition and penetration depth. Taking for example TiO_2 as one of the most widely studied nanoparticles, we see reports of penetration no further than the SC[78,86,91] but also several studies suggesting deeper penetration (basal cell layer) and even penetration into the dermis[63,84] although this is often reported as being a very small fraction/infrequent. Another compositional issue in relation to nanoparticles and in particular TiO_2 is the crystalline structure. TiO_2 is often used in either its anatase or rutile form or as mixture of both. Within the literature, there are studies using both the anatase form[86,94], the rutile form[91,114] or a mixture[84,114] although we were unable to find any studies which appear to systematically evaluate the role of crystal form in TiO_2 absorption into the skin.

Such conflicting results within compositional types can also be seen within the literature for gold nanoparticles, quantum dots, polymers and ZnO (the other compositions have far less of a body of evidence to note such variability). In relation to ZnO, the main evidence for transdermal penetration is systemic availability as shown by detection of the stable isotope ^{68}Zn in blood/urine and as the authors of these studies point out, it is not clear if this is a result of particulate penetration or solubilisation.[98,99] As the variability in terms of physicochemical characteristics *within* a composition group is apparent (although it must be said, the majority of studies do not show penetration past the SC into viable cell layers), looking between groups could be more useful although this similarly is likely to be heavily confounded by variability in multiple particle characteristics. Taking this approach, it appears on balance that there is a slightly higher frequency of studies reporting dermal penetration through the SC for quantum dots although this is followed closely by TiO_2 and ZnO. Interestingly, taking a similar broad view, polystyrene/polymer particles

are reported as penetrating through the SC with a lower frequency whilst in relation to the number of studies available, silica particles are reported as penetrating into viable cell layers more often than other particle compositions. However a major caveat in this approach is that numbers of studies for each particle composition are often very small and both within and between compositions, the particle properties vary widely, confounding analysis and negating any definitive conclusions being reached. Therefore, based on the current knowledge it is impossible to state conclusively if particle composition has a significant impact on penetration properties. Given, however, the physical interaction between a particle and the skin, it is more likely that physicochemical properties such as size, hydrophobicity (and thus surface modifications/chemistry, which will be discussed later) and surface charge are able to influence penetration due to the nature of interactions between particles, cell layers (gaps), cell membranes and lipids.

A challenge when considering composition and how this may affect penetration/ absorption is considering the solubility of a nanoparticle. This is because whilst an insoluble particle or sub-portion thereof may be prevented from absorbing through skin and may only penetrate upper layers, the soluble fraction may penetrate further and become systemically available. An often mentioned example of a soluble nanoparticle of relevance to consumer exposure is that of silver. As stated by Christensen *et al.* "*It is generally not known whether the silver uptake occurs solely as ions or whether also uptake of the silver nanoparticles themselves can occur*"[119] and this is certainly the case in relation to dermal exposure. Within the literature Vlachou *et al.* conducted a small study of burn victims using Acticoat™ dressings containing nanocrystalline silver and measured serum silver levels using inductively coupled plasma mass spectroscopy before, during and at discontinuation of the use of the dressings, as well as at 3 and 6 months following completion of treatment.[112] They detected serum levels of silver and noted that the median time to the maximum serum silver level was 9 days. However, the intention of this study was to look at systemic availability of silver (and markers of systemic toxicity) rather than silver *particle* absorption and therefore the methods used could not differentiate between soluble silver ions released systemically and particles penetrating the skin and becoming systemically available. This same issue has been described above in relation to the study by Gulson *et al.* using ZnO which is another nanoparticle which is known to be soluble.[99]

The absence of actual particle absorption does not mean that the soluble fraction should be discounted or is harmless but instead, this should be considered in relation to the nature of the dose (soluble ions) and the kinetics of the dose in terms of distribution and metabolism which is likely to differ markedly from systemically available nanoparticulates. Therefore, in order to understand if absorption occurs as particle or due to a release of a soluble fraction more than one methods of particle detection is needed and one of which must be able to detect particulates rather than an ionic species or tracer.

In addition to the bulk composition as considered above, other compositional components could also play a role in the biological activity of a nanoparticle on the skin. In toxicity terms, this is well established especially in relation to transition metals and reactivity leading to toxicity and is suggested to play a substantial role in the pathogenesis of several particle mediated diseases. There are numerous examples of this for larger, non-nanoparticles in the literature and of these metals, iron is often cited as having a leading role in reactive oxygen species (ROS) generation and

subsequent oxidative stress and damage.[120-126] Aust summarised the hypothesis behind the toxic effect of transition metal contamination of particulates as "*bioavailable transition metals from inhaled airborne particulates catalyze redox reactions in human lung epithelial cells, leading to oxidative stress and increased production of mediators of pulmonary inflammation*".[120] This was further demonstrated by the reduction in coal fly ash (CFA) activity of over 90% after the addition of the metal chelator desferrioxamine, which they concluded strongly suggests that transition metal(s), probably iron, were responsible.[120]

Whilst the role of metals, contaminating or otherwise, in the biological activity of particles[126,127] and also nanoparticles such as metal oxides[128] and carbon nanotubes[123] has been well studied in relation to the lung, there is a deficit of information on the role metals may play in the biological activity of nanoparticles to the skin. One of the few studies addressing this issue was that of Murray *et al.* who compared the *in vitro* toxicity of unpurified and partially purified single walled carbon nanotubes which as a result, differed in the quantity of contaminating iron (residual catalyst).[129] They found that the unpurified single walled carbon nanotubes (SWCNT) induced greater cytotoxicity, depletion of anti-oxidants, and inflammation *in vitro* (mouse dermal cell line JB6 P+) to a greater extent than partially purified sample. They also noted significant induction of inflammatory cytokines in the reconstructed dermal model EPiSkin as well as thickening of skin when applied to SKH-1 mice at a dose of 40 µg/mouse, 80 µg/mouse, or 160 µg/mouse.[129] Such results are similar to those noted by McNeilly and colleagues who examined the role of soluble transition metals in respiratory toxicity using *in vitro* and later, *in vivo* models. The application of standard welding fume samples with high levels of several transition metals such as iron, nickel, and chromium led to toxicity and chelation of these metals or reduction *via* washing reduced the toxicity *in vitro* and *in vivo* markedly.[130,131] This similarity of response due to iron suggests a common oxidative stress mechanisms and also suggests that other transition metals may cause similar problems. This was also shown by Cohen *et al.* who noted after application of CuO to an explant human skin organ culture, toxicity (inflammatory cytokine secretion and necrosis) was observed and that nanoparticles were more toxic than micro-sized particles.[87] Whilst the study of Murray *et al.* did not address penetration and therefore only informs us in relation to dermal toxicity, Cohen *et al.* did evaluate penetration using light microscopy and TEM. They noted that whilst dense objects which they suggested were possibly CuO nanoparticles were seen only rarely and within or next to the SC. This suggested that physical penetration of CuO nanoparticles into the tissue was limited, even when most of the cornea was removed. This led the authors to hypothesise that the toxicity observed may have resulted from adherence of the particles to the skin which then react with the local acidic environment, and generate soluble ions that can penetrate the skin and cause localised toxicity.[87]

Active metal components of nanoparticles, such as that described by Murray above, have received a great deal of attention for their role in particle-mediated toxicity. However, there are other biologically active contaminants that can also play a role in particle mediated toxicity as has been demonstrated numerous times. The presence of other biologically active contaminants such as poly-aromatic hydrocarbons (PAHs) have long been associated with toxicity of diesel engine exhaust particles as well as other combustion derived particles[132] in relation to inhalation toxicology[133,134] and in particular carcinogenicity[134-136] meaning that the presence of such contaminants in manufactured nanomaterials could be consider a potential hazard.[137] However in relation to dermal effects of particulates, the presence of biologically active components such as

PAHs has received little attention. This is somewhat striking as concerns about such substances have been raised in relation to the petrochemical industry[138] as well as other exposed populations[139,140] suggesting that if such contamination occurs, this could be delivered onto or into the skin. This issue of chemical substances adsorbed to the surface of particles and as such, gaining entry into the skin (should the particle penetrate the skin) is interesting and somewhat understudied. Chemical substances could adsorb as a result of product processes (e.g. PAHs) or may result from environmental contamination of the particles or be adsorbed from the vehicle formulation used to apply the particle. This could result in a 'Trojan Horse' scenario whereby the nanoparticle facilitates entry or concentration of substance within the skin which may not normally occur. This has been suggested in relation to causing toxicity *via* the release of metal species intracellularly after nanoparticle uptake,[141] but mainly in relation to the lung.

Overall, the results appear to show that composition has little effect on dermal penetration although composition, either in terms of bulk or as a contaminant, should be considered in relation to dermal *toxicity*. Soluble metal (oxide) nanoparticles such as silver and ZnO show penetration of silver and zinc, but it is yet unknown whether this penetration mainly or exclusively occurs as metal ions or as nanoparticles.

3.2.1 Role of surface chemistry

Several published studies indicate that particle size is not the only influencing factor on the level of dermal penetration of engineered nanomaterials. There is some evidence to suggest that the surface chemistry of a nanoparticle may also play a role in determining its ability to penetrate the layers of the skin. The term surface chemistry is often used in the context of surface chemical composition, and is somewhat a broad and non-specific term which does not predispose itself to 'quantitative' characterisation according to a single comparable metric or measurand. Surface chemistry is closely linked with surface properties, including solubility equilibrium, catalytic properties, surface charge, and surface adsorption and desorption of molecules from solution, amongst others. Most of these properties are functions of the atomic or molecular composition of the surface and the physical surface structure. The surface chemistry is influenced by chemical purity, functionalisation (typically *via* covalent bonding) and surface coating (often *via* weaker Van der Waal forces) and extent of coverage of applied and/or acquired surface coatings.

The role of surface chemistry in determining the level of dermal penetration of nanoparticles has not been widely or comprehensively studied in research published to date. A number of studies in the available literature have assessed the level of dermal penetration of nanoparticles with different surface coatings, functionalisations and charges, but in many cases these properties have not been fully characterised. It is important to highlight that these properties are inherently linked. In all of the studies that suggest the surface charge may influence the dermal penetration of a particular nanoparticle type, the charge of the nanoparticle has been altered through the addition of a coating with charged functional groups. Quantitative characterisation of the surface charge through, for example, zeta potential measurements has not always been undertaken or reported. In addition, several studies have assessed uncoated and coated forms of the same nanoparticle, without making reference to the potential surface charge of each form. In our discussion of this literature, we have focussed on studies where a comparison has been made between nanoparticles of the same type

with different surface coatings and at least a qualitative indication of the surface charge of the various forms has been provided.

The skin is amphoteric in nature, capable of ionising both as an acid and base, and therefore has an isoelectric point. The isoelectric point is defined as the pH at which a particular molecule or surface carries no net electrical charge. Below its isoelectric point, a particular molecule/surface will carry a net positive charge; above its isoelectric point the molecule/surface will carry a net negative charge. The skin has an isoelectric point which is typically between 3 and 4. At physiological pH (7.4), the skin is therefore considered to be negatively charged.[142] This makes the skin selectively permeable to cations, as has been demonstrated for human and porcine skin in a number of studies using bulk compounds, such as that by Marro *et al.*[143]

A number of studies have been published which provide some support for the hypothesis that nanoparticles with cationic coatings may enhance electrostatic interactions with negative charges on the cell membrane and favour uptake, however results are not conclusive and have been contradicted by other studies.

Rancan *et al.* investigated the skin penetration and cellular uptake of amorphous silica particles of different sizes and surface charges both *in vitro* and *ex vivo*.[90] Uncoated (neutral) and (3-amino-propyl)-trimethoxysilane (APS)-coated (cationic) fluoresceine isothiocyanate (FITC)-labelled silica particles of four different sizes (42, 75, 190 and 291 nm) were synthesised and their zeta potential in water (pH 7.0) and phosphate buffered saline (pH 7.4) determined, as summarised in the table provided in Appendix 2. *In vitro,* primary epidermis cells (ECs) and Langerhans cells (LCs) were incubated with non-functionalised and APS-functionalised silica particles and compared with untreated control cells. Uptake of silica particles by both cell types, as indicated by flow cytometry, was found to be size dependent and enhanced when the particle surface was functionalised with positively charged APS ligands. However, particle colloidal stability, which is associated with surface charge, was found to strongly affect particle aggregation and cellular uptake. For example, in LC, the extent of particle association was positively influenced by APS-functionalization, with the exception of 190 nm particles, where the high tendency of APS-functionalised particles to form aggregates resulted in a reduction of cellular uptake for this larger particle. In an *ex vivo* study, where the various silica nanoparticles were topically applied to human skin explants with a partially disrupted SC, only the 42 nm particles were found to be associated with epidermal cells and dendritic cells. APS-functionalisation, despite enhancing uptake *in vitro*, was found to have no significant influence on the level of cellular uptake.

Ryman-Rasmussen *et al.* studied the penetration of intact skin by quantum dots with varying shape and surface charge.[109] Spherical 4.6 nm core/shell diameter QD 565 and ellipsoid 12 nm (major axis) by 6 nm (minor axis) core/shell diameter QD 655 with neutral (polyethylene glycol [PEG]), anionic (COOH) or cationic (PEG-NH_2) coatings were topically applied to porcine skin in flow-through diffusion cells at the pHs of the solutions provided by the QD manufacturer: 8.3 (PEG, PEG-NH_2) and 9.0 (COOH). Whilst this buffer is slightly more alkaline than physiological pH, it should still maintain an overall negative charge on the skin according to the aforementioned theory. The precise surface charge of the various QD was not quantified in this study. Confocal microscopy revealed the level of penetration to be both shape and surface charge dependent. Spherical QD 565

of each surface coating penetrated the SC, with PEG- and COOH-coated QD localised in the epidermal layers and PEG-amine coated QD localised in the deeper dermal layers. For ellipsoid QD 655, PEG- and PEG-amine coated QD were localised within the epidermal layers by 8 h. No penetration of COOH-coated ellipsoid QD 655 was evident until 24 h, at which time localisation in the epidermal layers was observed. Thus, although QD with cationic coating penetrated to a greater extent in both cases, some penetration of QD with neutral- and anionic-coating was also reported.

Prow *et al.* investigated quantum dot penetration in viable human skin, assessing the level of penetration at neutral pH as well as at the pHs investigated by Ryman-Rasmussen *et al.*[66] In this study, spherical 4.6 nm core/shell diameter quantum dot fluorescent nanoparticles with three surface modifications, PEG-, PEG-NH_2 and COOH coatings, were evaluated for human skin penetration from aqueous solutions at pH 7.0 and at pHs of solutions provided by the QD manufacturer: 8.3 (PEG, PEG-NH_2) and 9.0 (PEG-COOH). In this case, the zeta potential of the particles was measured at the various pHs, as summarised in the table provided in Appendix 2. Some penetration into intact viable epidermis of the skin was observed for PEG-QD at pH 8.3, but not at pH 7.0. Neither PEG-NH_2- nor COOH-modified QD penetrated appreciably into the viable epidermis of intact skin at all pH groups tested. Upon tape stripping 30 strips of SC, all QD at all pH tested entered the upper layers of the viable epidermis within 24 h.

Labouta *et al.* undertook a study to investigate the influence of particle size and polarity of gold nanoparticles in terms their penetration through human skin.[144] The permeation level of four types of gold nanoparticles (AuNP) was assessed in a Franz diffusion cell using excised human skin, specifically: AuNP1 (6 nm, uncharged, thiol coated and dispersed in toluene); AuNP2 (6 nm, negatively charged, lecithin coated and dispersed in water); AuNP3 (14.9 nm, negatively charged, citrate ion coated and dispersed in water); and AuNP4 (14.9 nm, uncharged, cetrimide coated and dispersed in toluene). The nanoparticles with more hydrophobic character, uncharged AuNP1 and AuNP4, showed skin penetration into deeper layers. Further skin penetration experiments were undertaken to assess whether the increased permeation of these particles was due to an effect of toluene on the barrier function of the skin, and results indicated that the level of penetration did not depend mainly on the vehicle. However, the authors acknowledge that it is a complex mechanism depending on several factors.

In contrast, several studies have been identified which provide results reporting negatively-charged nanoparticles to penetrate the skin to a greater extent than neutral or positively-charged particles, which is in direct contradiction to the expected cation preselectivity of the skin. An example of this is provided in the study by Kohli *et al.*, who investigated the effect of size and charge on the permeation of nanoparticles through the skin in an *ex vivo* study as a first step in designing a transdermal vaccine delivery system.[82] Fluorescent latex copolymer nanoparticles ranging in size (50, 100, 200 and 500 nm) and charge (neutral, positive, and negative) were applied to the surface of full thickness pig skin in a diffusion chamber. The receptor fluid was assayed to determine penetration and the skin was visualised after the experiments using fluorescence microscopy. The authors reported that only the 50 nm and 500 nm negatively charged particles permeated the skin and were detectable in the lower chamber, with no appreciable difference in the magnitude of permeation between the two sizes except on first application. Micrographs of both particles showed an accumulation in the SC and the viable epidermis. The remaining particles that were tested (i.e.

positively charged and neutral nanoparticles of all sizes, as well as negatively charged 100 nm and 200 nm nanoparticles) were not observed to permeate the skin. The authors conclude that the charge of the particles plays an important role in overcoming the skin's barrier, with negatively charged particles of sufficient charge showing greater permeation.

To explain the observed differences in permeation between negatively charged particles of different sizes, Kohli *et al.* suggested that it may be the overall charge density of contact with the skin that is important.[82] The authors suggested that 50 and 500 nm negatively charged nanoparticles allow a high charge density of contact with the skin; 50 nm through their large surface area resulting from their small size, and 500 nm through a high number of charged groups within the latex copolymer required to charge a large particle. The net effect of these characteristics, the authors suggested, may result in a greater concentration of charge per unit area of skin from the 50 and 500 nm particles as compared to the other particle sizes. Kohli *et al.* speculated that the observed permeation of negatively charged nanoparticles may be due to repulsive forces acting between negatively charged lipids in the skin and the nanoparticles at the surface, which could result in the temporary initiation of channels within the skin allowing for particle permeation. The authors suggest that a threshold charge may exist which must be reached to stimulate the adequate repulsion lipids to allow permeation of species through the skin.

This favourable permeation of negative nanoparticles is supported by Lee *et al.* in their investigation of the effect of surface charge of gold nanorods (GNs) on skin permeation.[104] Rod-shaped GNs (length:diameter 40:18 nm) varying in surface charge (cetyltrimethyl ammonium bromide [CTAB]-coated positive GNs and CTAB-PSS- and CTAB-PDADMAC-coated negative GNs) were synthesised, and their zeta potential determined in distilled water as summarised in Appendix Table 2. Following 8 h dermal exposure using an EpiDerm model as a human skin equivalent, TEM analysis revealed that the area density of the electron-dense dots of GNs which penetrated into the SC significantly increased for the negatively charged GNs compared to those which a positive charge ($P < 0.01$). In order to further investigate the effect on surface charge on percutaneous absorption, *in vitro* skin permeation studies were carried out using hair-less mouse skin in Franz-type diffusion cells (FDCs). Receptor chambers were filled with PBS and GNs applied to the donor chambers. No significant differences were observed at 8 h and 24 h after exposure. At 48 h after exposure however, negative GNs were more frequently detected in samples of receptor fluid consistent with the TEM observations.

In conclusion, surface chemistry has been indicated by several studies to be an important factor influencing the ability of nanoparticles to penetrate into the skin, with surface charge (through the modification of surface coating/functionalisation) being the most investigated aspect. Altogether, it is however difficult to elucidate a clear relationship between the many aspects of surface chemistry and dermal penetration at this stage. As highlighted previously, the level of characterisation of the surface chemistry of the nanoparticles investigated is often poor and in many cases only a qualitative indication is provided. Results at this stage are complex and inconclusive, with studies showing varying levels of penetration for a range of different types of nanoparticles with neutral, cationic or anionic coatings, although evidence seem to indicate that positive surface charge may enhance penetration. The interaction of charged nanoparticles with the skin has also been shown to be influenced by a range of factors, including the nature of the vehicle and its pH, particle colloidal

stability and aggregation potential. It remains to be elucidated whether the results of such investigations are due to a charge effect, or another aspect of altering the surface chemistry of the nanoparticle.

3.2.2 Role of shape

Shape has long been considered an important component in determining the toxicity of particulates and this has stemmed primarily from early work on the toxicity of asbestos fibres and in particular, the seminal studies of Stanton and colleagues in determining the role of length (and diameter) in fibre pathogenicity.[145] These studies and importance of shape has mainly been considered for inhalation toxicity, whereby shape can influence clearance rates of particulates[146] and activation of cells[147] within the lung. The relevance of shape of particulates in dermal toxicity has not been overly considered. This in itself is not overly surprising since an important factor in lung toxicity is biopersistence (i.e. the residence time of particulates in the lung) which can lead to particle accumulation to a toxic dose[148] and, as mentioned, this can be markedly effected by shape due a negative impact on clearance mechanisms. Because the skin has no such clearance mechanisms and due to the high quality barrier function of the epidermis, repeated exposure does not necessarily mean accumulation of dose in sensitive regions as it can with the lung. Therefore shape may have little impact on the normal barrier function of the skin.

Taking further the example of asbestos, possibly the most studied fibrous particle, we can see there is remarkably little information on the toxicity of asbestos towards the skin (the combined terms of "asbestos" AND "dermal toxicity" lead to 3 articles in PubMed (as of 12/05/2013)). Indeed, the most common documented dermal effect is the formation of asbestos corns which are defined by the Health and Safety Executive (HSE) as "*discrete nodules in the skin caused by implantation of asbestos fibres. Although sometimes painful, they are usually self-limiting and do not have any serious consequences*".[149] However, whilst relatively little research has been conducted in terms of the effects of asbestos on the skin, there are approximately 9 publications available for review that had considered the role of shape in dermal penetration of nanomaterials.

One of the first was that of Ryman-Rasmussen[109] who used ellipsoid quantum dots (6 nm x 12 nm) which were commercially available and spherical (4.6 nm diameter) quantum dots of differing coatings. These were topically applied to porcine skin in flow-through diffusion cells at an occupationally relevant dose for 8 h and 24 h. The dose used was 62.5 pmoles per cm^2 and the stated "*occupational relevance*" of this dose related to an exposure scenario in which one 40 µL drop of quantum dots at 1 µM concentration is applied to 0.64 cm^2 of skin and remains during the course of a work day or overnight.[109] The results showed that PEG–coated spherical and ellipsoid quantum dots penetrated through the intact SC barrier and were localized primarily in the epidermal layers by 8 h. The main point of difference was when comparing the spherical and elliptical carboxylic acid-coated quantum dots as they found that the spherical form penetrated through the SC and localised within the epidermis by 8 h, whilst this was only apparent for the elliptical quantum dots at 24 h. These appeared to be concentration dependent and were consistent with a passive diffusion mechanism of penetration which was suggested to be *via* an intercellular route of passage between adjacent corneocytes. When discussing the results, the authors noted that the most apparent physicochemical difference between the samples analysed was shape. From this

they recommended caution from speculating that spherical carboxylic acid–coated quantum dots penetrate skin more rapidly than ellipsoid-shaped quantum dots as previous studies had showed conflicting results (lower spherical carboxylic acid–coated nanoparticle penetration). Indeed the results did seem to show a far greater role of surface coating than shape in determining differences in penetration of quantum dots.

In a later study the authors also analysed their interactions with keratinocytes *in vitro.*[150] As this study uses a single cell model in culture, it does not provide information as to the effect of shape on penetration of the skin but it does provide some insight into the potential for a differential response. For example, in comparison to a 4.6 nm spherical quantum dot (both of which have a PEG coating), the elliptical quantum dot did show small yet significant increased cytotoxicity (measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay) whilst the spherical sample did not. The importance of these results given the limited magnitude of a differential response, the fact that the surface coating had a much greater effect (as dealt with in Section 3.1.4) and the rather limited difference between a 4.6 nm particle and a 12 nm (at longest axis), should not be overstated.

In a similar study as that of Ryman-Rasmussen, Zhang *et al.* applied shorter 'nail-shaped' coated quantum dots (see Figure 7) with a length of 8.4 nm and a diameter of 5.78 nm to porcine skin in a diffusion cell as well as human epidermal keratinocytes *in vitro*[151]. Similarly to the results of Ryman-Rasmussen[109], the authors noted penetration of the uppermost layers of the SC as well as around hair follicles but did not detect cadmium in the perfusate suggesting no permeation through the porcine skin. This occurred more quickly and to a greater extent in the Ryman-Rasmussen study for which several reasons were suggested such as differences in vehicle (discussed in Section 3.2.5) but also the larger and more irregular nature of the "nail-shaped" quantum dots.

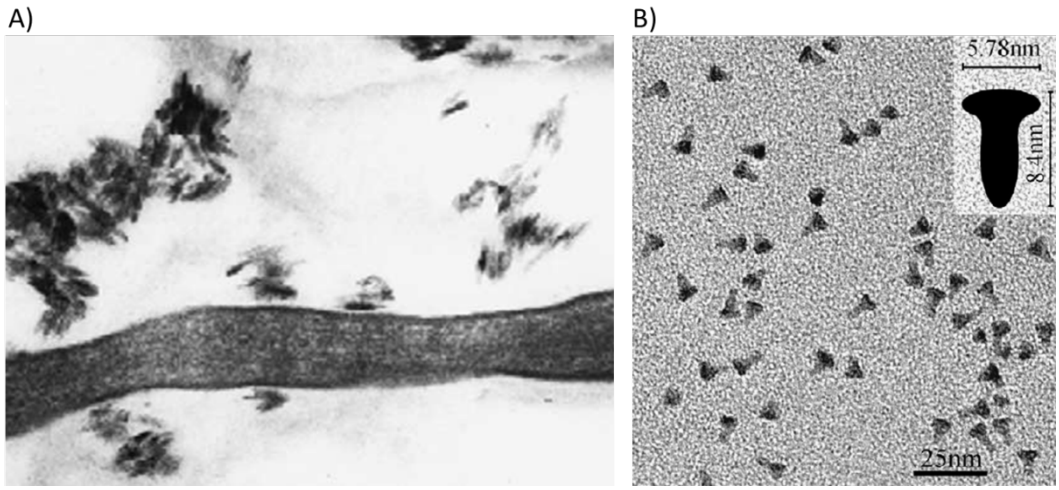

FIGURE 7: EXAMPLES OF ELONGATED NANOPARTICLES COMMONLY USED IN DERMAL PENETRATION STUDIES. The relatively short nature of elongated nanoparticles is exemplified by the TEM images from Gontier *et al.* (A)[114] and Zhang *et al.* (B).[151]

Within the peer reviewed literature, there are several studies considering the penetration of non-spherical TiO_2. One of the earliest was that of Gontier and colleagues comparing the commonly used, 21 nm spherical TiO_2 sample P25 produced by Degussa (now Evonik), and a 20 nm x 100 nm

rod-shaped sample with the trade name Eusolex T-2000 (produced by Merck KGaA). These samples were applied to ex-vivo porcine skin or human skin biopsies or human skin biopsies grafted onto severe combined immune-deficient (SCID) mice. In the porcine model, they found that TiO_2 particles of either shape were exclusively localised on the surface of the outermost SC layer and they noted that the TiO_2 particles sometimes appeared as individual particles, but more frequently agglomerate to clusters of different sizes.[114] Whilst not an experimental objective of the study, the authors noted that in one case after cleaning the surface with ethanol, the SC was severely disrupted resulting in TiO_2 nanoparticles being observed to a much greater depth. Looking at the human skin model, the authors noted that titanium was found to penetrate into a 10 µm thickness layer of the SC only, but no titanium was detected in the SS and in the SCID mice experiments. The TiO_2 particles appeared attached to the corneocyte layers yet no TiO_2 particles were observed close to the SG. The nature of the presentation of the results and discussion of the findings by the author mean that a definitive comparison of shape and its relation to penetration depth is not possible and whilst not explicit, the level of penetration appears to be similar for both shaped preparations. A deficiency of this study was the use of different vehicles during the application. The P25 sample was suspended in carbomergel whilst the Eusolex T-2000 was suspended in hydrophobic basisgel, or polyacrylategel. In addition, whilst P25 is an anatase/rutile mix, Eusolex T-2000 is rutile TiO_2 and was coated resulting in the following composition: 76-82% TiO_2, 8-11% Al_2O_3 and 1-3% SiO_2. These differences therefore hinder the drawing of firm conclusions.

A later study by Furukawa *et al.* looked at the carcinogenic potential of topically applied spindle shape TiO_2 particles with a long axis of 50–100 nm and short axis of 10-20 nm in a mouse model. The results showed that coated and uncoated TiO_2 nanoparticles topically applied at doses up to 20 mg did not increase the development of skin nodules. They noted that whilst foreign bodies on the skin were evident, histopathological examination revealed that the foreign bodies (suggested to be TiO_2) were detected only on the surface of the mouse skin.[113] This led the authors to conclude that significant amounts of TiO_2 did not penetrate to the mouse dermis and that this suggests there is no systemic risk due to percutaneous absorption. These findings must however be taken in light of the experimental approach used, specifically that skin samples were embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin for histopathological examination and so the resolution from a light microscope in terms of particle detection would be very low.

In a study by Monterio-Riviere,[91] both elongated TiO_2 (20 x 100 nm) and ZnO (cuboidal shape up to 200 nm) were analysed for the penetration into pig skin (*in vivo*) exposed to UVB resulting in moderate sunburn or in flowthrough diffusion cells over 24 h. They observed that TiO_2 nanoparticles penetrated 13 layers into UVB-damaged SC, yet only 7 layers in normal skin and that the ZnO nanoparticles were localized to the upper one to two SC layers and that there was no transdermal permeation detected in the *in vitro* diffusion cell. The results of a lack of penetration by elongated ZnO through a diffusion cell were also confirmed by Song *et al.*[117]

Similar to the previous studies, the aim of this study was not explicitly to compare the role of shape on penetration efficiency and therefore, no solid conclusions can be drawn however they do state that the study of Schulz *et al.*,[152] using micronised TiO_2 in oil/water emulsions applied to human forearms for 6 h showed that neither particle shape, formulation, nor exposure had a significant impact on penetration and that it was solely deposited on the outermost surface of the SC.[152] Again,

in a later study by Lee *et al.*,[104] whilst they did evaluate the penetration efficiency of 18 x 40 nm rod-shaped gold nanoparticles using a Franz diffusion cell with mouse skin, the aim of the study was not to evaluate the role of shape, rather the effect of surface charge was of primary interest. Whilst results were interesting and showed percutaneous absorption of negatively charged gold nano-rods, the role of shape cannot be fully established but appears secondary to particle charge.

Based on the evidence presented above from the current literature, it appears that particle shape has a minimal impact on the penetration efficiency of nanoparticles. However this issue of a shape difference, yet still relatively small length (< 100 nm), as shown in Figure 7, makes it difficult to assess the role of shape in true terms. To put this into perspective, in relation to more conventional fibres, the World Health Organisation defines a fibre[153] for the purposes of exposure monitoring (inhalation) as being a particle with a length greater than 5 µm, a diameter a less than 3 µm and a length to width ratio (its aspect ratio) of greater than 3:1. Based on such a definition, if applied to particles in general, irrespective of the route of exposure as a bench mark, it is evident that the effects of fibrous shape has not yet been rigorously investigated within the peer reviewed literature. This is because those studies identified as addressing shape, either explicitly as an aim of the study or by identification of the use of a non-spherical particle, address only elliptical particles and not fibres as defined above.

One of the few types of nanoparticles evaluated for dermal penetration that may in some cases meet the definition of a fibre are carbon nanotubes although this may not always be the case as exemplified by Degim *et al.*,[154] who investigated multi-walled and double-walled carbon nanotubes 100–200 nm in length and 2 nm in diameter. Other studies have used longer carbon nanotubes between 0.1-10 µm in length,[129,155,156] however all of these have focused on the toxicity of carbon nanotubes towards the skin and not on penetration. In one *in vitro* study with cultured human epidermal keratinocytes, multi-walled carbon nanotubes were shown to be capable of entering human keratinocytes as well as eliciting a biological effect (IL-8 release).[157] However as the authors point out, the mechanism of penetration and effect was unknown and the relevance of the results to exposed persons is limited as keratinocyte cultures lack the protective SC barrier seen with intact skin[157]. Therefore, no definitive conclusions as to the penetration efficiency of carbon nanotubes can be drawn.

The studies detailed above show that whilst there are limited examples of non-spherical nanoparticles being evaluated within the literature, there is no study which critically evaluates in a systematic way the role of particle shape/length on penetration. This is evidently a gap within the current knowledge but the importance of this gap must be seen in light of the fact that other physicochemical properties such as surface charge as shown by Ryman-Rasmussen[150] seem to have a far greater impact on penetration with little or no role for shape. If we consider longer, more needle-like nanoparticles such as nanowires or nanotubes, the ability of the particles to penetrate the skin may be increased. This is because it has been suggested in several publications that fibrous materials such as carbon nanotubes can cell pierce membranes[158] and Nagai *et al.* found that fibre diameter and rigidity played a major role in mesothelial cell penetration (and subsequent injury) by carbon nanotubes.[159] However, if this hypothesis is correct and longer, rigid nanofibres can penetrate to a greater efficiency, the length of the fibre is likely to limit the fibre's ability to become systemically available and instead remain lodged in the skin. If this is the case, one could consider

the consequence of interaction of such fibres with the lower layers of the dermis etc., yet by returning to the effect of asbestos on the skin, which is a highly pathogenic fibre capable of causing tumour formation, we see little evidence of adverse effects over and above localised inflammation (e.g. asbestos corns).

In addition to the lack of 'true' fibrous-shaped particles being critically evaluated for the ability of shape to influence penetration, there is an absence of other shaped particles being evaluated. An example of a non-spherical particle in the nano-dimension that is receiving a great deal of attention is graphene. Studies have suggested that the plate-like shape may play an important role in the inhalation and respiratory toxicity of graphene.[160,161] However, the flat plate-like structure in this case, may hinder effective penetration of the skin although this has yet to be tested.

3.2.3 Conclusions

When assessing the literature on the physicochemical properties effecting dermal penetration of nanomaterials it becomes evident that there are considerable challenges in drawing conclusions due to either limitations on the reporting of physicochemical data and/or the alteration of multiple experimental parameters in a non-systematic way. It is widely acknowledged that adequate characterisation of nanomaterials is necessary to accompany toxicological studies whether that be for the purposes of toxicity assessment or toxicokinetics. However the most challenging aspect is this issue of alteration of multiple experimental parameters in a non-systematic way. Being able to alter a single physiochemical property without significantly altering others has always been a challenge and whilst nanotechnology provides even more control of the physicochemical characteristics of particulates than ever before, it still remains a challenge. Whilst this a challenge faced by all branches of nanotoxicology, the issue appears especially prevalent within the dermal literature whereby multiple characteristics such as shape, charge, coating, size can all be changed meaning that little meaningful comparison of results can be made even within the experimental study, let alone between studies.

Despite these issues, some conclusions can be drawn. In relation to size, the results seem to suggest that penetration of particles in the nano-range into the skin is possible although occurs to a rather low degree and that this is greater than for larger particles. Bulk composition appears to have little effect on dermal penetration/ absorption although composition, either in terms of bulk or as a contaminant, should be considered in relation to dermal *toxicity*. Observed absorption following applications with nano-ZnO and nano-silver may be due to solubilisation and thus ion absorption rather than particle absorption. Shape similarly appears to have little effect on penetration or absorption although truly high aspect ratio long (i.e. > 5-10 µm) nanofibres have not been fully evaluated for dermal penetration nor has other shapes. Of the physicochemical properties that influence particle penetration, surface chemistry has been indicated by several studies to be an important factor yet by reviewing the array of results, it is apparent that at this stage the results are complex and inconclusive, although with a slight tendency towards greater uptake of positively-charged particles.

3.3 Experimental assessment of dermal penetration

3.3.1 Introduction

There are several techniques commonly used to assess the dermal penetration of compounds *in vitro*, *ex vivo*, *in vivo* and *in silico* models. These models will be described and their relevance and challenges associated with their use to assess dermal absorption of nanomaterials will be discussed.

3.3.2 In vitro/ex vivo test methods to assess dermal penetration

Non-animal methods to study dermal penetration of compounds have been published by the OECD in the form of OECD TG 428, Skin Absorption: *In vitro* Method.[162] This document describes the method to study dermal absorption and all the variations that can be included in the protocols. In simple terms, the test consists of applying a test substance onto the surface of a skin preparation that separates two chambers: donor and receptor chambers. After exposure for the desired amount of time under the chosen conditions, the test sample is cleaned from the skin and the receptor fluid is analysed for the test compound or its metabolites. Ideally, additional measurements are made on the other parts of the test system in order to have a total recovery study and therefore a better understanding of the compound's interaction with the skin. There are many options to choose from in terms of skin preparations. The skin preparation can be of human ("gold standard")[100] or other animal origin (usually pig). The skin sample can be complete or constituted of only viable or non-viable skin layers. Fresh and frozen skin samples can be used but again this could modify the results obtained and these parameters need to be clearly recorded. Finally, the development of tissue engineering has recently allowed the commercialisation of several reconstituted three dimensional human skin models such as EpiDerm® and EpiSkin®.[163]

Two *in vitro* dose regimes can be used for skin absorption studies: a finite dose or an infinite dose. The choice depends on the purpose of the experiment, i.e. infinite dose is preferred for studies to determine maximal flux which is used to estimate the permeability coefficients. Two different experimental models exist: static diffusion cells (Franz type) or flow-through systems (Bronaugh type). The static cells have a receptor chamber with a fixed volume from which samples can be removed during experiments to allow kinetic studies. The removed receptor fluid is then replaced with fresh receptor fluid. Sink conditions required for estimation of permeability parameters can be ensured by a relatively large volume of the receptor chamber compared to the skin area available for penetration. The flow through system, or Bronaugh type, uses a peristaltic pump and has the advantage that it mimics better the *in vivo* "sink conditions" as the receptor fluid is continuously renewed like the presence of blood vessels in physiological conditions. The two experimental models are equally well accepted for regulatory purposes. Importantly, the integrity of the skin sample before and after the assay is run needs to be assessed, as this is critical to validate the results. Several possibilities exist to check the integrity of the skin preparation, such as the use of tritiated water, transepidermal water loss, capacitance, or Transepidermal electrical resistance (TEER) can be used[6]. Both experimental models can use rodent, pig, as well as human skin, either full thickness or dermatomed.

The choices in terms of receptor chamber sampling and analysis must be carefully decided according to the test compound. Radio-labelling of the test substance or other analytical method can be used to analyse the dermal absorption of a compound. Detection system to assess skin absorption will be discussed in Section 3.2.4.

Many experimental parameters influence the rate of absorption through the skin and need to be properly controlled and/or recorded such as test model, volatility of the compound, dose, surface area, skin integrity, time of exposure, choice of the skin preparation, choice of vehicle, choice of receptor fluid. Most of these parameters and their importance have been discussed in the OECD Guidance Document for the Conduct of Skin Absorption Studies.[164] Overall, the guideline is non-specific in terms of protocol and experimental set-up, and it can therefore be difficult for the inexperienced to compare data produced by different experimental set-ups due to the many parameters governing skin absorption of compounds.

Explant skin tissues grown in cell culture dishes can also be used to study to some extent the dermal absorption of compounds. For example, Cohen *et al.*[87] cultured human skin tissue in Petri dishes and compared the toxicity of copper oxide particles according to two different mode of exposure: topical application in the centre of the skin explant or "systemic" treatment in the cell culture medium. However, this model to study dermal absorption is not ideal since it can be hypothesised that the topical application might permeate *via* the edges of the skin explant where there is no SC and bias the results. Therefore, this type of model may be difficult to control and results subsequently, difficult to interpret with confidence and as such this may not be an appropriate model to study dermal absorption.

Finally, the isolated perfused porcine skin flap (IPPSF) is another *in vitro* model using perfused porcine skin. Due to its high physiological relevance this model is of high value to study skin toxicology and in particular skin absorption.[165] However, this model is rather costly and requires a high level of expertise to be set-up and therefore has not been widely used yet to assess dermal absorption of nanomaterials.

In addition to complex dermal models, the use of single cell culture monolayer of keratinocytes is sometimes found in the literature to study the dermal penetration of compounds.[108,150,166,167] However, the results obtained from these studies although interesting to analyse the interaction of nanomaterials with viable cells are not relevant in terms of dermal absorption, since dermal absorption is complex and determined by the whole structure of the skin as an organ. Therefore, data obtained from single cell culture is of little relevance in terms of dermal absorption assessment.

3.3.3 In vivo *test methods to assess dermal penetration*

In terms of skin absorption tests, the OECD have published OECD TG 427 relative to *in vivo* testing.[168] In simple terms, the test sample is applied on the clipped skin of animals for the desired amount of time, under occlusive, semi-occlusive or non-occlusive conditions. The animals are kept in metabolism cages in order to study the complete metabolic profile of the compound tested. The rat is the most commonly used animal for these studies, and hairless strains are also available. Dry material or liquid preparation can be tested on the skin of animals, the guideline suggesting "*1-5 mg/cm² for a solid or up to 10 µL/cm² for liquids*". In terms of exposure duration, the guideline suggests 6 and 24 h as classic duration. Importantly, the test sample needs to be prepared the same as or as close as possible to the preparation humans could be exposed to. In order to follow the absorption characteristics of the test compound, radio-labelling is usually recommend as a tracing

method. Screening of the literature has shown several publications using animals to study skin penetration/ absorption of nanomaterials, for example TiO_2 in rodents[78,86,94,113], TiO_2 in pigs[84,91] and ZnO in pigs.[91]

Other animal models also exist, such as human skin grafts onto immune-deficient mice (SCID mice)[169], which was used as part of the FP7 NANODERM project[170] for example and other publications.[114] This model presents the advantage of studying human skin, which is structurally and chemically relevant for human risk assessment and to keep it into a physiologically relevant environment. This represents an alternative to human studies.

Human studies are the next level of dermal penetration/ absorption testing and considered the "gold standard". Several nanomaterials have already been tested on humans such as TiO_2,[114,171] ZnO,[77,98,99,116] quantum dots,[71,75] polymer nanoparticles[15] and silver nanoparticles.[112] Due to anatomical variations in terms of skin structure and chemistry, the site of exposure of the skin in human studies could influence the results obtained. *In vivo* studies often use tissue from the forearm, leg, or back and part of the project Reference Group have compared stomach and breast tissues with no differences observed.[172]

Moreover, age, sex, genetic background, skin "history" etc. are amongst the many factors that influence the level of dermal absorption for a given compound.[6] Therefore, inter-individual variations should be taken into account, at least by recording them in as much detail as possible. Finally, the number of individuals involved in the study will also influence the strength of the data obtained. For example, the number of human volunteers was 20 in Gulson *et al.*[90], yet only 3 in Gontier *et al.*[114] and in Gulson *et al.*,[116] 2 in Jeong,[71] and 8 in Ravichandran,[75] suggesting that the data gathered needs to be interpreted with caution before generalisation due to the small number of individuals tested. All these considerations regarding the methods to assess the dermal penetration of compounds, especially nanomaterials, will be discussed in more details in the next section.

3.3.4 Relevance and limitations of dermal penetration/ absorption models

Several technical concerns surrounding the use of *in vitro*, *ex vivo* and *in vivo* models to assess dermal absorption of compounds and more specifically nanomaterials have been highlighted from the literature. Since these matters are fundamentally similar for *in vitro* and *in vivo* systems they will be considered in conjunction within the next section.

3.3.4.1 Species

In a recent review of dermal penetration/ absorption of inorganic nanoparticles, Labouta *et al.* noted that 51% of studies (*in vitro* and *in vivo*) were performed using human material, and 47% of the dermal penetration studies performed on inorganic nanomaterials used animal models (rat, mouse and pig mainly) with the 2% not described within the article.[100] However, as mentioned previously, the dermal penetration/ absorption of compounds in dependent on their physicochemical characteristics and also on various skin characteristics. Due to species differences in terms of skin structure, especially dermal layer thickness, lipid composition, and pelage density, differences in terms of dermal penetration/ absorption can differ markedly.[6,100] It is generally

considered that the dermal permeability between species can be ranked as follows from the more permeable to the less permeable:[173]

Rabbit > Rat > Pig > Monkey > Human

Depending on the compound studied, these differences in terms of skin permeability could range from more than 4 times for the pig and up to 9 times more permeable for the rat compared to human skin.[173] Therefore in terms of animal models, rodents, which have a dense pelage, are not necessarily a relevant model to predict dermal penetration/ absorption in humans, since these species generally present a higher permeability. Overall, human skin remains the "gold standard" to evaluate dermal penetration/ absorption of compounds for humans, although its availability to conduct experiments is sometime problematic hence the common use of animal models.[100] The best animal alternative seems to be pig skin which displays the most common characteristics with humans and as such, pigs and minipigs are commonly accepted as models for dermal absorption studies to extrapolate results to humans, although as mentioned previously their permeability can be up to 4 times higher than human skin. In terms of experiments with nanomaterials, where deposition (if not penetration/ absorption) occurs along the hair follicles, the density of hair on the skin may also be expected to be important. In addition, it is important to consider that the use of animal and human skin, either *in vitro* or *in vivo*, is subject to ethical considerations.[162]

3.3.4.2 Model Characteristics

OECD TG 428 allows the researcher the choice between various animal species and skin models. Importantly, *ex vivo* experimentation using skin, still present limitations as the exposed skin may interact in a different manner *ex vivo* as it would *in vivo*.[6] Moreover, as mentioned previously, the origin, in terms of species but also age and anatomical origin of the skin influence the structural parameters of the skin which has consequences in terms of dermal absorption of a test sample.[6] Finally, whilst the development of reconstituted three dimensional human skin models, a recent comparison of dermal absorption results obtained using these new models and human and animal skins showed that these reconstructed human epidermis were more permeable than human skin and therefore were also over estimating the dermal absorption of benchmark compounds.[163]

3.3.4.3 Integrity of the skin model used

The integrity of the skin used in *ex vivo* experiments can be altered. For example, some studies have used only viable or only non-viable layers of the skin to test dermal penetration/ absorption while other have used a whole skin. Although relevant in terms of mechanistic studies, these alterations would potentially give different results if the compound is metabolised by the cells or if the absent layers were critical in determining the absorption of the compound through the skin. Moreover, Senzui *et al.* stated concerns that the use of split skin as recommended by OECD TG 428 may over estimate permeation of nanoparticles as the hair follicle is cut and therefore may form a route into the receptor phase.[85] In addition, other modes of preparation such as skin stripping may be used. In a recent study, the use of cyanoacrylate stripping was justified as it has been stated that "*in excised skin the hair follicle orifices are often clogged and have a smaller volume due to the loss of the physiological skin elasticity. Therefore, in the case of excised skin, the cyanoacrylate pre-treatment represents a suitable way to get closer to the in vivo situation where most hair follicles*

are accessible to particles".[90] However, such pre-treatment may also damage the SC, which is its primary use in other studies.

Similarly, *in vivo*, pre-treatment of the skin such as shaving, depilation and clipping are commonly used to prepare skin for *in vivo* studies and may also affect the results obtain in terms of dermal absorption.[6] All these variations in term of densities of hair follicles, species and pre-treatment differences of skin models, should be taken into consideration when interpreting results of dermal absorption. This is especially relevant as it should be considered that such activities (e.g. shaving) are commonly performed by humans before applying skin lotions that contain nanoparticles, and so they are highly relevant to real world application of such materials. These considerations are particularly important for nanomaterials since it has been hypothesised that the follicular pathway could act as a size-selective and/or "pumping" system, especially when the skin is mechanically (movements: flexion, massage) stimulated for the dermal penetration/ absorption of nanomaterials.[100]

3.3.4.4 Effects of movements on dermal penetration

In vitro models to study dermal penetration are usually static. However, as mentioned previously, stimulation of the skin by natural movement of a living animal, or massage can influence the level of dermal penetration/ absorption of a compound. This seems to be particularly relevant for nanomaterials, since due to their particulate structure, diffusion may not be the primary mechanism driving the penetration of engineered nanomaterials through the skin. This has been suggested by many authors and was one of the main conclusions of the NANODERM report for TiO_2 nanoparticles.[100,170] Therefore, the classical diffusion cell testing may be rather simplistic to properly assess dermal absorption of nanomaterials. Several studies have now been published in the literature, using a diffusion cell and a flexing system, in order to create movement and therefore are more realistic and more relevant for nanomaterials dermal absorption studies.[100] Results published indicated that flexion in an *in vitro* system increased the penetration of particles through skin models as shown with fluorescent dextran particles and fullerenes.[92,102] The same considerations are relevant for *in vivo* studies, when materials are applied to living animals or to humans with or without skin massaging etc. This consideration is also important to take into account when studying the dermal absorption of a product that consumer should use as a topical application, for example a sunscreen versus other types of product containing nanomaterials, consumers may be exposed to *via* the skin.

3.3.4.5 "Sink effect"

In terms of diffusion cells, the system can be either static (Franz diffusion cell) or dynamic (Bronaugh type of cell) using a peristaltic pump. It is very important to keep in mind that both systems might give very different outcomes in terms of dermal penetration. The flow through system is closer to physiological conditions since it recreates the "sink" effect relative to the presence of blood vessels in the skin. Therefore, the kinetics of dermal penetration of a compound may be different in both systems. However, as mentioned previously, the dermal penetration/ absorption of nanomaterials may involve more of a mechanical phenomenon than a diffusion effect. If this is the case, penetration *via* a diffusion mechanisms is less important, and so the presence or absence of a "sink" effect *in vitro* might not be so relevant for nanomaterials.[100,170] Finally, the detection system would need to be extremely sensitive in the flow through system in order to pick

up the presence of test sample into a diluted environment over time. For these reasons, it is always advisable to perform additional measurements on the other parts of the test system in order to have a total recovery study and therefore a better understanding of the compound interaction with the skin.[162,164]

3.3.4.6 Formulation used to test dermal absorption

Dry powder materials represent a great concern in terms of respiratory exposure. By contrast, nanomaterials as a powder are regarded less as a risk in terms of skin exposure since in the absence of solvents nanomaterials are assumed not penetrate the skin.[1] By contrast, the nature of the solvent used to suspend nanomaterials plays a critical role in terms of dermal absorption since it may alter the structure of the skin hence increasing or decreasing its permeability and it also may influence the physicochemical characteristics of the nanomaterials such as agglomeration state, charge etc. that are important parameters in terms of dermal absorption.[1,100] This consideration is important for both *in vitro* and *in vivo* experimental design and data interpretation. Again, this is specifically relevant for nanomaterials dermal absorption and is also very important for risk assessment for consumer in terms of skin as a route of exposure.

3.3.4.7 Experimental considerations: surface area, numbers of applications, duration of exposure and sampling time, enhanced skin permeation techniques.

Many variations in the protocols to study dermal absorption of chemicals and even more for nanomaterials can affect the results obtained, thereby making it difficult to compare results and interpret data. Surface area and time of exposure as well as concentration of the test samples, volume and diffusion area of the system and finally sampling time are all critical in terms of dose applied to a skin model. However, due to the absence of specific and standardised methods even within the OECD TGs to test skin absorption, it is difficult to efficiently compare data obtained from different studies. There are a wide range of techniques that can be used to increase dermal penetration mainly in *in vitro* experiments such as temperature, ionophoresis, dermaporation, dermabrasion, ballistic bombardment etc.[100] These enhanced skin permeation techniques and most importantly their relevance in assessing dermal absorption of nanomaterials, especially for consumer products should be highlighted and data carefully interpreted.

Observations from the real-life-scenario study by Gulson *et al.*[99] may help to define some of these parameters for future studies. In this beach study, which used the highly sensitive technique of stable isotope tracing to assess dermal absorption of Zn from ZnO particles, levels of traceable ^{68}Zn from ^{68}ZnO particles in sunscreens applied to humans twice daily over 5 consecutive days were first detected at the end of the second day of the study and after four applications of sunscreen. In addition, levels of ^{68}Zn in blood continued to increase for at least 6 days after the final application of sunscreen, suggesting that ^{68}Zn was stored in the body and released to blood over time. Whether or not the ^{68}Zn was absorbed as ^{68}ZnO particles or soluble ^{68}Zn, these observations suggest that *in vivo* studies to assess dermal absorption of nanomaterials in humans may need to be conducted over at least 2 weeks, especially if the method for detecting systemic absorption is less sensitive than the stable isotope method used by Gulson *et al.*[99], or if the nanomaterial cannot be radiolabelled as recommended by OECD TG 427 (developed for chemicals).[168]

3.3.4.8 Other critical factors for dermal absorption studies

As part of good practice and robust study design the use of benchmark or standard compounds is recommended in order to validate results, compare data from different research groups and to make relevant interpretation of data.[163] The use of benchmark compounds, chemical and nanomaterials when studying the dermal penetration/ absorption of nanomaterials should be common practice. This is critical to validate results in terms of dermal penetration/ absorption and to further better understanding of the mechanisms driving it and is especially important for dermal penentration/ absorption since many parameters such as temperature, UV exposure, humidity etc. are known to influence the skin structure and the thermodynamics of the skin and compounds to be tested.[6,100] However, whilst this is the ideal situation, the standard approaches of assessing dermal penetration for nanomaterials need to be developed in order to provide the data supporting such 'standard materials'.

OECD TG 427 insists on the use of individual cages in order to avoid oral absorption as an experimental confounding due to animal grooming.[168] For example, Wu et. al.[94] in their study did not address this parameter when testing the penetration and toxicity of TiO_2 nanoparticles in mice and was therefore heavily criticised.[93] In addition, Elizabethan collars can be applied to animals in individual cages to minimise oral entry by personal grooming. Other confounding factors have also been highlighted by Gulson *et al.* when studying nanoparticle absorption from sunscreens applied to humans.[99] For example, these authors recorded UV levels throughout each day at the beach using a UV spectrophotometer, since UV exposure can modify the level of dermal penetration of a compound.[99] Finally, it has been shown that movement and temperature also influence greatly the level of dermal absorption and as such need to be controlled or at least appropriately monitored in *in vivo* studies.[174]

Importantly, as mentioned before, the permeability of the skin depends heavily on its anatomical region of origin.[6] The OECD TG 428 for the choice of skin sample only states: "*The selection of species, anatomical site and preparative technique must be justified*". By contrast, OECD TG 427 indicates "*an area of skin in the region of the shoulders and the back*"[168] of the animal is used to test dermal absorption. Use of this site makes personal grooming difficult, and hence minimises oral entry. Since the dermal absorption of compounds depends critically on the structure of the skin and hence its anatomical origin, the anatomical origin of the skin used for experiments should always be mentioned. Moreover, the choice of anatomical origin of the skin should be relevant for the compound tested.

Finally, as a good practice, attention to potential artefacts of preparation of the sample and/or analysis of the samples should be borne in mind. Indeed, there are still many technical challenges associated with the specificity, sensitivity, invasiveness of assays and detection methods. For example, NANODERM was one of the first projects that emphasised the need to pay attention to artefacts of preparation of skin samples for analysis especially for nanomaterials.[170] Similarly, it was suggested that the angle used to section skin samples is critical for the quality of the data obtained. For example, Sadrieh *et al.* in relation to their 2010 study stated that:

"To avoid inadvertently dragging TiO_2 from the epidermis into the dermis with instruments, we initiated cuttings from the dermis side proceeding into the epidermis and blades were changed between each sectioning".

Detection methods and the challenges associated with their used for dermal absorption studies are described and discussed in Section 3.2.6.

3.3.5 Intact versus damaged skin models

It is evident from the literature that a large proportion of studies are conducted using models representative of healthy skin, yet a very significant fraction of the working population has compromised skin, either for intrinsic reasons (e.g. atopic dermatitis patients) or due to chemical exposures, wet work, sunburnt skin or physical damage to the skin including from regular shaving (eg facial hair for men, leg hair for women). These individuals represent a more susceptible group than people with intact skin in relation to dermal exposures. This is a general statement, but may also be expected to count for exposures to nanomaterials, although available data to support the latter are still very limited.

Considering the effect of dermal barrier impairment on the penetration of chemicals, it is evident from the publication of Kezic and Nielsen[175] that this varies widely depending on the nature of the skin damage, the penetrant and the model. This publication summarises in a table the enhancement factor that solvents such as acetone, surfactants such as sodium lauryl sulphate (SLS) and mechanical damage such as tape stripping can have. The enhancement can vary from a factor 2 for acetone to as high 1,300 for tape stripping although this should be considered within a range as for tap stripping, the following enhancement factors were recorded – 4, 30, 157, 440-1,300. Variability may be caused not only by differences between enhancement factors but also by different experimental methods applied. However, looking at the study of Benfeldt,[176] the author uses salicylic acid as a penetrant and the same experimental model with human volunteers, but 3 different dermal impairment methods: acetone, SLS and tape stripping. For each of these dermal impairment methods different enhancement factors were observed: acetone – 2x, SLS – 46x and tape stripping – 157x.[176] Thus, different methods used for enhancement significantly affect the experimental results.

In addition to the study of artificial dermal impairment and perhaps most importantly, Kezic and Nielsen also looked at the enhancement factor of intrinsically compromised skin (i.e. diseased skin) addressing conditions such as dermatosis, eczema, and atopic dermatitis.[175] Interestingly the enhancement factors noted in skin diseases were lower than that of artificially compromised models (e.g. tape stripping).

Such a comparative summary of the effects of dermal impairment on the penetration enhancement of nanomaterials is not evident in the literature and this could be seen as a gap but may also not yet be possible due to the limited information available and the conflicting and confounding nature of such studies for nanomaterials. However despite this, there are numerous studies which have considered the effect of dermal impairment on nanomaterial penetration.

We can consider dermal damage in respect of how it occurs or the deficit in the dermal barrier it is trying to recreate. For example, there are several studies considering the effect of UV exposure on dermal integrity and what role this may have on penetration of nanomaterials such as those found in sunscreen which may be applied to such skin. Another approach is tape or cyanoacrylate stripping whereby the SC is completely or in part removed and this along with other methods such as scoring or puncture could be seen as mimicking a physical injury or, in the case of stripping, loss of dermal integrity as seen in skin conditions such as eczema.

An early such study considering direct dermal barrier damage (tape stripping or abrasion with sandpaper) is that of Zhang *et al.* who looked at the penetration efficiency of quantum dots through rat skin held in a diffusion cell.[110] They noted that intact skin did not show penetration at 8 or 24 h and that tape stripping which removed the SC showed quantum dots only on the surface of the viable epidermis. This was in contrast to the abraded skin which did show penetration into the viable dermal layers at both 8 and 24 h which is perhaps non-surprising as whilst the tape stripping involved stripping 10 times with Scotch Magic™ to remove the SC, abrasion involved 60 applications of sandpaper *"until the skin was bright red but not bleeding and blood vessels could be seen on the surface"* indicating substantial barrier impairment.

Similar to the study of Zhang *et al.*, Gopee *et al.* also evaluated the penetration of quantum dots into intact, tape stripped, acetone treated, or dermabraded mouse skin although this time *in vivo*.[107] The effect of tape stripping can be seen clearly in Figure 8 with removal of upper layers with progressive stripping.

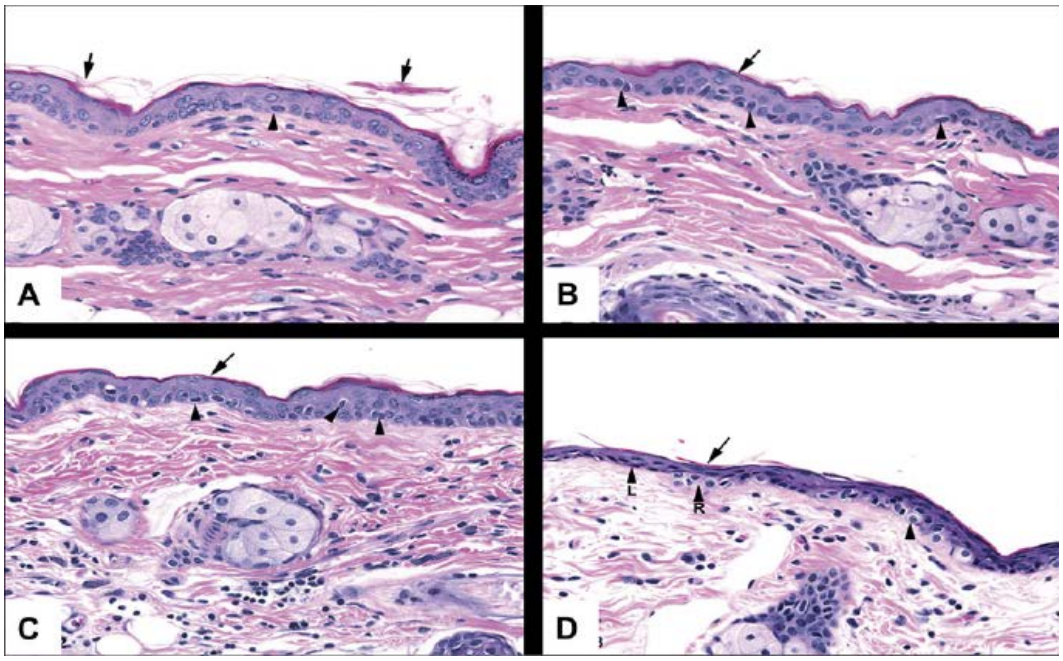

FIGURE 8: PHOTOMICROGRAPHS OF SKIN FROM SKH-1 HAIRLESS MICE FOLLOWING THE APPLICATION AND REMOVAL OF TAPE STRIPS 0 (A), 5 (B), 10 (C), AND 15 (D) TIMES. Reproduced from [107]. The skin was stained with hematoxylin and eosin. The arrows point to partially desquamated keratin, and the arrow heads refer to cells in the stratum basale with perinuclear halos. Magnification is at x20.

Similar to Zhang *et al.* when Gopee *et al.* applied quantum dots to the intact or tape stripped skin they did not find evidence of the migration into or through the skin to the regional lymph nodes or liver (as measured by tissue cadmium levels and confocal microscopy). In contrast, dermal abrasion led to a significant increase in the level of cadmium in the lymph nodes and livers of mice. Interestingly, Gopee calculated that based on the level of cadmium measured in the sentinel organs, around 1.96% of the topically applied dose reached the liver in 24 h in dermabraded skin.[107] This result of a lack of a significant increase in penetration of nanomaterials into skin that has been tape stripped was also supported by Senzui *et al.*[85] In addition, the observation of increased penetration after a more invasive dermal disruption is also supported by the findings of several studies by Filon *et al.* Within studies evaluating penetration of silver[177], gold[80] and cobalt[101] nanoparticles, Filon *et al.* found significant increase in penetration through human skin (held in a diffusion cell) damaged by scoring with a 19-gauge hypodermic needle. In the case of silver nanoparticles, the level of penetration was found to 5 times greater than that of intact skin.

Due to the high consumer relevance, the effect of UV exposure on dermal penetration has received a great deal of attention. One study addressing this issue is that of Monteiro-Rivere *et al.* who undertook a comprehensive study looking at dermal penetration *in vitro* (using a diffusion cell with pig skin) and *in vivo* in pigs with and without sunburn. The comparison of normal and sunburned skin marks this study out as being of particular interest as the authors demonstrated that sun burned skin does show a higher degree of particle penetration than healthy skin. They found that *in vivo*, TiO_2 could penetrate 13 layers into UV damaged skin yet only 7 layers into undamaged skin. Whilst this does signify a difference, it does not show large scale penetration dramatically further into the viable skin than for undamaged skin and within their diffusion model, they did not detect particulates in the lower chamber suggesting no systemic absorption[91].

Such an increase in penetration after UV exposure was also noted by Mortensen *et al.* and similarly to Monteiro-Rivere *et al.*, this was shown to be minimal. In their experiment 14-15 nm quantum dots were applied to the backs of mice 3.5-4.5 days post exposure to UV (or not) at a dose of 30 µL of 3.5 µM quantum dots over 6 cm^2. After 24 h, the mice were sacrificed for skin and organ analysis. Low-level skin penetration in intact or UV damaged skin was noted as well as quantum dot accumulation in folds and hair follicles. In the case of UV exposed skin, there was an increased presence of QD below the SC away from hair follicles although this was still rare. In addition, whilst liver concentrations of cadmium showed no increase in the exposed mice without UV exposure, it did show a significant increase in the livers of UV exposed mice although again this was very low and accounted for 0.0035% of the dose applied.[65] It should also be considered that the duration between UV exposure and application of nanoparticles could affect results as the response to UV damage is transient loosening of intercellular adhesions (possibly assisting permeation of nanoparticles) which allows for a subsequent cellular proliferative response and thickening of the SC (possibly inhibiting penetration).[88] Therefore, such detail in describing methodology should be apparent in future studies to facilitate adequate comparability.

In relation to the effect of damage to the skin on dermal penetration of nanomaterials, it is apparent that this does have the effect of increasing dermal penetration. However this appears minimal in all but the most severe forms of damage (i.e. abrasion) and does not appear to lead to significant breakdown of barrier protection and large scale penetration. However, further studies ideally

utilising intrinsically compromised skin (i.e. diseased skin) addressing conditions such as dermatosis, eczema, and atopic dermatitis are needed to verify the above data. Another issue not dealt with in the literature is that where there is skin damage *in vivo*, this would likely be associated with localised inflammation consisting of localised swelling, oedema and influx of inflammatory cells. These changes may have an effect on dermal integrity and permeability which would not be reflected within *in vitro* models of damaged dermal barrier due to the absence of inflammation. Two human studies are relevant here, although neither specifically addressed the effect of damaged skin on dermal penetration of nanoparticles. One subject in the human study of Gulson et al.[99] had a reaction to the sunscreen applied to her back, and this subject had levels of traceable Zn (from the ZnO nanoparticles in the sunscreen) in her blood and urine samples at elevated levels compared with all other subjects. In the study by Tan et al[18], in which sunscreen containing TiO_2 nanoparticles was applied daily for 2-6 weeks to skin adjacent to lesions in patients scheduled for skin surgery, levels of titanium were higher in the epidermis and dermis of the patients than in the epidermis and dermis found in controls.

As noted by Prof. Bo Neilsen (personal communication), the degree to which the penetration of nanomaterials would be affected/enhanced by an induced damage to the SC would be expected to depend on whether the SC is the rate limiting factor. Present knowledge indicates very limited (or absent) penetration of nanomaterials through the SC, but the question is to what extent nanomaterials would be able to cross the dermis if the nanomaterial was allowed to get through to the dermis. These studies are lacking. If the dermis should turn out to be equally difficult to penetrate for the nanomaterials as the SC, then damage to the SC would not be expected to have much impact. However, at present we are missing robust data to support any conclusion.

3.3.6 Nanoparticle detection and quantification

Quantification of nanoparticles in dermal penetration studies presents a great challenge. As highlighted by Labouta *et al.* in their recent review, the sparse concentration of nanoparticles able to penetrate the skin with regard to the detection limit, as well as the integrity of the particulate nature, limits the available techniques or requires the combination of at least two approaches.[100] A range of techniques have been employed in studies conducted to date for the detection and quantification nanoparticles in skin samples post exposure, as can be seen in the table presented in Appendix 3. The most commonly employed techniques are further detailed in Table 7 below, including an outline of their relative advantages and limitations, including particular focus on issues relevant for nanomaterials.

TABLE 7: COMMONLY USED ANALYTICAL METHODS FOR DETECTING THE DERMAL PENETRATION OF NANOPARTICLES

Detection Method	Brief Description	Advantages	Limitations
Confocal laser scanning microscopy (CLSM) / Fluorescence confocal microscopy	Classical technique for obtaining images from cell or tissue samples by means of laser scanning on an optical platform.	• Images are obtained at a higher resolution with depth selectivity compared to conventional optical microscopy; • Allows in-focus images to be acquired from selected depths (optical sectioning); • 3-D imaging; • Allows elimination or reduction of background information away from the focal plane; • Can be performed on living tissue (*ex vivo*/*in vitro*; • Suitable for time-sequence observation as no fixation or preparation that is detrimental to the specimen survival is required; • Contamination can be eliminated using cryo-sectioning or optical sectioning within the tissue; • Relatively fast data acquisition; • Widely available.	• Loss of laser power with increasing depth of skin; • Nanoparticles appear non-spherical, possibly due to a reconstruction artefact derived from the software or laser scanning vibration; • Images may not reflect the true size or shape of nanoparticles; • When using fluorescently labelled nanoparticles this may cause problems in terms of label stability as well as fundamentally altering the surface properties.
Electro thermal atomic absorption spectroscopy (ETAAS)	Spectro-analytical procedure for the quantitative determination of chemical elements employing the absorption of optical radiation (light) by free atoms in the gaseous state.	• Quantitative technique.	• Allows determination of the elemental composition of the particles, not the particles themselves, which may result in interference problems.
Inductively coupled plasma mass spectrometry (ICP-MS) / Inductively coupled plasma optical emission spectrometry (ICP-OES)	Type of mass spectrometry for quantitative multi-elemental analysis and stable isotopic ratio calculation. Ionises the sample with inductively coupled plasma and then uses a mass spectrometer to separate and quantify those ions. Used in combination with isotopic labelling, whereby nanomaterials are highly enriched with a stable isotope of an element present in the material.	• Quantitative techniques; • Specific and sensitive; • Enables measurement of isotopic ratios in samples • Can be used to detect elements within larger volumes (e.g. blood/urine); • Greater speed, precision and sensitivity compared to atomic absorption techniques; • Widely available; • Isotope labelling allows the detection of dermal penetration of nanoparticles from a consumer product applied to a species, even if the species were exposed to natural particles of the same element from other sources; • Enrichment of the stable isotope is within the internal	• Allows determination of the elemental composition of the particles; • May have interference problems and thus require a purification step; • Susceptible to trace contaminants; • Standard sample processing dissolves particulates, and so cannot determine the form of the element that penetrates (i.e. as nanoparticles, or as soluble ions formed by partial dissolution of the nanoparticles on the skin); however, single-particle ICP-MS methods being developed are suitable for particles not dissolved by sample processing; • Resolution in terms of cell layer is limited to accuracy of specimen preparation (e.g. removal and selective

Detection Method	Brief Description	Advantages	Limitations
		structure of the nanomaterial, and so "labelling" does not alter surface properties.	analysis of the SC from the rest of the epidermis); • Cannot distinguish an element present in the nanoparticles against high background concentrations of that element, unless stable isotope labelling is used; • Isotope labelling requires the integration of a stable isotope within the nanomaterials (e.g. incorporation of C^{13} during carbon nanotube synthesis) and is therefore not suitable for all nanomaterials, and can be expensive; • Further analysis is required for quantitative determination of absorbed nanoparticles (the change in stable isotope ratios of the element and the total concentration of that element are both needed to calculate the actual concentration of absorbed nanoparticles in the sample); • The quality of the isotopic measurements is constrained by mass spectrometer effects.
Multi-collector ICP-MS/Thermal ionisation MS (TIMS)	Types of mass spectrometry in which simultaneous measurement of multiple isotopes of a specific element can be made, allowing isotope ratios of that element to be determined with very high precision and accuracy	• Same advantages as for ICP-MS (above) but highly specific and sensitive; • Dermal penetration of nanoparticles enriched with a stable isotope of an element can be detected, at low concentrations and with very high precision, against a high background concentration of that element by determining changes in isotope ratios in biological samples.	• Same limitations as for ICP-MS (above), and usually requires a purification step; • Not widely available; • Use of multi-collector ICP-MS and TIMS can be expensive.
Multi-photon laser scanning microscopy / Multi-photon fluorescence microscopy (MPM)	Technique based on the non-linear process of 2-photon excitation of endogenous fluorophores, which can be used to acquire horizontal optical sectioning of intact biological tissue samples	• Provides high-resolution fluorescence imaging, allowing visualisation of cellular and subcellular structures of the epidermis and upper dermis; • Non-invasive; • High depth profiling; • Can be used on living skin, cell cultures and *ex vivo* samples ; • Relatively fast data acquisition; • Can be performed on living tissue (*ex vivo*/*in vitro*) with less toxicity due to lower energy; • Suitable for time-sequence observation as no fixation or preparation that is detrimental to the specimen survival is required; • Combined "multi-photon pixel analysis" method allows semi-quantitative analysis.[178]	• As the technique detects fluorescence within a specimen, it can detect only florescent nanoparticles (e.g. quantum dots) or fluorescently labelled nanoparticles which may cause problems in terms of label stability as well as fundamentally altering the surface properties; • Resolution at the nanoscale is limited; • Expensive; • Loss of laser power with increasing depth of skin.
Nuclear	Includes Scanning transmission ion	• These 3 methods can be carried out either	• Individual nanoparticles cannot be resolved (in STIM

Detection Method	Brief Description	Advantages	Limitations
microscopy	microscopy (STIM), Rutherford Backscattering Spectrometry (RBS) and Particle-induced X-ray emission (PIXE): STIM is based on the measurement of the energy loss of individual particles in the beam passing through thin sample sections; PIXE is based on the emission of X-rays after the collision of incident protons with the constitutive atoms; RBS is based on the energy measurement of backscattered protons after elastic collision with sample atom nuclei.	simultaneously or successively on the same sample region; • Well adapted to the measurement of the major, minor, and trace element concentrations in most biological tissues of all origins (human, animals or plant); • STIM gives high resolution cross sectional views based on the material density contrast, both rapidly and non-destructively; • STIM maps allow a reliable determination of the different strata of the epidermis; • PIXE provides not only the chemical composition of the sample, but also 2D- elemental maps; • Simple sample preparation without fixation and staining; • A large observable area with the option to zoom into regions of interest; • Easy quantification of concentrations at a level of sensitivity of a few parts per million; • Possible to check for possible preparation artefacts such as spilling micrometer-sized detached fragments of the SC during cross-sectioning onto the cross-section or contamination of the microtome-blade with formulation containing nanoparticles.	the best lateral resolution thus far is about 100 nm); • Allows determination of the elemental composition of the particles, not the particles themselves, which may result in interference problems; • Not widely available; • Expensive.
Optical (light) microscopy	Uses visible light and a system of lenses to magnify images of small samples. Used to perform analysis of stained skin samples (histology).	• Widely available; • Cost-effective; • Easy technique; • Larger sections of tissue can be viewed.	• Limited resolution making it impossible to view single particles or small agglomerates < 200 nm; • Limited depth profile (without sequential sectioning); • As tissue fixation, preparation and sectioning is required, it is not suitable for analysis of living specimens; • Tissue preparation and in particular, sectioning may cause particles to be moved thereby introducing inaccuracy.
Scanning Electron Microscopy (SEM)	Produces images of a sample by scanning it with a focused beam of electrons.	• High resolution making it possible to view larger nanoparticles and smaller agglomerates; • Provides detailed images of surface topography; • If coupled with Energy-dispersive X-ray spectroscopy it can allow confirmation of particle composition; • Larger areas of tissue can be analysed relatively rapidly; • Relatively widely available;	• Without Energy-dispersive X-ray spectroscopy it is difficult to prove nanoparticle presence; • Limited depth profile although this can be improved to some extent by use of backscatter electron signals;[179] • Where tissue preparation has required sectioning, artefacts such as particle drifting may occur.

Detection Method	Brief Description	Advantages	Limitations
		• Cost-effective.	
Transmission Electron Microscopy (TEM), TEM-EDX	Microscopy technique whereby a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen. The image is magnified and focused onto an imaging device.	• High resolution – possible to detect individual nanoparticles; • Enables visualisation of cell interior and thus a detailed evaluation of penetration pathways and cell interactions; • If coupled with Energy-dispersive X-ray spectroscopy it can allow confirmation of particle composition; • Relatively widely available.	• Substantial sample preparation involved with the possible risk of observing preparation artefacts; • Without Energy-dispersive X-ray (EDX) spectroscopy it is difficult to prove nanoparticle presence; • Limited depth profile (without sequential sectioning); • Where tissue preparation has required sectioning, artefacts such as particle drifting may occur; • Analysis is typically confined to very small areas and so sampling may not be representative or, where multiple sections from a wide area are taken, analysis is slow.
Secondary Ion Mass Spectrometry (SIMS)	Based on the mass spectrometric analysis of ions, which are generated by the interaction of a primary ion beam with a liquid or solid sample. Provides images of the tissue or cellular distribution of both stable and radioactive atoms.	• Sensitive; • Allows mapping of nanoparticle distributions throughout tissue sections.	• Nanoparticles must be labelled with either a stable isotope or a radioactive isotope, which may cause problems in terms of label stability and alteration of nanoparticle surface properties; • Not widely available; • Expensive.

The majority of studies conducted to date have employed qualitative microscopic visualisation methods for assessing the level of dermal penetration. These include scanning electron microscopy (SEM), transmission electron microscopy (TEM), optical microscopy of stained skin samples (histology), nuclear-, confocal- and multi-photon microscopy. Whilst electron microscopy methods such as TEM and SEM provide a readily available and cost-effective method, it is vital that they are used in combination with an elemental profiling method such as energy dispersive X-ray spectroscopy (EDX) in order to fully confirm the presence of nanoparticles within the dermal tissue and enable an assessment of level of penetration. For example, in their study of the toxicity and penetration of TiO_2 nanoparticles in hairless mice and porcine skin after sub-chronic dermal exposure, Wu *et al.* performed TEM alone to image of punch biopsies taken from the centre of the test area in the skin.[94] The authors claim that the resultant TEM micrographs confirm the presence on TiO_2 nanoparticles in the SC, stratum granulosum, prickle cell later and basal cell layer, however the published images, reproduced in Figure 9, provide very little evidence to support this and no further quantification was reported.

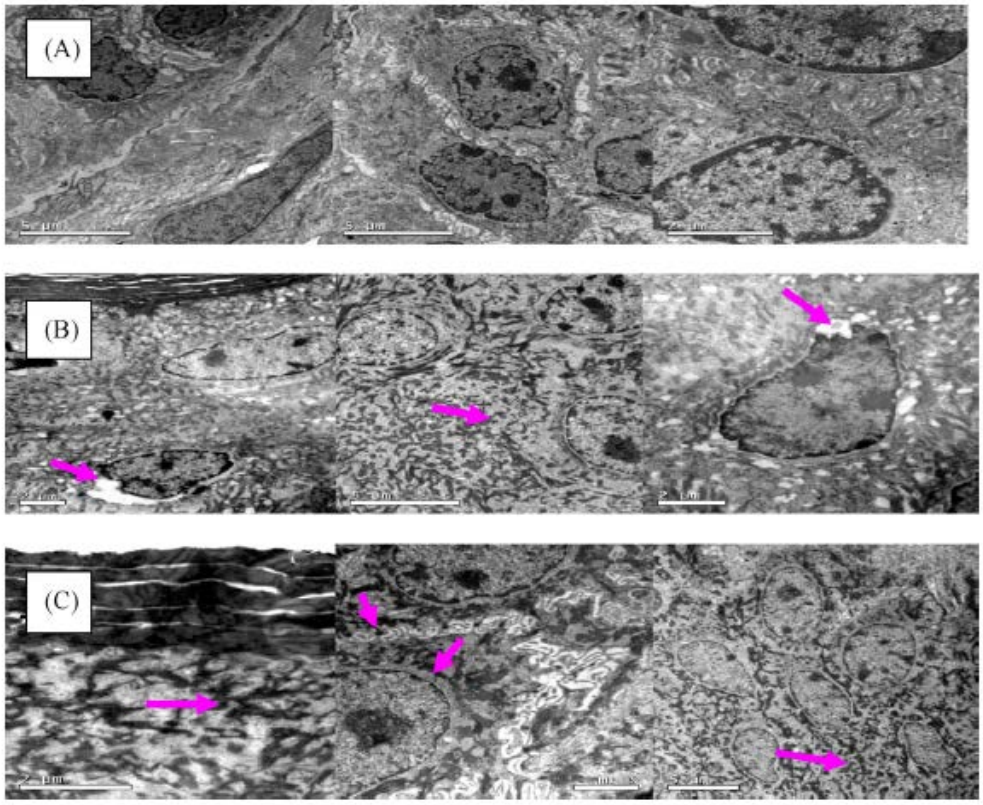

FIGURE 9: TEM MICROGRAPH OF THE PORCINE EAR SKINS AFTER *IN VIVO* PENETRATION TESTS, where a) a placebo formulation without TiO_2 was topically applied; b) a formulation containing 5% 4 nm nano-TiO_2 particles were topically applied for 30 days; c) a formulation containing 5% 60 nm nano-TiO_2 particles were topically applied for 30 days. Reproduced from [94].

Another key problem with methods such as optical and electron microscopy is that they require some kind of tissue preparation prior to analysis which may include sectioning and fixation of skin samples. This has the potential to produce artefacts and alter the distribution and locations of the nanoparticles, thus influencing observations. Nuclear (ion beam) microscopy (STIM/PIXE/RBS), which was investigated by the NANODERM project,[170] offers a complementary technique which

overcomes some of these problems, requiring very few sample preparation steps, thus reducing the risk for preparation artefacts, and allowing easy identification artefacts should they occur and a large observable area with the option to zoom to areas of interest. A disadvantage is that individual nanoparticles cannot be visualised.

The development of advanced microscopy methods such as confocal and multi-photon laser scanning microscopy was seen as one of the most promising ways to address these problems, allowing three-dimensional information on the distribution of nanoparticles in different dermal layers to be obtained by way of optical sectioning *in vivo* thus avoiding artefacts due to sectioning and sample preparation. Indeed, these methods are the most frequently applied for analysis of nanoparticles in the skin within the reviewed literature, with authors such as Zhang *et al.* promoting the merits of such methods for assessing the interactions of nanomaterials with the skin.[180] However, these methods are limited by loss of laser power and resolution with increasing depth inside the skin tissue. In addition, the use of fluorescently-labelled nanoparticles for such methods may introduce potential problems in terms of label stability and alterations to the surface properties of the nanoparticle. There is the potential for noble metal nanoparticles, such as gold nanoparticles, to be visualised by multi-photon-absorption-induced luminescence (MAIL), where the absorption of multiple photons from a near-infrared (NIR) pulsed femtosecond laser can lead to robust luminescence of metal particles thus avoiding the use of fluorophore-labelled particles.[178,181,182] However, this method is only applicable to noble metal nanoparticles.

A number of attempts have been made to develop and apply a quantitative approach to allow a better understanding of skin penetration of nanoparticles and provide a sound scientific basis for health risk assessment. For example, Labouta *et al.* have developed a combined multi-photon imaging-pixel analysis approach for the semi-quantitation of gold nanoparticle population in different skin locations in terms of pixels, from which the weighed number of particles could be calculated.[178] However, this method remains to be validated by the aid of other analytical techniques and more widely investigated. Several examples of the application of more common quantitative analytical methods such as inductively coupled plasma mass spectrometry (ICP-MS) or inductively coupled plasma optical emission spectrometry (ICP-OES) have been identified in the available literature, primarily for the detection of nanoparticles in the receptor fluid of diffusion cell studies. The approach of stable isotope tracing has also shown utility in the assessment of dermal absorption of zinc from ZnO nanoparticles from sunscreens in human volunteer studies by Gulson *et al.*, whereby multi-collector ICP-MS has been used to detect levels of ^{68}Zn tracer in blood and urine samples following application of sunscreen containing ^{68}ZnO.[116,183] This method is highly sensitive and able to detect low levels of absorption *in vivo*, but this also makes it susceptible to trace contaminants; in addition, nanoparticles highly enriched with a stable isotope can be very expensive.

An *in vivo* non-invasive technique would be the “gold standard” for any analytical technique. Novel approaches are beginning to appear in the literature. For example, Lin *et al.* have demonstrated the use of time-correlated single photon counting (TCSPC) for the simultaneous monitoring of zinc oxide nanoparticles and the metabolic state of human volunteer skin with altered barrier function.[184] However, this method suffers from several limitations most notably that it relies on the

use of multiphoton-excited photoluminescense signal to quantify ZnO nanoparticles which is limited to the superficial skin and excludes the dermis.

It is important to note that different techniques will suit different types of nanoparticles and, given limitations associated with each of the methods, no individual technique can satisfy a fully comprehensive level of detection and quantification. It is important that multiple techniques are used where possible, preferably with different methods of sample preparation, in order to formulate an appropriate understanding of the level of nanoparticle penetration. The optimum set of required techniques should be selected based on the specific nanoparticle type under investigation. Indeed, a number of studies published to date employ a combination of methods for monitoring nanoparticle skin penetration which is encouraging.

3.3.7 Role of vehicle and other excipients

In addition to physicochemical parameters, the nature of the vehicle and presence of other excipients in the test preparation/formulation could play a role in influencing the level of dermal penetration of the nanoparticles. Commercially available formulations may range from simple granule to complex multi-phase solutions, and the potential exists for the physical form or presence of additives and adjuvants to impact on the penetration/ absorption characteristics of the nanoparticle. The influence of the vehicle on dermal penetration/ absorption of conventional chemicals is well established, as outlined by the OECD in their guidance notes on dermal absorption.[185] For example, mineral oils and solvents have been shown to increase solubility of lipophilic permeants in vehicles, and can reduce the thermodynamic activity and skin permeation of lipophilic permeants; aprotic solvents such as dimethyl sulfoxide (DMSO), dimethylformamide (DMF) and decylmethyl sulfoxide (DCMS) have been shown to alter keratin and bilayer lipid, such that a high concentration can result in increased penetration of hydrophilic and lipophilic permeants and also cause skin irritation and damage; and surfactants may increase solubility within the SC and disrupt bilayer lipids resulting in a substantial increase of hydrophilic permeants.

The OECD guidance notes on dermal absorption,[185] as well as test guidelines from the OECD and U.S. EPA, recommend using test preparations that are either commercially available formulations (e.g. cosmetics), or for the test substance to be applied alone in a suitable vehicle which should closely match the proposed commercial formulation. Whilst this approach helps improve the relevance of studies to consumer products on an individual basis, it may also have the effect of introducing further diversity in experimental parameters thereby confounding results and hampering study comparisons. As can be seen in the table presented in Appendix 2, a wide range of vehicles have been employed in nanoparticle dermal penetration studies to date. Most frequently, studies are undertaken under biological conditions using aqueous or biocompatible dose formulations with water, buffer solutions (e.g. PBS or oil/water vehicle systems. Comparatively few studies have employed commercially available formulations, such as sunscreen formulations, depilatory agents or base formulations in commercial dermatological products, which can be considered to be more representative from a consumer exposure perspective. A number of studies have employed industrial solvents alone, such as octanoic acid (Pentalan 408), toluene, cyclohexane, and chloroform, yet it is questionable how representative these systems are for consumer exposure. Such studies may be more informative for considering dermal exposure of

workers undertaking industrial or pharmaceutical manufacturing processes that involve the handling and use of nanomaterials in liquid dispersions.

Despite awareness in the literature of the potential for the nature of the vehicle to influence dermal penetration/ absorption, to date there has been little research conducted on this issue in relation to nanoparticle penetration/ absorption. In their review of the interaction of inorganic nanoparticles with the skin barrier, Labouta *et al.* suggest that the nature of the vehicle could affect either: i) the barrier state of the skin, thus favouring enhanced penetration, and/or; ii) the physical state of the nanoparticles, a determinant of skin penetration as highlighted in the previous sections.

A typical example that shows how the presence of other excipients influences the state of nanoparticles inside the skin has been published by Bolzinger and colleagues.[186] Hydrophobic silica particles in aqueous suspension were found to penetrate the outermost layers of the SC, with electron microscopy images revealing well-dispersed silica particles deposited at the surface of corneocytes as shown in Figure 10 (a & b). A Pickering emulsion stabilised with the same silica particles also penetrated the first few layers of the SC, however silica particles observed at the surface of corneocytes were strongly agglomerated by the presence of the oil, as shown in Figure 10 (c & d). Although no difference in the level of penetration was observed in this case, this example clearly indicates that the vehicle can alter the physical state of the nanoparticles and highlights the need for physicochemical characterisation of nanoparticles both in the test formulation and after administration to enable informed interpretation of results.

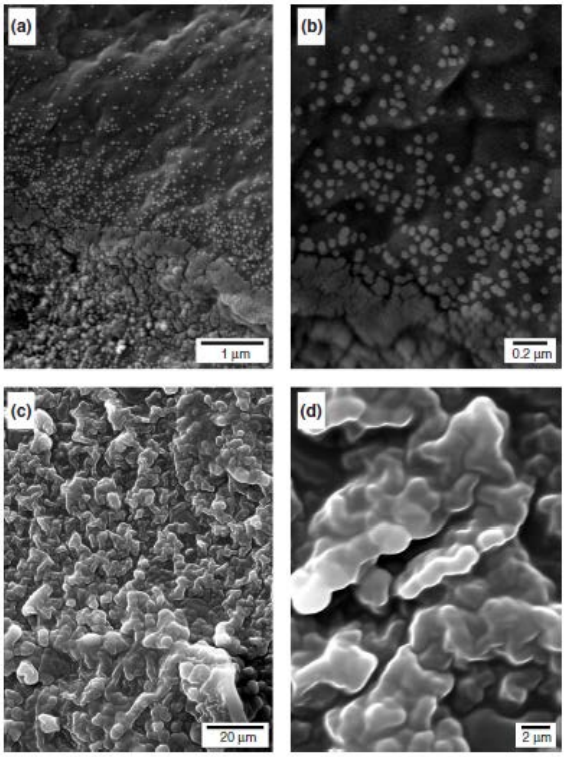

FIGURE 10: SCANNING ELECTRON MICROSCOPY OF TAPE STRIP OF SC **AFTER 24 HOUR EXPOSURE TO HYDROPHILIC SILICA IN AQUEOUS SUSPENSION (A AND B) AND PICKERING EMULSION (C AND D).** Reproduced from [186].

Two studies have been identified in the available literature undertaking comparisons of levels dermal penetration using different vehicle systems. However, both of these studies employ industrial solvents alone and are thus more relevant from the perspective of occupational exposure rather than consumer exposure.

Xia and colleagues explored the impact of solvents on skin penetration of fullerene (C_{60}) from different types of industrial solvents, specifically toluene, cyclohexane, chloroform and mineral oil.[103] Yorkshire weanling pigs were topically dosed with C_{60} (diameter 1 nm) in a given solvent for 24 h and re-dosed daily for four days to simulate the worst scenario in occupational exposures. The dose sites were tape-stripped and skin biopsies were taken after 26 tape-strips for quantitative analysis. When dosed in toluene, cyclohexane or chloroform, pristine C_{60} penetrated deeply into the SC. More C_{60} was detected in the SC when dosed in chloroform compared to toluene or cyclohexane. C_{60} were not detected in the skin when dosed in mineral oil. The penetration of C_{60} was verified using isolated SC *in vitro* using flow-through diffusion cell experiments in pig skin and the solvent effects on the SC penetration of C_{60} were found to be consistent with those observed *in vivo*. The authors conclude that solvent effects are crucial in the skin penetration of fullerenes. As the solubility of C_{60} in mineral oil is comparable to toluene and much higher in chloroform and cyclohexane, the observed solvent effects cannot be explained solely by the solubility of C_{60} in the various solvents. Xia *et al.* propose solvent flux, by which C_{60} diffused into the SC faster in the other solvents as compared to mineral oil, or super-saturation of C_{60} due to solvent evaporation as possible mechanisms. The relevance of this study to consumer exposures must be borne in mind as whilst it does show differences in penetration based on differences in particle solvent, these solvents are not of consumer relevance and instead would likely be of only research or occupational relevance.

In contrast, Labouta *et al.* studied the effect of toluene compared to water as a dispersion medium for four model gold nanoparticles of diameter 6 and 15 nm through human skin in a diffusion cell model and reported the level of penetration to be independent of the nature of the vehicle[144]. However, toluene was shown to have an effect on the barrier function of the SC in terms of lipid quantity, with approximately 8-17% of the total epidermal lipid content extracted by this solvent. Lipid composition, another determinant of barrier function, was not observed to change in the presence of toluene, however, suggesting that a drastic structural change in the skin barrier upon toluene application is unlikely. It is important to highlight that, in this study, all four gold nanoparticles possessed different surface chemistries and polarities, which could potentially confound investigation of the role of vehicle as a single parameter.

In summary, despite known potential for the nature of the vehicle and presence of excipients in test formulations to influence dermal absorption profiles of conventional chemicals, this area remains largely unconsidered in relation to the dermal absorption of nanoparticles. The few studies published to date focussing on this aspect have indicated that the vehicle employed may influence the physical state of the nanoparticles, the barrier function of the skin, and the level of penetration of the nanoparticles into the SC. There is need for further studies to more clearly elucidate the role of the vehicle and presence of other excipients in influencing the dermal absorption of nanoparticles. Such studies should focus on altering the vehicle as a single parameter wherever possible. In investigating consumer exposure, there is a need for further dermal penetration studies

which focus on the nanoparticles in commercially-relevant formulations. A comparative study of a particular nanoparticle in an aqueous dispersion versus the same nanoparticle in a commercially-relevant formulation would also allow further investigation of the influence of excipients. In addition, the ability of the vehicle to alter the physical state of the nanoparticle emphasises the need for thorough physicochemical characterisation of nanoparticles in the test formulation and, where possible, after administration.

3.3.8 In silico test methods/modelling

In silico toxicological test methods offer insight into diffusion of a substance through the different layers of the skin, beyond the laboratory. In addition, computational absorption modelling offers exploration into "what if?" cases which could not be simulated through *in vitro* or *in vivo* experimental methods.[115] *In silico* modelling removes the testing of animals or humans and provides a complementary method to *in vitro* penetration methods. Due to the availability and individual variability provided by both animal and human skin, complications for *in vivo* testing are avoided through the use of *in silico* modelling.[134] Of importance is that given the number of new chemicals, nanomaterials, and combinations, traditional *in vivo* or *in vitro* studies will not be able to meet the expectations for thorough hazard assessments due to limited testing facilities and financial constraints. Therefore, *in silico* methods are needed. The challenge is to feed the researchers developing these models with valid traditional experimental data to create valid computational models.

Akkermans and Wescott presented a study[134] which produced a multiscale mathematical model to complement *in vitro* studies. The method presented models human SC, at three levels of resolution: macroscopic, mesoscopic and microscopic. The macroscale model allows for the prediction of composite permeability and the mesoscale considers the structure and composition of the lipid phase (within the confinement of the cell envelope), whilst the microscale models the lipid-particle system at the atomistic level, which offers a potential understanding of the penetrant molecule's affinity within the lipid. The permeability of the test compound was calculated as a function of cell thickness, with changes in local humidity simulated. The study simulates SC penetration of TeCd quantum dot, in a ceramide bilayer at constant temperature and pressure, where the molecular dynamics were simulated. Akkermans and Wescott state:[115]

"*The presence of a nanoparticle has the effect of decreasing the lipid chain radius of gyration and minor alterations to the hydrogen bonding pattern, stabilizing the layer.*"

In addition, the authors noted the importance of such an *in silico* approach in taking the analysis of permeant-SC interaction one step further. Indeed they suggest that it affords "*an atomistic understanding as to how the SC properties may be altered by, in our example, the presence of a foreign body such as a nanoparticle and state that within their study a bulky inclusion will distort the lipid order and in all likelihood compromise the SC barrier*".[115] Whilst interesting, this still represents an early step in computational modelling as it is not immediately obvious if such observations have any bearing for other nanomaterials than TeCd quantum dots or inform us as to penetration rates of nanomaterials.

A number of *in silico* models have been developed to describe the absorption of chemicals and drugs over the skin. As far as is evident, only the publication of Akkaermans described above describes a model specifically for nanoparticles. One of the modelling tools that has been developed to estimate the dermal absorption of chemicals is IHSkinPerm.[187,188] The user can choose from a library of 137 substances already in the tools database, or can use their own but will need to provide all of the required input parameters. A second tool, developed by the US Centre for Disease Control (CDC), estimates the fluxes, skin concentration, and amounts absorbed from any dose applied partially or fully to the skin.[189-192] There are other models which have not necessarily been made into useable tools.

It may be possible to use these tools and models for nanoparticles but further information would be required for the various parameters of the model, which may be quite difficult to obtain and data will also need to be collected in order to test any such models that are developed. In addition, the fundamental principles on which these models are based may differ for nanoparticulates meaning they may or may not be relevant and as such require validation.

(Q)SAR predictive computational models have been developed to assess the skin permeability of drugs and chemicals based on molecular structure. As with other experimental methods, (Q)SAR models present limitations and variation between models. Indeed, these models make use of the physicochemical characteristics of the substances in order to estimate their skin permeability.[193,194] Although not yet applied specifically to dermal absorption, knowledge gaps required to accelerate the use of nano-QSAR methods have been identified in a workshop funded by the European Cooperation in Science and Technology held in 2011 and reported in a review which also summarised recent advances in the field of QSAR modelling of nanomaterial toxicity.[195] The challenge for QSAR modelling is the lack of relevant data on which to develop and test the models. A dedicated experimental program is needed to fast-track work in this area to provide appropriate data.

3.3.9 Conclusions

As a conclusion, in terms of experimental assessment of dermal penetration/ absorption of nanomaterials in consumer products several key issues have been highlighted. These drawbacks in terms of dermal absorption assays are general for chemicals and some are more specific for nanomaterials. One of the major difficulties highlighted from the literature search is the absence of clear guidelines and then the diversity of models and hence the difficulties in comparing results and therefore interpreting data. More relevant for the study of dermal penetration/ absorption of nanomaterials are the importance of flexion of skin in the experimental set-up and the importance of controlling parameters such as the formulation of the solvent used, and extending the study periods. In addition, there are still lots of unresolved technical challenges regarding the detection of compounds and their behaviour in the skin, in terms of artefacts, specificity, sensitivity, invasiveness of the techniques available.

The effect of impaired barrier function on dermal penetration of nanomaterials appears minimal in all but the most severe forms of damage (i.e. abrasion) and does not appear to lead to significant breakdown of barrier protection and large scale penetration. Also in relation to barrier function, the potential for the nature of the vehicle and presence of excipients in test formulations to influence

dermal absorption profiles of nanoparticles remains largely unconsidered in relation to the dermal absorption. There is need for further studies to more clearly elucidate the role of the vehicle and presence of other excipients in influencing the dermal absorption of nanoparticles.

Whilst *in silico* models and QSAR approaches offer perhaps the most cost effective and efficient way of assessing dermal penetration of nanomaterials, they are currently in their infancy although further development and validation of current models for chemicals may offer a rapid approach. However in terms of considering what data are needed, expected time frames and outputs, it is important to consider what an *in silico* model should provide for risk assessment. Algorithms that provide confident quantitative penetration rates for nanomaterials will likely take quite some time to develop and will require a large amount of information to allow their development and validation which is not currently available. A shorter term and more realistic goal may be a more qualitative approach whereby such models provide information if a nanomaterial may or may not penetrate or whether it will have a high or low penetration rate. Such a qualitative approach will still require data and time to develop but could be used as part of the initial hazard assessment process and as a first step to decide whether further studies using other methods are required or not.

4. Research Gaps

The following analysis considers the findings from the literature review of requirements, methods and approaches that may offer potential value for the assessment of dermal penetration of nanomaterials, and identifies gaps where further research and development (and validation, when appropriate) is necessary. These gaps are drawn from the review and discussions in the previous chapter and are recorded here in succinct form to *highlight* potential areas of research and development.

Physiochemical properties related to dermal absorption

As discussed, the current state of knowledge surrounding physicochemical properties of nanomaterials influencing or potentially influencing particle penetration is both complex and limited. From evaluating the literature, gaps within the current information may be summarised as follows:

- One of the biggest gaps in the literature is systematic evaluation of the effect physicochemical properties on dermal penetration by altering a limited number of experimental factors. It is evident that in many studies there are numerous confounding factors such as differing experimental models, species, doses, duration, but perhaps most challenging, particles of differing physicochemical characteristics.

- It is now widely acknowledged that adequate characterisation of a nanomaterial is necessary to accompany any toxicity study and forms an integral part of the risk assessment yet this is currently often incomplete. It is not just preferable but important that each toxicological assay should be accompanied by a detailed characterisation of all the physicochemical properties of the investigated material that could have *biological relevance.*

- To support this, a key gap is that a selection of key physicochemical parameters should be identified that is relevant to dermal penetration to form a *minimal characterisation dataset* that should accompany and be required for all studies on dermal penetration of nanomaterials.

- The development of a relevant short list of priority parameters for the characterisation of nanomaterials ideally should be based upon the relationship between their physicochemical properties and adverse health effects however such relationships form a significant gap in current understanding.

- Whilst characterisation of nanomaterials as-produced or as-supplied is the most direct and currently realistic approach to obtaining physicochemical information about the material

being studied, this data may not appropriately represent the properties of the material when in contact with the environment in which it is being observed. Therefore methods which allow *in situ* and ideally real-time characterisation are required. However whilst understanding the characterisation *in situ* would thus increase the understanding of how nanomaterials act within the skin, but would not generally be needed for testing the capability of causing toxicity and dermal penetration/absorption.

- As it is known that aggregates and agglomerates of nanomaterials can form in solution, powder and aerosol forms, and their presence is influenced by a number of factors including the method of synthesis, storage, handling and environmental conditions, it is important to consider what effect this has on experimental outcomes.

- A further gap is the issue of follicular versus non-follicular penetration and if follicular penetration should be seen as more or less of concern than non-follicular penetration.

- It is often not clear if for some nanoparticles, detection is of the particles or a soluble fraction and therefore if the result is particulate penetration or solubilisation. In addition, the relative toxicological significance of these two speciation's is not clear (i.e. is one more or less of concern that the other). This ambiguity is related to lack of analytical methods for proper investigation of the permetate.

- There is a deficit of information on the role metals or the presence of biologically active components such as PAHs' may play in the biological activity of nanoparticles to the skin.

- The relevance of shape of particulates in dermal toxicity has not been overly considered and as such, the effects of fibrous shape have not yet been rigorously investigated within the peer reviewed literature. In addition, there is an absence of other shaped particles being evaluated.

- The role of surface chemistry in determining the level of dermal penetration of nanoparticles has not been widely or comprehensively studied in research published to date. A number of studies in the available literature have assessed the level of dermal penetration of nanoparticles with different surface coatings, functionalisations and charges, but in many cases these properties have not been fully characterised.

Physiologically-relevant test methods for the assessment of dermal absorption

- There is a need for comparative studies for nanomaterials on the effects of flexion, UV exposure, temperature, solvent and such systematic comparisons are needed to better understand dermal absorption of nanomaterials.

- There is a lack of benchmark comparators used within studies (e.g. particles or even chemicals with *known* penetration efficiencies that are tested alongside new test materials to provide contextual comparators).

- As with most areas of toxicology, most experimental studies are performed using *in vitro* methods or using animal models. Ideally there needs to be further human studies and such studies should have a large enough group size to provide meaningful statistical analysis, but this should of course be carefully weighed against ethical issues.

- The information on the effect of compromised skin in relation to dermal penetration is limited but crucially, the models currently employed to mimic this (e.g. abrasion, tape stripping etc.) do not appear linked by evidence to show their relevance to sensitive populations such as those with dermatitis.

Role of vehicle and other excipients

- Few studies have employed commercially available formulations (e.g. sunscreens, dermatological products etc.) containing nanoparticles, which can be considered to be more representative from a consumer exposure perspective.

- Despite an awareness in the literature of the potential for the nature of the vehicle and presence of excipients to influence dermal absorption, to date there has been little research conducted on this issue in relation to nanoparticle absorption.

- There is a need for further studies to more clearly elucidate the role of the vehicle and presence of the excipients in influencing the dermal absorption of nanoparticles. Such studies are critical to enable understanding of the variability in observed penetration levels.

Detection and quantification of nanoparticle penetration and systemic distribution

- Available techniques are limited by the sparse concentration of nanoparticles able to penetrate the skin with regards to the detection limit and the integrity of the particulate nature.

- No individual technique has been developed that can satisfy a fully comprehensive level of detection and quantification. Challenge remains that the suitability of detection methods are dictated by the chemical nature of the nanoparticle (e.g. only some nanoparticles can be labelled with fluorophores or isotopes) and often several methods are needed to provide a clear picture.

- Methods are required which enable visualisation of individual nanoparticles without issues of artefacts/interference and good depth of penetration to be achieved, although the biological relevance of single nanoparticles in terms of toxicologically relevant doses also needs to be considered.

- Best practice guidance on the use of analytical methods for various nanomaterials and protocols is needed.

- Best practice guidance needed in relation to preparation of skin samples containing nanoparticles (e.g. sectioning and fixation) for common used microscopy methods, in

order to minimise the production of artefacts and maintain nanoparticle distributions and locations in the sample

- Validation of new methods (e.g. multi-photon imaging pixel analysis) are required.

- Combined approach required for human studies which allows detection of nanoparticles as well as isotope tracer by ICP-MS.

In vitro/*in silico* alternatives to *in vivo* testing

Alternatives to *in vivo* testing are required for the assessment of dermal absorption of nanomaterials due to a variety of reasons. These include: toxicity risk from the nanomaterial, the limited availability and expense of human skin for *in vivo* experiments, and a recent EU animal testing ban on cosmetics. The Cosmetics Regulation (EC) No 1223/2009), due to come into force on 11th July 2013, refers to a ban on the laboratory testing of both end product cosmetics and their ingredients, on animals. However, Directive 2010/63/EU specifically allows the use of animals for scientific purposes.

Currently, studies into *in vitro* or *in silico* alternatives to *in vivo* testing are limited. The Scientific Committee on Consumer Safety (SCCS) presented, in the 2012 revision of the Guidance on the Safety Assessment of Nanomaterials in Cosmetics report, alternatives to *in vivo* test methods. The report presents that alternative methods will require optimisation for each nanomaterial requiring testing, which offers considerable scientific challenges for the full replacement of animal testing of nanomaterials in cosmetics[38].

From evaluating the literature, gaps within the current information may be summarised as follows:

- *In vitro* alternatives to *in vivo* testing, at present, lie with diffusion cells and reconstructed skin models such as EpiSkin. With regards to diffusion cells, progression towards dynamic flexation systems from static systems improves the suitability as an alternative to *in vivo* methods, although limitations remain.

- *In silico* methods for dermal absorption of nanomaterials present a vast research gap as currently only the Akkermans and Wescott model refers to the dermal absorption of nanomaterials, as previously discussed in Section 3.3.8.

- According to the SCCS, the development of reliable *in silico* models for nanomaterials is hampered by the current lack of data regarding the relationship between physiological properties and the toxicological effects of nanomaterials[38].

There are several European working groups currently focussed on testing and validation of alternative methods to animal testing models, including the Alternative Testing Strategies Working Group, European Centre for Validation of Alternative Methods (ECVAM) and European Partnership for Alternative Approaches to Animal Testing (in collaboration with International Cooperation on Cosmetics Regulation (ICCR)).

Updating standard test methods

- Regarding the aforementioned EU cosmetics regulation, new test method standards are required for studies relating to the dermal absorption of nanomaterials in cosmetics. At present, there are no published test methods for alternatives to animal testing specifically for the dermal assessment of nanomaterials.

- In addition to the EU cosmetics regulation, new test methods are required as current OECD test guidelines refers to chemicals, not nanomaterials, and therefore require investigation into their relevance and suitability when testing in the nano form. Recent comments suggest that nanomaterials will need to be validated with all substances, and therefore a suggestion is that *in silico* methods may be the most appropriate due to the time consuming and costly nature of testing each substance.

- The OECD has compiled Expert Meetings to discuss this particular issue. Outcome of OECD Expert Meeting on Environmental Fate and Ecotoxicity, Physical and Chemical characterisation of nanomaterials concluded that there is a need for guidance on sample preparation for nanomaterials for all of the test methods, in addition to concluding that most methods developed for the testing of chemicals will need (at a minimum) modification for testing nanomaterials (note this based on personal communication[97] as the proceedings of these meeting have not yet been released). One particular OCED Expert Meeting regarding *Toxicokinetics and Mechanic Issues* was originally planned for December 2012 with the aim of addressing dermal penetration and absorption. This meeting was postponed to February 2014, with speculation of insufficient data to inform discussions from the OECD sponsorship programme. This highlights the issues regarding the transition from OECD test guidelines for chemicals to the testing of nanomaterials, not the least for dermal penetration studies.

5. Recommendations on Methods and Endpoints to Assess Dermal Absorption for Nanoparticles

Based on the identified research gaps in Section 4 and the current state of knowledge, the key project recommendations are the following:

- In relation to understanding the physicochemical determinant(s) with highest impact on the penetration of nanoparticles, a specific research question needs to be asked and a clear hypothesis generated rather than a simple screening approach which may or may not allow comparability or show relationship between physicochemical property and penetration/absorption efficiency. Such an experimental hypothesis then must be tested using a robust and systematic experimental design. For example within other areas of nanotoxicology, approaches to research questions such as the role of transition metals in toxicity have been investigated by developing particles differing in metal content[123,127] and testing them to see what the effect is and where the borderline between activity and inactivity.[196] This has also been done for fibre length to approach the long standing question of threshold length for fibre effects.[197,198] This approach to taking a base, model particle and altering a single physicochemical property in various ways and then performing a comparison with all experimental parameters kept the same (model, vehicle, dose, duration etc.) is challenging yet is likely to be fruitful. Therefore, future directed studies into the dermal penetration of nanomaterials should potentially be more prescriptive in terms of harmonization at every level, including particle sources, vehicles used, concentrations applied between differing research projects with common aims. Indeed, there is a need to conduct systematic evaluations of nanoparticle physicochemical properties to define quantitatively the role such properties play in dermal penetration. This needs to be done with minimal confounding of results and may require a different approach that involves the study of 'tailor made' nanoparticles developed to answer specific research questions that can then inform judgements on those particles found in consumer products. Such a tailor made approach may wish to consider as a priority the role of surface charge, hydrophobicity and size.

- In order to address the above point in the most robust, efficient and cost effective way the key question to be addressed first is what are the appropriate models, vehicles, doses, and durations relevant to dermal absorption for nanomaterials in humans? This report

provides a basis for this and further considerations and recommendations are given below. However, once the "test guidelines" for model, vehicle, dose and duration are developed, they should be agreed on by the scientific/regulation/industrial community, and then systematic investigations can begin. It should be noted that such "test guidelines" are not a prerequisite for systematic investigations of physicochemical properties as such an approach is used in numerous studies within particle and nanoparticle toxicology without "test guidelines". Instead, such guidelines enhance the utility, comparability and hence worth of determined study results making them more cost effective.

Sample preparation

- Most recently, the International Committee on Standardisation (ISO) has published a guidance document[49] outlining a list of physicochemical properties for detailed description of manufactured nano-objects subject to toxicological testing. These properties, as well as proposed methods for their characterisation, are outlined in Table 4 and should be considered as part of a greater harmonisation of study outputs. Future characterisation should also provide information on any changes in the nanomaterial characteristics during formulation, and similar care is needed during toxicological evaluations especially in relation to agglomeration state and how this relates to actual use.
- As a minimum, characterisation of nanoparticles in relevant vehicle formulations as applied to a test model should be sought and this could consist of:
    - Particle Size/Agglomeration status;
    - Particle Size Distribution;
    - Particle Surface Charge;
    - Particle Surface Chemistry (e.g. alteration and adsorption of compounds to the particle surface).

Dermal models

- Whilst there exists (and detailed above in Sub-Section 3.2.4.1) information on relative penetrability of chemicals through skin for different animals, this information does not yet exist for nanomaterials which hampers a clear decision on the most appropriate model species. Therefore in the absence of such information, human skin (being the target species) is the most appropriate model. The use of human *in vivo* studies offers closer representation of normal human activities especially where the use of a product (e.g. sun lotion or eye make-up) is taken into account. Note that for sunscreens, this would include shaving sections of the skin prior to applying the product, possible sweating (especially if there is a hot sun, or exertion from playing sport), possible dehydration from exposure to wind and sun, skin swelling from swimming in water, abrasion/massage from lying in sand or from clothes, and normal activities such as walking or jogging to permit skin flexing.

- In considering *in vivo* human exposure, one area that raises considerable ethical challenges but is critically important to mention here due to the absence of data is dermal absorption in children. Sunscreens are often applied fastidiously to children during periods of strong sun and with great frequency in hotter countries such as Australia where

sunscreen may be applied several times per day and therefore represents considerable exposure to a potentially sensitive population.

- As such *in vivo* human studies are often expensive (see sub-section on "Cost") and also raise ethical as well as logistical challenges. Therefore, where such studies are conducted it would be preferable to include *in vitro* studies using human skin together with the *in vivo* study. This is because if the *in vitro* study did not give the same results as the *in vivo* study, then the *in vitro* model would need to be reconsidered for its suitability and adjusted. This could be done with greatest efficiency and relevance due to comparative nature of the study.

- In relation to developing more suitable and representative *in vitro* models, there is a definite need to develop standard approaches for *in vitro* models employing a more physiologically relevant flexion system to study dermal absorption of nanomaterials.

- Other considerations on "model" are gender, age, skin type of subjects. These variables can be accommodated by using a large cohort in relation to *in vivo* studies but credence to this issue should also be given to *in vitro* models.

- There is a need for a stronger link in the relevance between nanomaterials studied and the anatomical origin of the skin model used (e.g. mucous membranes versus forearms).

- A clear delineation should be made between studies aiming to look at effects during consumer exposure (and therefore these should replicate faithfully these conditions as recommended by the OECD TGs') and those wishing to evaluate the role of physicochemical properties in relation dermal penetration of nanoparticles. The latter should be encouraged to use a standard simple vehicle and perhaps not try to achieve both aims.

Nanoparticle vehicle

- Studies should focus on altering the vehicle as a single parameter wherever possible to more fully elucidate the role in the vehicle in influencing dermal absorption of nanoparticles.

- In investigating consumer exposure, dermal penetration studies should focus on the application of nanoparticles in commercially-relevant formulations preferably in the simplest possible yet most widely used formulation however such information would need to be gained from commercial producers of such formulations. However, it is important to note that this will introduce considerable study variability. From a consumer exposure perspective, the use of industrial solvents as vehicles is not considered relevant.

- The ability of the vehicle to alter the physical state of the nanoparticles emphasises the need for thorough physicochemical characterisation of nanoparticles as produced, in the test formulation and, where possible and needed, after administration.

- In relation to nanoparticle toxicity, there is a deficit of information on the role metals and other biologically active components such as PAHs' may play in the biological activity of nanoparticles to the skin and this should be addressed. In addition, the ability of nanoparticles to transmit other chemical compounds within the environment or product into the skin where they themselves may cause an effect (rather than the nanoparticle *per se*) also needs further consideration.

Dose

- Dose is a critical parameter within toxicology and is the most critical parameter in terms of understanding the relevance of study outcomes and therefore relative risk to consumer if penetration/absorption is detected and quantified. Therefore an important question is what products are in use in the Danish market, what is the recommended application and what are the actual application/frequency? This will of course differ with different products with, for example deodorants being applied more frequently than sunscreen or certain cosmetics being applied over differing sized areas at differing doses and most often by women. Taking the example of sun lotion, 2 mg/cm^2 is recommended yet people in different countries and in particular more northerly countries may apply less than this amount, with less frequency and perhaps not to all areas of exposed skin (e.g. sunscreen is not applied close to the hairline, or close to clothing)[97]. Whilst in warmer climates the application dose may be considerable more in terms of quantity, area and frequency and in the study by Gulson *et al.* the overall mean of 4.3 mg/cm^2 for each application was used[99], likely reflecting the higher usage and attitude to sunscreen in Australia.

- Studies frequently employ 1-2 applications of test substance however skin applications of consumer products tend to occur more frequently and may be used on a daily basis for many years meaning a considerable difference in both dose and duration. Therefore numerous applications of preparations should be applied and this is especially the case where a very large single dose is to be used (in the absence of usage information to justify such a large single dose). Instead, repetitive application of smaller doses resulting in the accumulation of a larger total dose is likely to be more representative of actual application and accumulation (the same could be said of respiratory exposure to particles)

Duration

- It has been noted in several studies that there can be a considerable lag time between application of a test substance and appearance in the circulation indicating absorption[199]. For example, Gulson *et al.* noted a lag time of ~30 h before the first detection of ^{68}ZnO (by a highly sensitive method) indicating that tracer^{68}Zn was in systemic circulation[99]. Therefore study designs should be aware of the potential for lag-times in dermal absorption and this must be taken into account when taking the biological samples. Preferably study designs (*in vivo* and *in vitro*) should consist of a sufficient post-exposure duration to account for potential lag-times (rather than concluding no absorption over a short time frame) as well as frequent sampling to gauge absorption kinetics. Such frequent

sampling however will likely have an impact on study costs due to greater analysis required but also potential non-compliance by human subjects.

Nanoparticle detection and quantification

- Multiple techniques should be used, preferably with different methods of sample preparation, in order to formulate an appropriate understanding of the level of penetration. This should employ as a minimum of two highly-sensitive methods, one of which must be able to detect the actual particle, rather than simply a tracer or elemental fraction in order to remove the issues associated with loss of label, or dissolution of nanoparticles.

- Electron microscopy methods (e.g. TEM, SEM) should be used in combination with an elemental profiling method (e.g. EDX) in order to fully confirm the presence of nanoparticles within the dermal tissue and enable an assessment of level of penetration.

- Complementary techniques such as nuclear microscopy (STIM/PIXE/RBS) are recommended to be used in support of high resolution electron microscopy, as they require few sample preparations steps, thus reducing the risk for preparation artefacts and allow a larger observable area.

- Whilst new techniques improve in terms of resolution and the ability to identify low levels of penetration and single nanoparticles, the relevance of single particle detection, in terms of cost and ease, and most importantly how this relates to actual biologically relevant dose needs to be considered. Currently, there appears no cut-off as to what is considered significant penetration and what is considered insignificant penetration and such a decision appears arbitrarily reached by study authors. Indeed, when one considers potential risks resulting from penetration, we must understand the potential effects (*disease* endpoints) and at what dose these may occur at.

Cost

- Using appropriate parameters for model, vehicle, dose and duration (and multiple detection methods) is likely to incur considerable cost, as for every variable (*N*) subsequently investigated using these parameters will require a budget close to *N* x unit cost. To put this into context, in 2009 the Gulson *et al.* study[99] had planned to look at uncoated and coated ZnO nanoparticles of two sizes, and also two different formulations with and without penetration enhancers, but the cost of the study (in 2009) with just two uncoated particles was close to $AUS 1 million, and so the coated particles and the variations in formulation had to be reconsidered[200].

- However, such costs should be considered in relation to the importance of obtaining relevant information and how this relates to continue spending lesser amounts on a large number of less-expensive, non-systematic studies that may result in the need for costly repetition or production of low value data.

Overall, there is a clear need to subsequently perform the following studies. To ensure their relevance to dermal absorption in humans, data from these studies can be referenced against the robust data generated from the *in vivo* human studies described above.

(i) Relative nanoparticle penetrability of skin *in vivo* from different animals compared with humans – need to report anatomical location, skin thickness, hair follicle density;

(ii) As in point (i) but *in vitro*;

(iii) Once data for (i) and (ii) have been obtained, systematic variation of physicochemical properties of nanomaterials, vehicle composition, skin conditions, environmental conditions use of benchmark chemicals and materials agreed amongst the scientific community etc. can be explored in the most optimal system.

6. Recommendations for Relevant and Priority Candidate Nanomaterials

Across the current literature concerning dermal penetration (and effects) of nanomaterials, a wide variety of inorganic nanoparticles have been studied. However most attention has either been placed on those nanoparticles which are considered to be in *greatest use* in consumer products, specifically TiO_2 and ZnO nanoparticles in topical sunscreens, or those particles which can be detected more easily such as quantum dots, thereby providing a more straightforward route to detecting dermal penetration. Even amongst these more heavily studied particles, there is considerable variability in the physicochemical properties and sources of these particles meaning that in actuality, the same nanoparticles are rarely studied by independent researchers. This is in stark contrast to the study of chemicals whereby one study assessing the dermal penetration of a chemical compound can more easily be compared to another as the chemical structure should be the same. This enables a body of evidence to be generated considering the *same substance* which is more difficult and rare within nanotoxicology generally. Therefore it is difficult to categorically state if one nanoparticle type has been studied sufficiently whilst another, less so.

This situation shall hopefully be improved by the wider use of the inauguration in February 2011 of the Joint Research Centre's (JRC) Institute for Health and Consumer Protection (IHCP) repository of representative nanomaterials which are also being used within the OECD sponsorship program. Whilst this issue is encountered throughout the discipline of nanotoxicology, it could be said that it is especially challenging for dermal exposure to nanoparticles due to the sheer variety of excipients used, additional ingredients encountered in consumer products and exposure conditions (e.g. massage, flexation etc.) which is often not the case in, for example, respiratory toxicology where passive deposition of a 'pure' nanoparticle aerosol/suspension is most often considered. Therefore, even with the use of standard nanoparticles, variability in test substance characteristics (e.g. particle, vehicle and additional additives) is still likely to be an issue.

In order to recommend those nanomaterials which are to be considered as a priority for future testing, it is important to first consider the nature of the question in terms of what is the purpose of theses future studies. Specifically, are such studies intended: a) to test nanoparticles to which consumers are exposed; b) to understand the routes by which nanoparticles may penetrate the skin, or; c) to test and identify structure activity relationships between nanoparticles and their dermal penetration efficiency (and/or toxicity)? These different endpoints whilst not mutually exclusive, do differ in their aims and therefore may differ in which nanoparticles should ideally be selected as priority test materials to meet such aims.

If the purpose is to identify those particles to which consumers are in contact with due to their use in products (current or future) and ensure their safety then the recommendation can only be based on a detailed understanding of the nanoparticles currently on or in development for the Danish market. Such a review is out of the scope of the current project, but several studies are on-going, which together with a foreseen Danish nano-product database will inform on this issue. In this context there are several important issues that should be considered and some further analysis needed for informing the choice of nanomaterials:

- Independent verification of the nano-component should be made to verify if the "nano" component exists (as opposed to a marketing claim) and therefore if potential exposure to nanomaterials would be possible.

- Where verification is made, physicochemical characterization of the particles should be conducted with emphasis on size distribution, composition and surface charge.

- Description of the concentration of nanoparticles within the product.[100]

- A detailed description of the composition of the vehicle used with special emphasis on penetration enhancers.

- Details of other "active-ingredients" which may alter the penetration profile or dermal effects. This is especially important in consideration of toxicity to rule out the false ascribing of toxicity to the nano-component.

- Description of the use characteristics, specifically intended area of use (e.g. face, forearm, mucous membranes etc.), size of area of application, application method (to be massaged in or spread), frequency of application, and exposure duration.[100]

The approach of identifying those nanoparticles (and specific physicochemical characteristics) consumers are actually likely to be exposed to as priority materials for testing does provide a high degree of specificity and therefore validity of results to consumer exposure which would not be apparent in a blanket approach (e.g. testing each of the JRC standard materials). However there are several possible drawbacks to this approach such as the disproportionate analysis potentially required to identify and characterize nanomaterials in consumer products and the potential unwillingness of companies to divulge commercially sensitive product information, within current or future products. In addition, if the aim is to protect consumers then by the time a product has been identified as containing nanomaterials and a similar formulation/nanoparticle type tested then the product is likely to have been on the market for quite some time.

Questions b) and c) above are linked yet differ as question b) asks a more research centred question aimed at looking at the mechanisms of penetration. This whilst interesting and useful in a research sense, it may be of more limited utility in a risk assessment and health protection sense. Instead, an approach gaining considerable momentum in most fields of nanotoxicology is identifying which structural characteristics of nanomaterials rather than specific nanomaterials that influence penetration and to do this in a rigorous manner that allows quantification of penetration efficiency

or rate based on knowledge physicochemical properties of a nanoparticle in question. The benefit of this approach is that by taking a targeted and systematic approach, potentially using nanoparticles of *low* relevance to the current consumer market but of *high* relevance to confidently quantifying the role of a certain property on penetration (e.g. tailor made nanoparticles to answer a specific research question), is that it enables the development of future (quantitative) structure activity relationships. This in turn allows rapid evaluation of nanoparticles placed on (or approaching) the consumer market in a cost effective manner. In addition, by using physicochemical properties as the basis of evaluation (rather than a pristine particle) it may be possible to account for changes to a particles behaviour on the skin due to interactions with and resultant changes in the physicochemical characteristics resulting from the product vehicle.

In broad terms, based on the evidence and what could be expected from looking at the effects of physicochemical properties in conventional particles (e.g. fibres) the physicochemical properties that seem to have the greatest impact on particle absorption are size and surface chemistry and therefore should be considered as being of high priority. Of these two collections of properties size has been investigated reasonably well, but not systematically and so of the two, surface chemistry and in particular aspects such as surface charge and hydrophobicity is of highest priority in terms of its potential to effect nanoparticle absorption and lack of information.

7. Conclusions

There is a diverse literature base surrounding the issue of dermal penetration/ absorption of nanomaterials. However despite the relative abundance of publications, there is a limitation on the reporting of physicochemical data and/or the alteration of multiple experimental parameters in a non-systematic way that hampers true comparisons of nanomaterials or their physicochemical properties and the drawing of robust conclusions. Indeed, similar to this conclusion, it has been noted in a previous review article that "experimental data do not allow clear cut conclusions because experimental conditions lack consistency".[186] This is a common issue within nanotoxicology and more recently, in terms of undertaking and reporting of rigorous physicochemical data, there have been significant improvements in this area as journals increasingly consider such characterisation a prerequisite for consideration of publication. The confounding of experimental results within studies by non-systematic and non-sequential alteration of particle physiochemical properties is challenging yet can be addressed by robust experimental design and pursuit of clear hypothesis driven research rather than a screening approach *per se*. This would likely require addressing a hypothesis (e.g. effect of positive charge on dermal penetration/ absorption) by taking a base, model particle which may, in itself, not be a commercially relevant particle but possess the characteristics of interest that can be extrapolated to commercial preparations. This would then be altered, by a single physicochemical property sequentially in various ways and compared whilst all other experimental parameters kept the same (model, vehicle, dose, duration etc.). There are several such articles in the literature and these are highly relevant and very useful in understanding dermal absorption of nanomaterials and the role of various physicochemical properties in influencing this.

Of those properties considered within this report, size and surface chemistry appear to play the most significant roles in dermal penetration whilst particle composition and shape tend to have little effect. However one prominent challenge relating to composition is the effect of solubility on detection of absorption whereby if a particle is soluble, such as silver, then it may be detected in the receiving fluid etc. (e.g. blood serum, Franz cell receptor chamber) either as a particle or as a soluble fraction such as the ionic form. Indeed detection methods and especially those employed for large samples of complex composition such as blood or urine tend not to be able to differentiate between particulate and ionic forms thereby negating the ability to conclude if *particle* absorption does or does not occur. Whilst this is a quandary and hinders the drawing of a definitive conclusion, one way of looking at it is that although it *may* not show absorption of a particle, it *does* show absorption of an exogenous substance with potentially adverse effects. The main difference in terms of particle vs. soluble fraction after absorption is likely to be in terms of toxicokinetics as the soluble and particulate forms may have different distribution, metabolisation and excretion pathways within the body.

As indicated, our result showed that the role of size is considered to be a critical component of dermal penetration but this is still subject to the influences of other properties as well as changes in particle size in a dynamic fashion (i.e. agglomeration/ aggregation state). Overall, the conclusion that can be drawn is that penetration of particles in the nano-range into the skin is possible and that this may be greater than for larger particles although this still occurs only at low levels. However there is considerable variability outside of this broad view with little evidence of large deviations in absorption efficiency or distribution profile within the skin across different size fractions within the nano-range and this may depend on various factors such as surface chemistry and experimental conditions etc. In terms of dermal absorption, there is very little robust evidence showing systemic availability of *nanoparticles in vivo* although particle components (silver, cadmium, ^{68}Zn) have been shown in systemic circulation which may indicate particle or soluble ion absorption. Therefore further research is needed to establish if such positive results show particle or ion absorption and what the toxicological impact of this may be.

In addition to size, surface chemistry is a key group of physicochemical properties dictating dermal interaction with surface charge seen as the most investigated property within this group. In relation to surface charge, there is a slight tendency towards greater uptake of positively charged particles although there are conflicting studies in relation to this. Such conflicting information is even more apparent in the less well studied aspects of surface chemistry meaning that elucidating a clear relationship between surface chemistry components and dermal absorption/penetration is difficult. This is often confounded by often poor characterisation and multiple alterations of surface properties within the same experiment meaning that it is not possible to predict the effect of one single property on dermal absorption. Indeed a key issue is that surface chemistry can be both complex and highly variable especially in relation to interactions with the local environment such as excipient used or cell culture media (e.g. presence of proteins). Therefore, proper characterisation of surface chemistry addressing the various characteristics that comprise surface chemistry (e.g. surface charge, hydrophobicity, catalytic properties etc.) and how this changes with alteration in coating, different experimental conditions etc. is important and often lacking.

Looking across the evidence surrounding dermal absorption of nanomaterials, there are numerous methodical approaches that have been used to evaluate absorption ranging in terms of the nature of the model (e.g. *in vitro* or *in vivo*), species, sample type, sample preparation, vehicle, duration of experiment, doses used and methods of detection. Within these different methodological considerations there is considerable variability in approach and an overriding theme evident from a holistic view of the literature is the need for harmonisation of experimental approaches (e.g. models), methods (e.g. sample preparation, vehicles, and doses) and reporting (e.g. in terms of penetration depth, particle frequency etc.). This is likely to be best facilitated by the development of suitable “test guidelines” for nanomaterials, particularly in relation to sample preparation and is a prominent recommendation from this study. However this in itself is not a straightforward task and will likely require a great deal of collaborative work between the scientific, regulatory and industrial communities to develop guidelines that are both *relevant* to real use consumer exposure to nanomaterials, but also *usable* by these communities.

Such guidelines represent an important step to facilitating the gathering of harmonised and comparable data sets that may allow the further development of *in silico* approaches such as

quantitative structure activity relationships (QSAR) to predict dermal absorption which is still very much within their infancy for nanomaterials. Such *in silico* approaches represent a fundamental aim of future intelligent testing and so mechanisms to support their development should be considered in aspects of current and future experimental testing and analysis.

In relation to identifying relevant and priority candidate nanomaterials for future testing, this is not straightforward and, if considered in relation to future consumer exposure, is likely to be highly variable depending on market forces and new technological advancements. Therefore a more comprehensive approach that may be less susceptible to such variances in consumer usages would be to consider priority candidate physicochemical properties rather than a single or group of nanomaterials. Taking this approach, size and surface chemistry currently should be considered as priority physicochemical properties.

8. References

1 Yokel, R. A. & Macphail, R. C. (2011). Engineered nanomaterials: exposures, hazards, and risk prevention. *Journal of occupational medicine and toxicology* **6**, 7

2 Commission, E. (2011) Commission Recommendation of 18 October 2011 on the definition of nanomaterial (Official Journal of the European Union,

3 Zartarian, V., Bahadori, T. & McKone, T. (2005). Adoption of an official ISEA glossary. *Journal of exposure analysis and environmental epidemiology* **15**, 1-5

4 Tobin, D. J. (2006). Biochemistry of human skin-our brain on the outside. *Chemical Society Reviews* **35**, 52-67

5 Elder, A., Vidyasagar, S. & DeLouise, L. (2009). Physicochemical factors that affect metal and metal oxide nanoparticle passage across epithelial barriers. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*

6 Chilcott, R. P., Price, S. & Interscience, W.(2008) *Principles and practice of skin toxicology*. (Wiley Online Library).

7 Fuchs, E. (2008). Skin stem cells: rising to the surface. *The Journal of Cell Biology* **180**, 273-284

8 Robertson, T. A., Sanchez, W. Y. & Roberts, M. S. (2010). Are commercially available nanoparticles safe when applied to the skin? *Journal of biomedical nanotechnology* **6**, 452-468

9 ObservatoryNano. (2009) An overview of nanotechnology in cosmetics. (

10 ObservatoryNano. (2010) Focus Report 2010: Medical Textiles, Sport/Outdoor Textiles. (

11 Robert E. Geertsma, C. B., Susan W.P. Wijnhoven, Adriënne J.A.M. Sips. (2011) ObservatoryNano Technology Sector Evaluation: Health, Medicine & Nanobio. (

12 Blume-Peytavi, U. & Vogt, A. (2011). Human hair follicle: reservoir function and selective targeting. *The British journal of dermatology* **165 Suppl 2**, 13-17

13 Jung, S. *et al.* (2009). Strategy of topical vaccination with nanoparticles. *Journal of Biomedical Optics* **14**, 021001

14 Lademann, J. *et al.* (2006). Hair follicles - a long-term reservoir for drug delivery. *Skin pharmacology and physiology* **19**, 232-236

15 Lademann, J. *et al.* (2007). Nanoparticles--an efficient carrier for drug delivery into the hair follicles. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V* **66**, 159-164

16 Lee, W. R. *et al.* (2012). Skin Permeation of Small-Molecule Drugs, Macromolecules, and Nanoparticles Mediated by a Fractional Carbon Dioxide Laser: The Role of Hair Follicles. *Pharmaceutical research*

17 Bos, J. D. & Meinardi, M. M. (2000). The 500 Dalton rule for the skin penetration of chemical compounds and drugs. *Exp Dermatol* **9**, 165-169

18 Tan, M. H., Commens, C. A., Burnett, L. & Snitch, P. J. (1996). A pilot study on the percutaneous absorption of microfine titanium dioxide from sunscreens. *Australas.J Dermatol.* **37**, 185-187

19 Higgins, J. P., Green, S. & Collaboration, C.(2008) *Cochrane handbook for systematic reviews of interventions*. Vol. 5 (Wiley Online Library).

20 Zhang, Z., Kong, F., Vardhanabhuti, B., Mustapha, A. & Lin, M. (2012). Detection of engineered silver nanoparticle contamination in pears. *Journal of Agricultural and Food Chemistry* **60**, 10762-10767

21 Mamot, C. *et al.* (2012). Tolerability, safety, pharmacokinetics, and efficacy of doxorubicin-loaded anti-EGFR immunoliposomes in advanced solid tumours: a phase 1 dose-escalation study. *The lancet oncology* **13**, 1234-1241

22 Campbell, C. S., Contreras-Rojas, L. R., Delgado-Charro, M. B. & Guy, R. H. (2012). Objective assessment of nanoparticle disposition in mammalian skin after topical

exposure. *Journal of controlled release : official journal of the Controlled Release Society* **162**, 201-207

23 Borm, P. J. *et al.* (2006). The potential risks of nanomaterials: a review carried out for ECETOC. *Particle and fibre toxicology* **3**, 11

24 Aillon, K. L., Xie, Y., El-Gendy, N., Berkland, C. J. & Forrest, M. L. (2009). Effects of nanomaterial physicochemical properties on in vivo toxicity. *Advanced Drug Delivery Reviews*

25 Klimisch, H. J., Andreae, M. & Tillmann, U. (1997). A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory toxicology and pharmacology : RTP* **25**, 1-5

26 Card, J. W. & Magnuson, B. A. (2010). A method to assess the quality of studies that examine the toxicity of engineered nanomaterials. *Int J Toxicol* **29**, 402-410

27 Monteiro-Riviere, N. A. 1-S edn 6.

28 Ryman-Rasmussen, J. P., Riviere, J. E. & Monteiro-Riviere, N. A. 1-S edn 168.

29 Santamaria, A. B. & Rachman, N. J. 1-S edn 133.

30 Warheit, D. B., Borm, P. J., Hennes, C. & Lademann, J. (2007). Testing strategies to establish the safety of nanomaterials: conclusions of an ECETOC workshop. *Inhalation toxicology* **19**, 631-643

31 Nohynek, G. J., Lademann, J., Ribaud, C. & Roberts, M. S. (2007). Grey goo on the skin? Nanotechnology, cosmetic and sunscreen safety. *Critical reviews in toxicology* **37**, 251-277

32 Nohynek, G. J., Dufour, E. K. & Roberts, M. S. (2008). Nanotechnology, cosmetics and the skin: is there a health risk? *Skin pharmacology and physiology* **21**, 136-149

33 Tsuji, J. S. *et al.* (2006). Research strategies for safety evaluation of nanomaterials, part IV: risk assessment of nanoparticles. *Toxicological sciences : an official journal of the Society of Toxicology* **89**, 42-50

34 Jani, P., Halbert, G. W., Langridge, J. & Florence, A. T. (1990). Nanoparticle uptake by the rat gastrointestinal mucosa: quantitation and particle size dependency. *J Pharm.Pharmacol.* **42**, 821-826

35 Geiser, M. & Kreyling, W. G. (2010). Deposition and biokinetics of inhaled nanoparticles. *Particle and fibre toxicology* **7**, 2

36 Zuin, S., Pojana, G., Marcomini, A. .(2007) in *Nanotoxicology: Characterization, Dosing and Health Effects* (ed N.A.; Tran Monteiro-Riviere, C.L.) (Informa Healthcare, New York).

37 Boverhof, D. R. & David, R. M. (2010). Nanomaterial characterization: considerations and needs for hazard assessment and safety evaluation. *Analytical and bioanalytical chemistry* **396**, 953-961

38 SCCS. (2012) Guidance on the safety assessment of nanomaterials in cosmetics. (Scientific Commission on Consumer Safety,

39 Oberdorster, G. *et al.* (2005). Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. *Part Fibre Toxicol* **2**, 8

40 SCENIHR. (2006) Modified Opinion (after public consultation) on: The appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies. (Scientific Committee on Emerging and Newly Identified Health Risks,

41 SCENIHR. (2009) Risk assessment of products of nanotechnologies. (Scientific Committee on Emerging and Newly Identified Health Risks,

42 VCI. (2008) Responsible Production and Use of Nanomaterials. (Verband der Chemischen Industrie e.V. , Frankfurt, Germany).

43 OECD. (2008) List of Manufactured Nanomaterials and List of Endpoints for Phase One of The OECD Testing Programme., (Organisation for Economic Co-operation and Development, Paris, France).

44 OECD. (2010) List of manufactured nanomaterials and list of endpoints for phase one of the Sponsorship Programme for the testing of manufactured nanomaterials: revision. (Organisation for Economic Co-operation and Development, Paris, France).

45 RIVM. (2009) Nanomaterials under REACH: Nanosilver as a case study. (National Insitute for Public Health and the Environment Bilthoven, The Netherlands).

46 EFSA. (2011) Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain. 2140 (European Food and Safety Authority,
47 Hankin, S. M. P., S.A.K.; Poland, C.A.; Foss Hansen, S.; Holmqvist, J.; Ross, B.L.; Varet, J.; Aitken, R.J. (2011) Specific Advice on Fulfilling Information Requirements for Nanomaterials under REACH (RIP-oN 2) – Final Project Report. (
48 Warheit, D. B. (2008). How meaningful are the results of nanotoxicity studies in the absence of adequate material characterization? *Toxicol Sci* **101**, 183-185
49 ISO. (2012) Nanotechnologies — Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment. Report No. ISO/TR 13014:2012(E). (International Organization on Standardization,
50 ISO.(2013) *Online Browsing Platform (OPB)*, <https://www.iso.org/obp/ui/>.
51 Stone, V. H., S.; Aitken, R.; Aschberger, K.; Baun, Anders; Christensen, F.; Fernandes, T.; Hansen, Steffen Foss; Hartmann, Nanna Isabella Bloch; Hutchinson, G.; Johnston, H.; Micheletti, C.; Peters, S.; Ross, B.; Sokull-Kluettgen, B.; Stark, D.; Tran, L (2010) Engineered nanoparticles: Review of health and environmental safety (ENRHES). Project Final Report. . (
52 Tran, C. L. H., S.M.; Ross, B.; Aitken, R.J.; Jones, A.D.; Donaldson, K.; Stone, V.; Tantra, R. . (2008) An outline scoping study to determine whether high aspect ratio nanoparticles (HARN) should raise the same concerns as do asbestos fibres. (UK).
53 Linsinger T, R. G., Gilliland D, Calzolai L, Rossi F, Gibson P, Klein K. (2012) Requirements on measurements for the implementation of the European Commission definition of the term 'nanomaterial'. (
54 Sayes, C. M. & Warheit, D. B. (2009). Characterization of nanomaterials for toxicity assessment. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* **1**, 660-670
55 OECD. (2012) Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials. (Organisation for Economic Co-operation and Development, Paris, France).
56 EC. (2004) Guidance document on dermal absorption. (
57 Oberdorster, G., Ferin, J. & Lehnert, B. E. (1994). Correlation between particle-size, in-vivo particle persistence, and lung injury. *Environmental health perspectives* **102**
58 Renwick, L. C. (2004). Increased inflammation and altered macrophage chemotactic responses caused by two ultrafine particle types. *Occupational and Environmental Medicine* **61**, 442-447
59 Abdelhalim, M. A. (2011). Exposure to gold nanoparticles produces cardiac tissue damage that depends on the size and duration of exposure. *Lipids in health and disease* **10**, 205
60 Kim, T. H. *et al.* (2012). Size-dependent cellular toxicity of silver nanoparticles. *Journal of biomedical materials research. Part A* **100**, 1033-1043
61 Carlson, C. *et al.* (2008). Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. *The journal of physical chemistry. B* **112**, 13608-13619
62 Pan, Y. *et al.* (2007). Size-dependent cytotoxicity of gold nanoparticles. *Small* **3**, 1941-1949
63 Kimura, E., Kawano, Y., Todo, H., Ikarashi, Y. & Sugibayashi, K. (2012). Measurement of skin permeation/penetration of nanoparticles for their safety evaluation. *Biological & pharmaceutical bulletin* **35**, 1476-1486
64 Nabeshi, H. *et al.* (2011). Systemic distribution, nuclear entry and cytotoxicity of amorphous nanosilica following topical application. *Biomaterials* **32**, 2713-2724
65 Mortensen, L. J. *et al.* (2012). Quantification of quantum dot murine skin penetration with UVR barrier impairment. *Nanotoxicology*
66 Prow, T. W. *et al.* (2012). Quantum dot penetration into viable human skin. *Nanotoxicology* **6**, 173-185
67 Baroli, B. (2010). Skin absorption and potential toxicity of nanoparticulate nanomaterials. *Journal of biomedical nanotechnology* **6**, 485-496
68 Gratieri, T. *et al.* (2010). Penetration of quantum dot particles through human skin. *Journal of biomedical nanotechnology* **6**, 586-595
69 Wu, X., Price, G. J. & Guy, R. H. (2009). Disposition of Nanoparticles and an Associated Lipophilic Permeant following Topical Application to the Skin. *Molecular Pharmaceutics* **6**, 1441-1448

70 Baroli, B. *et al.* (2007). Penetration of metallic nanoparticles in human full-thickness skin. *Journal of Investigative Dermatology* **127**, 1701-1712
71 Jeong, S. H. *et al.* (2010). Assessment of penetration of quantum dots through in vitro and in vivo human skin using the human skin equivalent model and the tape stripping method. *Biochemical and biophysical research communications* **394**, 612-615
72 Labouta, H. I. *et al.* (2011). Gold nanoparticle penetration and reduced metabolism in human skin by toluene. *Pharmaceutical research* **28**, 2931-2944
73 Liu, D. C. *et al.* (2012). The Human Stratum Corneum Prevents Small Gold Nanoparticle Penetration and Their Potential Toxic Metabolic Consequences. *Journal of Nanomaterials*, 721706-721706
74 Szikszai, Z. *et al.* (2010). Nuclear microprobe investigation of the penetration of ultrafine zinc oxide into intact and tape-stripped human skin. *Nuclear Instruments & Methods in Physics Research Section B-Beam Interactions with Materials and Atoms* **268**, 2160-2163
75 Ravichandran, S., Mortensen, L. J. & DeLuise, L. A. (2011). Quantification of human skin barrier function and susceptibility to quantum dot skin penetration. *Nanotoxicology* **5**, 675-686
76 Samberg, M. E., Oldenburg, S. J. & Monteiro-Riviere, N. A. (2010). Evaluation of silver nanoparticle toxicity in skin in vivo and keratinocytes in vitro. *Environmental health perspectives* **118**, 407-413
77 Szikszai, Z. *et al.* (2011). Nuclear microprobe investigation of the penetration of ultrafine zinc oxide into human skin affected by atopic dermatitis. *Nuclear Instruments & Methods in Physics Research Section B-Beam Interactions with Materials and Atoms* **269**, 2278-2280
78 Xu, J. *et al.* (2011). Lack of promoting effect of titanium dioxide particles on ultraviolet B-initiated skin carcinogenesis in rats. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* **49**, 1298-1302
79 Alvarez-Roman, R., Naik, A., Kalia, Y. N., Guy, R. H. & Fessi, H. (2004). Skin penetration and distribution of polymeric nanoparticles. *Journal of controlled release : official journal of the Controlled Release Society* **99**, 53-62
80 Filon, F. L. *et al.* (2011). Human skin penetration of gold nanoparticles through intact and damaged skin. *Nanotoxicology* **5**, 493-501
81 Sonavane, G. *et al.* (2008). In vitro permeation of gold nanoparticles through rat skin and rat intestine: effect of particle size. *Colloids and surfaces.B, Biointerfaces* **65**, 1-10
82 Kohli, A. K. & Alpar, H. O. (2004). Potential use of nanoparticles for transcutaneous vaccine delivery: effect of particle size and charge. *Int.J Pharm.* **275**, 13-17
83 Larese, F. F. *et al.* (2009). Human skin penetration of silver nanoparticles through intact and damaged skin. *Toxicology* **255**, 33-37
84 Sadrieh, N. *et al.* (2010). Lack of significant dermal penetration of titanium dioxide from sunscreen formulations containing nano- and submicron-size TiO2 particles. *Toxicological sciences : an official journal of the Society of Toxicology* **115**, 156-166
85 Senzui, M. *et al.* (2010). Study on penetration of titanium dioxide (TiO(2)) nanoparticles into intact and damaged skin in vitro. *The Journal of toxicological sciences* **35**, 107-113
86 Adachi, K., Yamada, N., Yamamoto, K., Yoshida, Y. & Yamamoto, O. (2010). In vivo effect of industrial titanium dioxide nanoparticles experimentally exposed to hairless rat skin. *Nanotoxicology* **4**, 296-306
87 Cohen, D. *et al.* (2013). Evaluation of topically applied copper(II) oxide nanoparticle cytotoxicity in human skin organ culture. *Toxicology in vitro : an international journal published in association with BIBRA* **27**, 292-298
88 Mortensen, L. J., Oberdorster, G., Pentland, A. P. & Delouise, L. A. (2008). In vivo skin penetration of quantum dot nanoparticles in the murine model: the effect of UVR. *Nano letters* **8**, 2779-2787
89 Vogt, A. *et al.* (2006). 40 nm, but not 750 or 1,500 nm, nanoparticles enter epidermal CD1a+ cells after transcutaneous application on human skin. *The Journal of investigative dermatology* **126**, 1316-1322

90 Rancan, F. *et al.* (2012). Skin penetration and cellular uptake of amorphous silica nanoparticles with variable size, surface functionalization, and colloidal stability. *ACS nano* **6**, 6829-6842
91 Monteiro-Riviere, N. A. *et al.* (2011). Safety Evaluation of Sunscreen Formulations Containing Titanium Dioxide and Zinc Oxide Nanoparticles in UVB Sunburned Skin: An In Vitro and In Vivo Study. *Toxicological Sciences* **123**, 264-280
92 Tinkle, S. S. *et al.* (2003). Skin as a route of exposure and sensitization in chronic beryllium disease. *Environmental health perspectives* **111**, 1202-1208
93 Jonaitis, T. S., Card, J. W. & Magnuson, B. (2010). Concerns regarding nano-sized titanium dioxide dermal penetration and toxicity study. *Toxicology letters* **192**, 268-269
94 Wu, J. *et al.* (2009). Toxicity and penetration of TiO2 nanoparticles in hairless mice and porcine skin after subchronic dermal exposure. *Toxicology letters* **191**, 1-8
95 Chourasia, R. & Jain, S. K. (2009). Drug targeting through pilosebaceous route. *Current Drug Targets* **10**, 950-967
96 Mahe, B. *et al.* (2009). Nanoparticle-based targeting of vaccine compounds to skin antigen-presenting cells by hair follicles and their transport in mice. *The Journal of investigative dermatology* **129**, 1156-1164
97 McCall, M. (2013). Gulson Study Contamination Comments. (Personal Communication,09/05/2013, Craig A. Poland).
98 Gulson, B. *et al.* (2008). Dermal absorption of ZnO nanoparticles in sunscreen using the stable isotope approach. *Toxicology letters* **180**, S222-S222
99 Gulson, B. *et al.* (2010). Small amounts of zinc from zinc oxide particles in sunscreens applied outdoors are absorbed through human skin. *Toxicological sciences : an official journal of the Society of Toxicology* **118**, 140-149
100 Labouta, H. I. & Schneider, M. (2013). Interaction of inorganic nanoparticles with the skin barrier: current status and critical review. *Nanomedicine : nanotechnology, biology, and medicine* **9**, 39-54
101 Larese Filon, F. *et al.* (2013). Human skin penetration of cobalt nanoparticles through intact and damaged skin. *Toxicology in vitro : an international journal published in association with BIBRA* **27**, 121-127
102 Rouse, J. G., Yang, J., Ryman-Rasmussen, J. P., Barron, A. R. & Monteiro-Riviere, N. A. (2007). Effects of mechanical flexion on the penetration of fullerene amino acid-derivatized peptide nanoparticles through skin. *Nano letters* **7**, 155-160
103 Xia, X. R., Monteiro-Riviere, N. A. & Riviere, J. E. (2010). Skin penetration and kinetics of pristine fullerenes (C60) topically exposed in industrial organic solvents. *Toxicology and applied pharmacology* **242**, 29-37
104 Lee, O. *et al.* (2013). Influence of surface charge of gold nanorods on skin penetration. *Skin research and technology : official journal of International Society for Bioengineering and the Skin (ISBS) [and] International Society for Digital Imaging of Skin (ISDIS) [and] International Society for Skin Imaging (ISSI)*
105 Baik, K. Y. *et al.* (2008). Imaging of fluorescent nanoparticles distributed in mouse skin. *Journal of the Korean Physical Society* **53**, 880-885
106 Wu, X., Griffin, P., Price, G. J. & Guy, R. H. (2009). Preparation and in vitro evaluation of topical formulations based on polystyrene-poly-2-hydroxyl methacrylate nanoparticles. *Molecular pharmaceutics* **6**, 1449-1456
107 Gopee, N. V. *et al.* (2009). Quantitative determination of skin penetration of PEG-coated CdSe quantum dots in dermabraded but not intact SKH-1 hairless mouse skin. *Toxicological sciences : an official journal of the Society of Toxicology* **111**, 37-48
108 Mortensen, L. J., Ravichandran, S. & Delouise, L. A. (2012). The impact of UVB exposure and differentiation state of primary keratinocytes on their interaction with quantum dots. *Nanotoxicology*
109 Ryman-Rasmussen, J. P., Riviere, J. E. & Monteiro-Riviere, N. A. (2006). Penetration of intact skin by quantum dots with diverse physicochemical properties. *Toxicological sciences : an official journal of the Society of Toxicology*
110 Zhang, L. W. & Monteiro-Riviere, N. A. (2008). Assessment of quantum dot penetration into intact, tape-stripped, abraded and flexed rat skin. *Skin pharmacology and physiology* **21**, 166-180

111 Staroňová, K., Nielsen, J. B., Roursgaard, M. & Knudsen, L. E. Transport of SiO2 Nanoparticles through Human Skin. *Basic & clinical pharmacology & toxicology*
112 Vlachou, E. *et al.* (2007). The safety of nanocrystalline silver dressings on burns: a study of systemic silver absorption. *Burns : journal of the International Society for Burn Injuries* **33**, 979-985
113 Furukawa, F. *et al.* (2011). Lack of skin carcinogenicity of topically applied titanium dioxide nanoparticles in the mouse. *Food and Chemical Toxicology* **49**, 744-749
114 Gontier, E. *et al.* (2008). Is there penetration of titania nanoparticles in sunscreens through skin? A comparative electron and ion microscopy study. *Nanotoxicology* **2**, 218-231
115 Akkermans, R. L. C. & Wescott, J. T. (2011). Multiscale Modeling of Human Skin. *Current Nanoscience* **7**, 736-742
116 Gulson, B. *et al.* (2012). Comparison of dermal absorption of zinc from different sunscreen formulations and differing UV exposure based on stable isotope tracing. *The Science of the total environment* **420**, 313-318
117 Song, Z. *et al.* (2011). Characterization of optical properties of ZnO nanoparticles for quantitative imaging of transdermal transport. *Biomedical optics express* **2**, 3321-3333
118 Surekha, P. *et al.* (2012). Repeated dose dermal toxicity study of nano zinc oxide with Sprague-Dawley rats. *Cutaneous and ocular toxicology* **31**, 26-32
119 Christensen, F. M. *et al.* (2010). Nano-silver - feasibility and challenges for human health risk assessment based on open literature. *Nanotoxicology* **4**, 284-295
120 Aust, A. *et al.* (2002). Particle characteristics responsible for effects on human lung epithelial cells. *RESEARCH REPORT-HEALTH EFFECTS INSTITUTE*
121 Donaldson, K. *et al.* (1997). Free radical activity of PM10: Iron-mediated generation of hydroxyl radicals. *Environmental health perspectives* **105**, 1285-1289
122 Gilmour, P. S. *et al.* (1996). Adverse health effects of PM10 particles: involvement of iron in generation of hydroxyl radical. *Occupational & Environmental Medicine* **53**, 817-822
123 Kagan, V. E. *et al.* (2006). Direct and indirect effects of single walled carbon nanotubes on RAW 264.7 macrophages: role of iron. *Toxicology letters* **165**, 88-100
124 Wilson, M. R., Lightbody, J. H., Donaldson, K., Sales, J. & Stone, V. (2002). Interactions between ultrafine particles and transition metals in vivo and in vitro. *Toxicol.Appl.Pharmacol.* **184**, 172-179
125 Nagai, H. *et al.* (2011). Asbestos surface provides a niche for oxidative modification. *Cancer science* **102**, 2118-2125
126 Gazzano, E. *et al.* (2007). Iron-loaded synthetic chrysotile: a new model solid for studying the role of iron in asbestos toxicity. *Chemical Research In Toxicology* **20**, 380-387
127 Turci, F., Tomatis, M., Lesci, I. G., Roveri, N. & Fubini, B. (2011). The iron-related molecular toxicity mechanism of synthetic asbestos nanofibres: a model study for high-aspect-ratio nanoparticles. *Chemistry* **17**, 350-358
128 Fahmy, B. & Cormier, S. A. (2009). Copper oxide nanoparticles induce oxidative stress and cytotoxicity in airway epithelial cells. *Toxicology in vitro : an international journal published in association with BIBRA* **23**, 1365-1371
129 Murray, A. R. *et al.* (2009). Oxidative stress and inflammatory response in dermal toxicity of single-walled carbon nanotubes. *Toxicology* **257**, 161-171
130 McNeilly, J. D. *et al.* (2004). Soluble transition metals cause the pro-inflammatory effects of welding fumes in vitro. *Toxicol.Appl.Pharmacol.* **196**, 95-107
131 McNeilly, J. D. *et al.* (2005). Soluble transition metals in welding fumes cause inflammation via activation of NF-kappaB and AP-1. *Toxicology letters* **158**, 152-157
132 Valavanidis, A., Fiotakis, K. & Vlachogianni, T. (2008). Airborne particulate matter and human health: toxicological assessment and importance of size and composition of particles for oxidative damage and carcinogenic mechanisms. *Journal of environmental science and health. Part C, Environmental carcinogenesis & ecotoxicology reviews* **26**, 339-362
133 Gerlofs-Nijland, M. E. *et al.* (2009). Particle induced toxicity in relation to transition metal and polycyclic aromatic hydrocarbon contents. *Environmental science & technology* **43**, 4729-4736

134 Sharma, A. K. *et al.* (2007). Genotoxicity, inflammation and physico-chemical properties of fine particle samples from an incineration energy plant and urban air. *Mutation research* **633**, 95-111
135 Lewtas, J. (2007). Air pollution combustion emissions: characterization of causative agents and mechanisms associated with cancer, reproductive, and cardiovascular effects. *Mutation research* **636**, 95-133
136 Siddens, L. K. *et al.* (2012). Polycyclic aromatic hydrocarbons as skin carcinogens: comparison of benzo[a]pyrene, dibenzo[def,p]chrysene and three environmental mixtures in the FVB/N mouse. *Toxicology and applied pharmacology* **264**, 377-386
137 Lam, C. W., James, J. T., McCluskey, R., Arepalli, S. & Hunter, R. L. (2006). A review of carbon nanotube toxicity and assessment of potential occupational and environmental health risks. *Crit Rev.Toxicol.* **36**, 189-217
138 Boogaard, P. J. & van Sittert, N. J. (1995). Urinary 1-hydroxypyrene as biomarker of exposure to polycyclic aromatic hydrocarbons in workers in petrochemical industries: baseline values and dermal uptake. *The Science of the total environment* **163**, 203-209
139 Zhang, W. *et al.* (2011). Distribution and health-risk of polycyclic aromatic hydrocarbons in soils at a coking plant. *Journal of Environmental Monitoring* **13**, 3429-3436
140 Boeniger∗, M., Neumeister, C. & Booth-Jones, A. (2008). Sampling and Analytical Method Development and Hand Wipe Measurements of Dermal Exposures to Polycyclic Aromatic Hydrocarbons. *Journal of Occupational and Environmental Hygiene* **5**, 417-425
141 Cronholm, P. *et al.* (2013). Intracellular Uptake and Toxicity of Ag and CuO Nanoparticles: A Comparison Between Nanoparticles and their Corresponding Metal Ions. *Small*
142 Banga, A. K.(2006) *Therapeutic peptides and proteins: Formulation, processing and delivery systems*. Second Edition edn, (Taylor & Francis Group).
143 Marro, D., Guy, R.H., Delgado-Charro, M. B. (2001). Characterization of the iontophoretic permselectivity properties of human and pig skin. *Journal of Controlled Release* **70**, 213-217
144 Labouta, H. I., el-Khordagui, L. K., Kraus, T. & Schneider, M. (2011). Mechanism and determinants of nanoparticle penetration through human skin. *Nanoscale* **3**, 4989-4999
145 Stanton, M. *et al.* (1981). Relation of particle dimension to carcinogenicity in amphibole asbestoses and other fibrous minerals. *JNCI* **67**, 965 - 975
146 Oberdorster, G. (2002). Toxicokinetics and effects of fibrous and nonfibrous particles. *Inhal.Toxicol.* **14**, 29-56
147 Miller, K. (1978). The effects of asbestos on macrophages. *CRC critical reviews in toxicology* **5**, 319-354
148 Donaldson, K., Murphy, F., Duffin, R. & Poland, C. (2010). Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. *Particle and fibre toxicology* **7**, 5
149 HSE. (2012) Guidance for appointed doctors on the control of asbestos regulations 2012. (
150 Ryman-Rasmussen, J. P., Riviere, J. E. & Monteiro-Riviere, N. A. (2007). Surface coatings determine cytotoxicity and irritation potential of quantum dot nanoparticles in epidermal keratinocytes. *The Journal of investigative dermatology* **127**, 143-153
151 Zhang, L. W., Yu, W. W., Colvin, V. L. & Monteiro-Riviere, N. A. (2008). Biological interactions of quantum dot nanoparticles in skin and in human epidermal keratinocytes. *Toxicology and applied pharmacology* **228**, 200-211
152 Schulz, J. *et al.* (2002). Distribution of sunscreens on skin. *Advanced drug delivery reviews* **54 Suppl 1**, S157-163
153 WHO.(1997) (
154 Degim, I. T., Burgess, D. J. & Papadimitrakopoulos, F. (2010). Carbon nanotubes for transdermal drug delivery. *Journal of microencapsulation* **27**, 669-681
155 Ema, M., Matsuda, A., Kobayashi, N., Naya, M. & Nakanishi, J. (2011). Evaluation of dermal and eye irritation and skin sensitization due to carbon nanotubes. *Regulatory toxicology and pharmacology : RTP* **61**, 276-281
156 Kishore, A. S., Surekha, P. & Murthy, P. B. (2009). Assessment of the dermal and ocular irritation potential of multi-walled carbon nanotubes by using in vitro and in vivo methods. *Toxicol Lett* **191**, 268-274

157 Monteiro-Riviere, N. A., Nemanich, R. J., Inman, A. O., Wang, Y. Y. & Riviere, J. E. (2005). Multi-walled carbon nanotube interactions with human epidermal keratinocytes. *Toxicology letters* **155**, 377-384

158 Vakarelski, I. U., Brown, S. C., Higashitani, K. & Moudgil, B. M. (2007). Penetration of living cell membranes with fortified carbon nanotube tips. *Langmuir* **23**, 10893-10896

159 Nagai, H. *et al.* (2011). Diameter and rigidity of multiwalled carbon nanotubes are critical factors in mesothelial injury and carcinogenesis. *Proceedings of the National Academy of Sciences* **108**, E1330–E1338

160 Sanchez, V. C., Jachak, A., Hurt, R. H. & Kane, A. B. (2012). Biological interactions of graphene-family nanomaterials: an interdisciplinary review. *Chemical Research In Toxicology* **25**, 15-34

161 Schinwald, A., Murphy, F. A., Jones, A., MacNee, W. & Donaldson, K. (2012). Graphene-based nanoplatelets: a new risk to the respiratory system as a consequence of their unusual aerodynamic properties. *Acs Nano* **6**, 736-746

162 OECD. *Test No. 428: Skin Absorption: In Vitro Method*. (OECD Publishing).

163 Schafer-Korting, M. *et al.* (2006). Reconstructed human epidermis for skin absorption testing: results of the German prevalidation study. *Alternatives to laboratory animals : ATLA* **34**, 283-294

164 OECD. *Guidance Document for the Conduct of Skin Absorption Studies*. (OECD Publishing).

165 Riviere, J. E., Bowman, K. F., Monteiro-Riviere, N. A., Dix, L. P. & Carver, M. P. (1986). The isolated perfused porcine skin flap (IPPSF). I. A novel in vitro model for percutaneous absorption and cutaneous toxicology studies. *Fundamental and applied toxicology : official journal of the Society of Toxicology* **7**, 444-453

166 Saathoff, J. G., Inman, A. O., Xia, X. R., Riviere, J. E. & Monteiro-Riviere, N. A. (2011). In vitro toxicity assessment of three hydroxylated fullerenes in human skin cells. *Toxicology in vitro : an international journal published in association with BIBRA* **25**, 2105-2112

167 Shukla, R. K. *et al.* (2011). ROS-mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells. *Toxicology in vitro : an international journal published in association with BIBRA* **25**, 231-241

168 OECD. *Test No. 427: Skin Absorption: In Vivo Method*. (OECD Publishing).

169 Kiss, B. *et al.* (2008). Investigation of micronized titanium dioxide penetration in human skin xenografts and its effect on cellular functions of human skin-derived cells. *Exp Dermatol* **17**, 659-667

170 Butz, T. R., T.; Pinheiro, T.; Moretto, P.; Pallon, J.; Kiss, A.Z.; Stachura, J.; Dabros, W.; Stachura, Z.; Lekki, M.; Lekka, M.; Hunyadi, J.; Biro, T.; Sticherling., M.; Van Vaeck, L.; Van Royen, P., Surleve-Bazeille, J-E. (2007) NANODERM Final Report - Quality of Skin as a Barrier to Ultra-fine Particles. (

171 Popov, A. P., Priezzhev, A. V., Lademann, J. & Myllyla, R. (2006). Advantages of NIR radiation use for optical determination of skin horny layer thickness with embedded TiO2 nanoparticles during tape stripping procedure. *Laser Physics* **16**, 751-757

172 Nielsen, J. (2013). Comment on Tissue Differences (Personal Communication,08/05/2013, Craig A Poland).

173 Magnusson, B. M., Walters, K. A. & Roberts, M. S. (2001). Veterinary drug delivery: potential for skin penetration enhancement. *Advanced Drug Delivery Reviews* **50**, 205-227

174 Benech-Kieffer, F. *et al.* (2003). Percutaneous absorption of Mexoryl SX in human volunteers: comparison with in vitro data. *Skin pharmacology and applied skin physiology* **16**, 343-355

175 Kezic, S. & Nielsen, J. B. (2009). Absorption of chemicals through compromised skin. *International archives of occupational and environmental health* **82**, 677-688

176 Benfeldt, E. (1999). In vivo microdialysis for the investigation of drug levels in the dermis and the effect of barrier perturbation on cutaneous drug penetration. Studies in hairless rats and human subjects. *Acta dermato-venereologica. Supplementum* **206**, 1-59

177 Filon, F. L. *et al.* (2007). In vitro percutaneous absorption of silver nanoparticles. *Giornale italiano di medicina del lavoro ed ergonomia* **29**, 451-452

178 Labouta, H. I., Kraus, T., El-Khordagui, L. K. & Schneider, M. (2011). Combined multiphoton imaging-pixel analysis for semiquantitation of skin penetration of gold nanoparticles. *International journal of pharmaceutics* **413**, 279-282
179 Schinwald, A. & Donaldson, K. (2012). Use of back-scatter electron signals to visualise cell/nanowires interactions in vitro and in vivo; frustrated phagocytosis of long fibres in macrophages and compartmentalisation in mesothelial cells in vivo. *Particle and fibre toxicology* **9**, 34
180 Zhang, L. W. & Monteiro-Riviere, N. A. (2013). Use of confocal microscopy for nanoparticle drug delivery through skin. *Journal of Biomedical Optics* **18**, 061214
181 Labouta, H. I. *et al.* (2012). Depth profiling of gold nanoparticles and characterization of point spread functions in reconstructed and human skin using multiphoton microscopy. *Journal of biophotonics* **5**, 85-96
182 Labouta, H. I., Thude, S. & Schneider, M. (2013). Setup for investigating gold nanoparticle penetration through reconstructed skin and comparison to published human skin data. *Journal of Biomedical Optics* **18**, 061218
183 Gulson, B. & Wong, H. (2006). Stable isotopic tracing-a way forward for nanotechnology. *Environmental health perspectives* **114**, 1486-1488
184 Lin, L. L. *et al.* (2011). Time-correlated single photon counting for simultaneous monitoring of zinc oxide nanoparticles and NAD(P)H in intact and barrier-disrupted volunteer skin. *Pharmaceutical research* **28**, 2920-2930
185 OECD. (2011) Guidance Notes on Dermal Absorption. (OECD, Paris).
186 Bolzinger, M.-A., Brianc, S. & Chevalier, Y. (2011). Nanoparticles through the skin: managing conflicting results of inorganic and organic particles in cosmetics and pharmaceutics. *Wiley Interdisciplinary Reviews-Nanomedicine and Nanobiotechnology* **3**, 463-478
187 ten Berge, W. (2009). A simple dermal absorption model: derivation and application. *Chemosphere* **75**, 1440-1445
188 Wilschut, A., ten Berge, W. F., Robinson, P. J. & McKone, T. E. (1995). Estimating skin permeation. The validation of five mathematical skin permeation models. *Chemosphere* **30**, 1275-1296
189 Kasting, G. B. & Miller, M. A. (2006). Kinetics of finite dose absorption through skin 2: volatile compounds. *Journal of pharmaceutical sciences* **95**, 268-280
190 Kasting, G. B., Miller, M. A. & Bhatt, V. D. (2008). A spreadsheet-based method for estimating the skin disposition of volatile compounds: application to N,N-diethyl-m-toluamide (DEET). *J Occup Environ Hyg* **5**, 633-644
191 Miller, M. A. & Kasting, G. B. (2010). Toward a better understanding of pesticide dermal absorption: diffusion model analysis of parathion absorption in vitro and in vivo. *Journal of toxicology and environmental health. Part A* **73**, 284-300
192 Wang, T. F., Kasting, G. B. & Nitsche, J. M. (2007). A multiphase microscopic diffusion model for stratum corneum permeability. II. Estimation of physicochemical parameters, and application to a large permeability database. *Journal of pharmaceutical sciences* **96**, 3024-3051
193 Lee, P. H., Conradi, R. & Shanmugasundaram, V. (2010). Development of an in silico model for human skin permeation based on a Franz cell skin permeability assay. *Bioorganic & Medicinal Chemistry Letters* **20**, 69-73
194 Neely, B. J., Madihally, S. V., Robinson, R. L., Jr. & Gasem, K. A. (2009). Nonlinear quantitative structure-property relationship modeling of skin permeation coefficient. *Journal of pharmaceutical sciences* **98**, 4069-4084
195 Winkler, D. A. *et al.* (2012). Applying quantitative structure–activity relationship approaches to nanotoxicology: Current status and future potential. *Toxicology*
196 Pumera, M. & Miyahara, Y. (2009). What amount of metallic impurities in carbon nanotubes is small enough not to dominate their redox properties? *Nanoscale* **1**, 260-265
197 Schinwald, A., Chernova, T. & Donaldson, K. (2012). Use of silver nanowires to determine thresholds for fibre length-dependent pulmonary inflammation and inhibition of macrophage migration in vitro. *Particle and fibre toxicology* **9**, 47
198 Schinwald, A. *et al.* (2012). The threshold length for fiber-induced acute pleural inflammation: shedding light on the early events in asbestos-induced mesothelioma. *Toxicological sciences : an official journal of the Society of Toxicology* **128**, 461 - 470

199 Nielsen, J. B. & Nielsen, F. (2000). Dermal in vitro penetration of methiocarb, paclobutrazol, and pirimicarb. *Occupational and environmental medicine* **57**, 734-737

200 McCall, M. (2013). Cost of Dermal Absorption Study in Humans (Personal Communication,Craig A Poland).

Appendix 1: Sources Evaluated – Computational Results

ABDEL-MOTTALEB, M.M., MOULARI, B., BEDUNEAU, A., PELLEQUER, Y. and LAMPRECHT, A., 2012. Nanoparticles enhance therapeutic outcome in inflamed skin therapy. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V,* **82**(1), pp. 151-157.

ABDULKARIM, M.F., ABDULLAH, G.Z., CHITNENI, M., SALMAN, I.M., AMEER, O.Z., YAM, M.F., MAHDI, E.S., SATTAR, M.A., BASRI, M. and NOOR, A.M., 2010. Topical piroxicam *in vitro* release and *in vivo* anti-inflammatory and analgesic effects from palm oil esters-based nanocream. *International journal of nanomedicine,* **5**, pp. 915-924.

ABDULLAH, G.Z., ABDULKARIM, M.F., SALMAN, I.M., AMEER, O.Z., YAM, M.F., MUTEE, A.F., CHITNENI, M., MAHDI, E.S., BASRI, M., SATTAR, M.A. and NOOR, A.M., 2011. *In vitro* permeation and *in vivo* anti-inflammatory and analgesic properties of nanoscaled emulsions containing ibuprofen for topical delivery. *International journal of nanomedicine,* **6**, pp. 387-396.

ABE, Y., 2011. Safety studies of nanomaterials about intracellular distribution and genotoxicity. *Yakugaku zasshi : Journal of the Pharmaceutical Society of Japan,* **131**(2), pp. 215-219.

ABRAMOVIC, Z., SUSTARSIC, U., TESKAC, K., SENTJURC, M. and KRISTL, J., 2008. Influence of nanosized delivery systems with benzyl nicotinate and penetration enhancers on skin oxygenation. *International journal of pharmaceutics,* **359**(1-2), pp. 220-227.

ADACHI, K., YAMADA, N., YAMAMOTO, K., YOSHIDA, Y. and YAMAMOTO, O., 2010. *In vivo* effect of industrial titanium dioxide nanoparticles experimentally exposed to hairless rat skin. *Nanotoxicology,* **4**(3), pp. 296-306.

AGRAWAL, Y., PETKAR, K.C. and SAWANT, K.K., 2010. Development, evaluation and clinical studies of Acitretin loaded nanostructured lipid carriers for topical treatment of psoriasis. *International journal of pharmaceutics,* **401**(1-2), pp. 93-102.

AILLON, K.L., XIE, Y., EL-GENDY, N., BERKLAND, C.J. and FORREST, M.L., 2009. Effects of nanomaterial physicochemical properties on *in vivo* toxicity. *Advanced Drug Delivery Reviews,* .

AINBINDER, D., PAOLINO, D., FRESTA, M. and TOUITOU, E., 2010. Drug delivery applications with ethosomes. *Journal of biomedical nanotechnology,* **6**(5), pp. 558-568.

AKKERMANS, R.L.C. and WESCOTT, J.T., 2011. Multiscale Modeling of Human Skin. *Current Nanoscience,* **7**(5), pp. 736-742.

AL-HILLI, S.M. and WILLANDER, M., 2006. Optical properties of zinc oxide nano-particles embedded in dielectric medium for UV region: Numerical simulation. *Journal of Nanoparticle Research,* **8**(1), pp. 79-97.

ALLEMANN, E., BRASSEUR, N., BENREZZAK, O., ROUSSEAU, J., KUDREVICH, S.V., BOYLE, R.W., LEROUX, J.C., GURNY, R. and VAN LIER, J.E., 1995. PEG-coated poly(lactic acid) nanoparticles for the delivery of hexadecafluoro zinc phthalocyanine to EMT-6 mouse mammary tumours. *The Journal of pharmacy and pharmacology,* **47**(5), pp. 382-387.

ALLOUNI, Z.E., HOL, P.J., CAUQUI, M.A., GJERDET, N.R. and CIMPAN, M.R., 2012. Role of physicochemical characteristics in the uptake of TiO_2 nanoparticles by fibroblasts. *Toxicology in vitro,* **26**(3), pp. 469-479.

ALPHANDERY, E., FAURE, S., SEKSEK, O., GUYOT, F. and CHEBBI, I., 2011. Chains of magnetosomes extracted from AMB-1 magnetotactic bacteria for application in alternative magnetic field cancer therapy. *ACS nano,* **5**(8), pp. 6279-6296.

ALVAREZ-ROMAN, R., NAIK, A., KALIA, Y.N., GUY, R.H. and FESSI, H., 2004. Enhancement of topical delivery from biodegradable nanoparticles. *Pharmaceutical research*, **21**(10), pp. 1818-1825.

ALVAREZ-ROMAN, R., NAIK, A., KALIA, Y.N., GUY, R.H. and FESSI, H., 2004. Skin penetration and distribution of polymeric nanoparticles. *Journal of controlled release : official journal of the Controlled Release Society*, **99**(1), pp. 53-62.

ALVES, M.P., SCARRONE, A.L., SANTOS, M., POHLMANN, A.R. and GUTERRES, S.S., 2007. Human skin penetration and distribution of nimesulide from hydrophilic gels containing nanocarriers. *International journal of pharmaceutics*, **341**(1-2), pp. 215-220.

ARAYACHUKEAT, S., WANICHWECHARUNGRUANG, S.P. and TREE-UDOM, T., 2011. Retinyl acetate-loaded nanoparticles: dermal penetration and release of the retinyl acetate. *International journal of pharmaceutics*, **404**(1-2), pp. 281-288.

AROCKIANATHAN, P.M., SEKAR, S., KUMARAN, B. and SASTRY, T.P., 2012. Preparation, characterization and evaluation of biocomposite films containing chitosan and sago starch impregnated with silver nanoparticles. *International journal of biological macromolecules*, **50**(4), pp. 939-946.

ARZILLO, M., MANGIAPIA, G., PEZZELLA, A., HEENAN, R.K., RADULESCU, A., PADUANO, L. and D'ISCHIA, M., 2012. Eumelanin buildup on the nanoscale: aggregate growth/assembly and visible absorption development in biomimetic 5,6-dihydroxyindole polymerization. *Biomacromolecules*, **13**(8), pp. 2379-2390.

ASLAN, K., LAKOWICZ, J.R. and GEDDES, C.D., 2005. Nanogold plasmon resonance-based glucose sensing. 2. Wavelength-ratiometric resonance light scattering. *Analytical Chemistry*, **77**(7), pp. 2007-2014.

ASMATULU, E., TWOMEY, J. and OVERCASH, M., 2012. Life cycle and nano-products: end-of-life assessment. *Journal of Nanoparticle Research*, **14**(3), pp. 720.

AUFFAN, M., DECOME, L., ROSE, J., ORSIERE, T., DE MEO, M., BRIOIS, V., CHANEAC, C., OLIVI, L., BERGE-LEFRANC, J.L., BOTTA, A., WIESNER, M.R. and BOTTERO, J.Y., 2006. *In vitro* interactions between DMSA-coated maghemite nanoparticles and human fibroblasts: A physicochemical and cyto-genotoxical study. *Environmental science & technology*, .

AUFFAN, M., ROSE, J., ORSIERE, T., DE MEO, M., THILL, A., ZEYONS, O., PROUX, O., MASION, A., CHAURAND, P., SPALLA, O., BOTTA, A., WIESNER, M.R. and BOTTERO, J., 2009. CeO_2 nanoparticles induce DNA damage towards human dermal fibroblasts *in vitro*. *Nanotoxicology*, **3**(2), pp. 161-U115.

AZARBAYJANI, A.F., VENUGOPAL, J.R., RAMAKRISHNA, S., LIM, P.F., CHAN, Y.W. and CHAN, S.Y., 2010. Smart polymeric nanofibers for topical delivery of levothyroxine. *Journal of pharmacy & pharmaceutical sciences : a publication of the Canadian Society for Pharmaceutical Sciences, Societe canadienne des sciences pharmaceutiques*, **13**(3), pp. 400-410.

AZEEM, A., AHMAD, F.J. and TALEGAONKAR, S., 2009. Exploration of skin permeation mechanism of frusemide with proniosomes. *Die Pharmazie*, **64**(11), pp. 735-740.

AZEEM, A., TALEGAONKAR, S., NEGI, L.M., AHMAD, F.J., KHAR, R.K. and IQBAL, Z., 2012. Oil based nanocarrier system for transdermal delivery of ropinirole: a mechanistic, pharmacokinetic and biochemical investigation. *International journal of pharmaceutics*, **422**(1-2), pp. 436-444.

BABAEIJANDAGHI, F., SHABANI, I., SEYEDJAFARI, E., NARAGHI, Z.S., VASEI, M., HADDADI-ASL, V., HESARI, K.K. and SOLEIMANI, M., 2010. Accelerated epidermal regeneration and improved dermal reconstruction achieved by polyethersulfone nanofibers. *Tissue engineering.Part A*, **16**(11), pp. 3527-3536.

BABOOTA, S., SHAKEEL, F., AHUJA, A., ALI, J. and SHAFIQ, S., 2007. Design, development and evaluation of novel nanoemulsion formulations for transdermal potential of celecoxib. *Acta pharmaceutica (Zagreb, Croatia)*, **57**(3), pp. 315-332.

BAIK, K.Y., OGAY, V., SOH, K.S., LEE, Y.W., LEE, J.K., LEE, S.H. and KIM, Y.J., 2008. Imaging of fluorescent nanoparticles distributed in mouse skin. *Journal of the Korean Physical Society*, **53**(2), pp. 880-885.

BAL, S.M., DING, Z., KERSTEN, G.F., JISKOOT, W. and BOUWSTRA, J.A., 2010. Microneedle-based transcutaneous immunisation in mice with N-trimethyl chitosan adjuvanted diphtheria toxoid formulations. *Pharmaceutical research*, **27**(9), pp. 1837-1847.

BAL, S.M., SLUTTER, B., JISKOOT, W. and BOUWSTRA, J.A., 2011. Small is beautiful: N-trimethyl chitosan-ovalbumin conjugates for microneedle-based transcutaneous immunisation. *Vaccine*, **29**(23), pp. 4025-4032.

BAL, S.M., SLUTTER, B., VAN RIET, E., KRUITHOF, A.C., DING, Z., KERSTEN, G.F., JISKOOT, W. and BOUWSTRA, J.A., 2010. Efficient induction of immune responses through intradermal vaccination with N-trimethyl chitosan containing antigen formulations. *Journal of controlled release : official journal of the Controlled Release Society*, **142**(3), pp. 374-383.

BARBOSA-BARROS, L., BARBA, C., COCERA, M., CODERCH, L., LOPEZ-IGLESIAS, C., DE LA MAZA, A. and LOPEZ, O., 2008. Effect of bicellar systems on skin properties. *International journal of pharmaceutics*, **352**(1-2), pp. 263-272.

BARBOSA-BARROS, L., BARBA, C., RODRIGUEZ, G., COCERA, M., CODERCH, L., LOPEZ-IGLESIAS, C., DE LA MAZA, A. and LOPEZ, O., 2009. Lipid nanostructures: self-assembly and effect on skin properties. *Molecular pharmaceutics*, **6**(4), pp. 1237-1245.

BARBOSA-BARROS, L., DE LA MAZA, A., ESTELRICH, J., LINARES, A.M., FELIZ, M., WALTHER, P., PONS, R. and LOPEZ, O., 2008. Penetration and growth of DPPC/DHPC bicelles inside the SC of the skin. *Langmuir : the ACS journal of surfaces and colloids*, **24**(11), pp. 5700-5706.

BARBOSA-BARROS, L., RODRIGUEZ, G., BARBA, C., COCERA, M., RUBIO, L., ESTELRICH, J., LOPEZ-IGLESIAS, C., DE LA MAZA, A. and LOPEZ, O., 2012. Bicelles: lipid nanostructured platforms with potential dermal applications. *Small (Weinheim an der Bergstrasse, Germany)*, **8**(6), pp. 807-818.

BARICHELLO, J.M., MORISHITA, M., TAKAYAMA, K. and NAGAI, T., 1999. Absorption of insulin from pluronic F-127 gels following subcutaneous administration in rats. *International journal of pharmaceutics*, **184**(2), pp. 189-198.

BAROLI, B., 2010. Penetration of nanoparticles and nanomaterials in the skin: fiction or reality? *Journal of pharmaceutical sciences*, **99**(1), pp. 21-50.

BAROLI, B., 2010. Skin absorption and potential toxicity of nanoparticulate nanomaterials. *Journal of biomedical nanotechnology*, **6**(5), pp. 485-496.

BAROLI, B., ENNAS, M.G., LOFFREDO, F., ISOLA, M., PINNA, R. and ARTURO LOPEZ-QUINTELA, M., 2007. Penetration of metallic nanoparticles in human full-thickness skin. *Journal of Investigative Dermatology*, **127**(7), pp. 1701-1712.

BASAVARAJ, K.H., 2012. Nanotechnology in medicine and relevance to dermatology: present concepts. *Indian journal of dermatology*, **57**(3), pp. 169-174.

BASTIAN, S., BUSCH, W., KUEHNEL, D., SPRINGER, A., MEISSNER, T., HOLKE, R., SCHOLZ, S., IWE, M., POMPE, W., GELINSKY, M., POTTHOFF, A., RICHTER, V., IKONOMIDOU, C. and SCHIRMER, K., 2009. Toxicity of Tungsten Carbide and Cobalt-Doped Tungsten Carbide Nanoparticles in Mammalian Cells *in vitro*. *Environmental health perspectives*, **117**(4), pp. 530-536.

BATHEJA, P., SHEIHET, L., KOHN, J., SINGER, A.J. and MICHNIAK-KOHN, B., 2011. Topical drug delivery by a polymeric nanosphere gel: Formulation optimization and *in vitro* and *in vivo* skin distribution studies. *Journal of controlled release : official journal of the Controlled Release Society*, **149**(2), pp. 159-167.

BATTAH, S., BALARATNAM, S., CASAS, A., O'NEILL, S., EDWARDS, C., BATLLE, A., DOBBIN, P. and MACROBERT, A.J., 2007. Macromolecular delivery of 5-aminolaevulinic acid for photodynamic therapy using dendrimer conjugates. *Molecular cancer therapeutics*, **6**(3), pp. 876-885.

BATTAH, S., O'NEILL, S., EDWARDS, C., BALARATNAM, S., DOBBIN, P. and MACROBERT, A.J., 2006. Enhanced porphyrin accumulation using dendritic derivatives of 5-aminolaevulinic acid for photodynamic therapy: an *in vitro* study. *The international journal of biochemistry & cell biology*, **38**(8), pp. 1382-1392.

BEANI, J.C., 2012. Solar protection products: efficacy and risks. *Annales de Dermatologie et de Venereologie*, **139**(4), pp. 261-272.

BEHRENDT, M., SANDROS, M.G., MCKINNEY, R.A., MCDONALD, K., PRZYBYTKOWSKI, E., TABRIZIAN, M. and MAYSINGER, D., 2009. Imaging and organelle distribution of fluorescent InGaP/ZnS nanoparticles in glial cells. *Nanomedicine*, **4**(7), pp. 747-761.

BERKMAN, M.S. and YAZAN, Y., 2012. Solid lipid nanoparticles: a possible vehicle for zinc oxide and octocrylene. *Die Pharmazie*, **67**(3), pp. 202-208.

BERND, H., DE KERVILER, E., GAILLARD, S. and BONNEMAIN, B., 2009. Safety and tolerability of ultrasmall superparamagnetic iron oxide contrast agent: comprehensive analysis of a clinical development program. *Investigative radiology*, **44**(6), pp. 336-342.

BERRY, C.C., WELLS, S., CHARLES, S., AITCHISON, G. and CURTIS, A.S., 2004. Cell response to dextran-derivatised iron oxide nanoparticles post internalisation. *Biomaterials*, **25**(23), pp. 5405-5413.

BERUBE, D.M., 2008. Rhetorical gamesmanship in the nano debates over sunscreens and nanoparticles. *Journal of Nanoparticle Research*, **10**, pp. 23-37.

BETTONI, C.C., FELIPPI, C.C., DE ANDRADE, C., RAFFIN, R.P., JAGER, A., GUTERRES, S.S. and COSTAL, T.D., 2012. Isotretinoin-loaded nanocapsules: stability and cutaneous penetration by tape stripping in human and pig skin. *Journal of biomedical nanotechnology*, **8**(2), pp. 258-271.

BHALEKAR, M.R., POKHARKAR, V., MADGULKAR, A., PATIL, N. and PATIL, N., 2009. Preparation and evaluation of miconazole nitrate-loaded solid lipid nanoparticles for topical delivery. *AAPS PharmSciTech*, .

BHASKAR, K., ANBU, J., RAVICHANDIRAN, V., VENKATESWARLU, V. and RAO, Y.M., 2009. Lipid nanoparticles for transdermal delivery of flurbiprofen: formulation, *in vitro*, *ex vivo* and *in vivo* studies. *Lipids in health and disease*, **8**, pp. 6-511X-8-6.

BHASKAR, K., KRISHNA MOHAN, C., LINGAM, M., JAGAN MOHAN, S., VENKATESWARLU, V., MADHUSUDAN RAO, Y., BHASKAR, K., ANBU, J. and RAVICHANDRAN, V., 2009. Development of SLN and NLC enriched hydrogels for transdermal delivery of nitrendipine: *in vitro* and *in vivo* characteristics. *Drug development and industrial pharmacy*, **35**(1), pp. 98-113.

BHASKAR, K., KRISHNA MOHAN, C., LINGAM, M., PRABHAKAR REDDY, V., VENKATESWARLU, V. and MADHUSUDAN RAO, Y., 2008. Development of nitrendipine controlled release formulations based on SLN and NLC for topical delivery: *in vitro* and *ex vivo* characterization. *Drug development and industrial pharmacy*, **34**(7), pp. 719-725.

BHATI, I., PUNJABI, P.B. and AMETA, S.C., 2010. PHOTOCATALYTIC DEGRADATION OF FAST GREEN USING NANOSIZED CeCrO3. *Macedonian Journal of Chemistry and Chemical Engineering*, **29**(2), pp. 195-202.

BHATTACHARYYA, S.S., PAUL, S., DE, A., DAS, D., SAMADDER, A., BOUJEDAINI, N. and KHUDA-BUKHSH, A.R., 2011. Poly (lactide-co-glycolide) acid nanoencapsulation of a synthetic coumarin: cytotoxicity and bio-distribution in mice, in cancer cell line and interaction with calf thymus DNA as target. *Toxicology and applied pharmacology*, **253**(3), pp. 270-281.

BHATTACHARYYA, S.S., PAUL, S. and KHUDA-BUKHSH, A.R., 2010. Encapsulated plant extract (Gelsemium sempervirens) poly (lactide-co-glycolide) nanoparticles enhance cellular uptake and increase bioactivity *in vitro*. *Experimental biology and medicine (Maywood, N.J.)*, **235**(6), pp. 678-688.

BIANCHI, P., 2007. [Nanotechnologies: New hazards?].

BLASCHKE, T., KANKATE, L. and KRAMER, K.D., 2007. Structure and dynamics of drug-carrier systems as studied by parelectric spectroscopy. *Advanced Drug Delivery Reviews*, **59**(6), pp. 403-410.

BLUME-PEYTAVI, U. and VOGT, A., 2011. Human hair follicle: reservoir function and selective targeting. *The British journal of dermatology*, **165 Suppl 2**, pp. 13-17.

BOLZINGER, M., BRIANC, S. and CHEVALIER, Y., 2011. Nanoparticles through the skin: managing conflicting results of inorganic and organic particles in cosmetics and pharmaceutics. *Wiley Interdisciplinary Reviews-Nanomedicine and Nanobiotechnology*, **3**(5), pp. 463-478.

BOLZINGER, M., BRIANCON, S., PELLETIER, J. and CHEVALIER, Y., 2012. Penetration of drugs through skin, a complex rate-controlling membrane. *Current Opinion in Colloid & Interface Science*, **17**(3), pp. 156-165.

BOONEN, J., BAERT, B., LAMBERT, J. and DE SPIEGELEER, B., 2011. Skin penetration of silica microparticles. *Die Pharmazie*, **66**(6), pp. 463-464.

BOONME, P., JUNYAPRASERT, V.B., SUKSAWAD, N. and SONGKRO, S., 2009. Microemulsions and nanoemulsions: novel vehicles for whitening cosmeceuticals. *Journal of biomedical nanotechnology*, **5**(4), pp. 373-383.

BORM, P.J., ROBBINS, D., HAUBOLD, S., KUHLBUSCH, T., FISSAN, H., DONALDSON, K., SCHINS, R., STONE, V., KREYLING, W., LADEMANN, J., KRUTMANN, J., WARHEIT, D. and OBERDORSTER, E.,

2006. The potential risks of nanomaterials: a review carried out for ECETOC. *Particle and fibre toxicology*, **3**, pp. 11.

BOROWSKA, K., WOLOWIEC, S., RUBAJ, A., GLOWNIAK, K., SIENIAWSKA, E. and RADEJ, S., 2012. Effect of polyamidoamine dendrimer G3 and G4 on skin permeation of 8-methoxypsoralene--*in vivo* study. *International journal of pharmaceutics*, **426**(1-2), pp. 280-283.

BRAEM, C., BLASCHKE, T., PANEK-MINKIN, G., HERRMANN, W., SCHLUPP, P., PAEPENMULLER, T., MULLER-GOYMAN, C., MEHNERT, W., BITTL, R., SCHAFER-KORTING, M. and KRAMER, K.D., 2007. Interaction of drug molecules with carrier systems as studied by parelectric spectroscopy and electron spin resonance. *Journal of controlled release : official journal of the Controlled Release Society*, **119**(1), pp. 128-135.

BRANDT, O., MILDNER, M., EGGER, A.E., GROESSL, M., RIX, U., POSCH, M., KEPPLER, B.K., STRUPP, C., MUELLER, B. and STINGL, G., 2012. Nanoscalic silver possesses broad-spectrum antimicrobial activities and exhibits fewer toxicological side effects than silver sulfadiazine. *Nanomedicine*, .

BREIDENICH, J., PATRONE, J., KELLY, L., BENKOSKI, J., LE, H. and SAMPLE, J., 2009. Chemiluminescent Solid Lipid Nanoparticles (SLN) and Interactions with Intact Skin. *Biosensing Ii*, **7397**, pp. 73970L.

BRILLET, P.Y., GAZEAU, F., LUCIANI, A., BESSOUD, B., CUENOD, C.A., SIAUVE, N., PONS, J.N., POUPON, J. and CLEMENT, O., 2005. Evaluation of tumoral enhancement by superparamagnetic iron oxide particles: comparative studies with ferumoxtran and anionic iron oxide nanoparticles. *European radiology*, **15**(7), pp. 1369-1377.

BRITLAND, S., FINTER, W., CHRYSTYN, H., EAGLAND, D. and ABDELRAHIM, M.E., 2012. Droplet aerodynamics, cellular uptake, and efficacy of a nebulizable corticosteroid nanosuspension are superior to a micronized dosage form. *Biotechnology progress*, **28**(5), pp. 1152-1159.

BUSCH, W., BASTIAN, S., TRAHORSCH, U., IWE, M., KUEHNEL, D., MEISSNER, T., SPRINGER, A., GELINSKY, M., RICHTER, V., IKONOMIDOU, C., POTTHOFF, A., LEHMANN, I. and SCHIRMER, K., 2011. Internalisation of engineered nanoparticles into mammalian cells *in vitro*: influence of cell type and particle properties. *Journal of Nanoparticle Research*, **13**(1), pp. 293-310.

C, S.R., KUMAR, J., V, R., M, V. and ABRAHAM, A., 2012. Laser immunotherapy with gold nanorods causes selective killing of tumour cells. *Pharmacological research : the official journal of the Italian Pharmacological Society*, **65**(2), pp. 261-269.

CALDERILLA-FAJARDO, S.B., CAZARES-DELGADILLO, J., VILLALOBOS-GARCIA, R., QUINTANAR-GUERRERO, D., GANEM-QUINTANAR, A. and ROBLES, R., 2006. Influence of sucrose esters on the *in vivo* percutaneous penetration of octyl methoxycinnamate formulated in nanocapsules, nanoemulsion, and emulsion. *Drug development and industrial pharmacy*, **32**(1), pp. 107-113.

CAMERIN, M., MAGARAGGIA, M., SONCIN, M., JORI, G., MORENO, M., CHAMBRIER, I., COOK, M.J. and RUSSELL, D.A., 2010. The *in vivo* efficacy of phthalocyanine-nanoparticle conjugates for the photodynamic therapy of amelanotic melanoma. *European journal of cancer (Oxford, England : 1990)*, **46**(10), pp. 1910-1918.

CAMPBELL, C.S., CONTRERAS-ROJAS, L.R., DELGADO-CHARRO, M.B. and GUY, R.H., 2012. Objective assessment of nanoparticle disposition in mammalian skin after topical exposure. *Journal of controlled release : official journal of the Controlled Release Society*, **162**(1), pp. 201-207.

CAPPEL, M.J. and KREUTER, J., 1991. Effect of nanoparticles on transdermal drug delivery. *Journal of microencapsulation*, **8**(3), pp. 369-374.

CARLESSO, G., KOZLOV, E., PROKOP, A., UNUTMAZ, D. and DAVIDSON, J.M., 2005. Nanoparticulate system for efficient gene transfer into refractory cell targets. *Biomacromolecules*, **6**(3), pp. 1185-1192.

CARLOTTI, M.E., SAPINO, S., UGAZIO, E., GALLARATE, M. and MOREL, S., 2012. Resveratrol in Solid Lipid Nanoparticles. *Journal of Dispersion Science and Technology*, **33**(4), pp. 465-471.

CASTRO, G.A. and FERREIRA, L.A., 2008. Novel vesicular and particulate drug delivery systems for topical treatment of acne. *Expert opinion on drug delivery*, **5**(6), pp. 665-679.

CHABRI, F., BOURIS, K., JONES, T., BARROW, D., HANN, A., ALLENDER, C., BRAIN, K. and BIRCHALL, J., 2004. Microfabricated silicon microneedles for nonviral cutaneous gene delivery. *The British journal of dermatology*, .

CHAN-REMILLARD, S., KAPUSTKA, L. and GOUDEY, S., 2009. NANOTECHNOLOGY The Occupational Health and Safely Concerns. *Nanomaterials: Risks and Benefits*, , pp. 53-66.

CHAUDHRY, B., ASHTON, H., MUHAMED, A., YOST, M., BULL, S. and FRANKEL, D., 2009. Nanoscale viscoelastic properties of an aligned collagen scaffold. *Journal of materials science.Materials in medicine*, **20**(1), pp. 257-263.

CHEN, H., CHANG, X., DU, D., LIU, W., LIU, J., WENG, T., YANG, Y., XU, H. and YANG, X., 2006. Podophyllotoxin-loaded solid lipid nanoparticles for epidermal targeting. *Journal of controlled release : official journal of the Controlled Release Society*, **110**(2), pp. 296-306.

CHEN, H., XIAO, L., DU, D., MOU, D., XU, H. and YANG, X., 2010. A facile construction strategy of stable lipid nanoparticles for drug delivery using a hydrogel-thickened microemulsion system. *Nanotechnology*, **21**(1), pp. 015101-4484/21/1/015101. Epub 2009 Nov 30.

CHEN, H., ZHU, H., ZHENG, J., MOU, D., WAN, J., ZHANG, J., SHI, T., ZHAO, Y., XU, H. and YANG, X., 2009. Iontophoresis-driven penetration of nanovesicles through microneedle-induced skin microchannels for enhancing transdermal delivery of insulin. *Journal of controlled release : official journal of the Controlled Release Society*, **139**(1), pp. 63-72.

CHEN, X., FERNANDO, G.J., RAPHAEL, A.P., YUKIKO, S.R., FAIRMAID, E.J., PRIMIERO, C.A., FRAZER, I.H., BROWN, L.E. and KENDALL, M.A., 2012. Rapid kinetics to peak serum antibodies is achieved following influenza vaccination by dry-coated densely packed microprojections to skin. *Journal of controlled release : official journal of the Controlled Release Society*, **158**(1), pp. 78-84.

CHEN, Y., ZHOU, L., YUAN, L., ZHANG, Z.H., LIU, X. and WU, Q., 2012. Formulation, characterization, and evaluation of *in vitro* skin permeation and *in vivo* pharmacodynamics of surface-charged tripterine-loaded nanostructured lipid carriers. *International journal of nanomedicine*, **7**, pp. 3023-3032.

CHENG, J., ZHOU, Y., CHEN, B., WANG, J., XIA, G., JIN, N., DING, J., GAO, C., CHEN, G., MIAO, Y., LI, W., LIU, Z. and WANG, X., 2011. Prevention of acute graft-versus-host disease by magnetic nanoparticles of Fe(3)O(4) combined with cyclosporin A in murine models. *International journal of nanomedicine*, **6**, pp. 2183-2189.

CHENG, Y., MAN, N., XU, T., FU, R., WANG, X., WANG, X. and WEN, L., 2007. Transdermal delivery of nonsteroidal anti-inflammatory drugs mediated by polyamidoamine (PAMAM) dendrimers. *Journal of pharmaceutical sciences*, **96**(3), pp. 595-602.

CHEN-YU, G., CHUN-FEN, Y., QI-LU, L., QI, T., YAN-WEI, X., WEI-NA, L. and GUANG-XI, Z., 2012. Development of a quercetin-loaded nanostructured lipid carrier formulation for topical delivery. *International journal of pharmaceutics*, **430**(1-2), pp. 292-298.

CHIEH, J.J., TSENG, W.K., HORNG, H.E., HONG, C.Y., YANG, H.C. and WU, C.C., 2011. *In vivo* and real-time measurement of magnetic nanoparticles distribution in animals by scanning SQUID biosusceptometry for biomedicine study. *IEEE transactions on bio-medical engineering*, **58**(10), pp. 2719-2724.

CHIRIO, D., GALLARATE, M., TROTTA, M., CARLOTTI, M.E., GAUDINO, E.C. and CRAVOTTO, G., 2009. Influence of alpha- and gamma- cyclodextrin lipophilic derivatives on curcumin-loaded SLN. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, **65**(3-4), pp. 391-402.

CHOI, J.S. and YOO, H.S., 2010. Pluronic/chitosan hydrogels containing epidermal growth factor with wound-adhesive and photo-crosslinkable properties. *Journal of Biomedical Materials Research Part a*, **95A**(2), pp. 564-573.

CHOI, M.J., KIM, J.H. and MAIBACH, H.I., 2006. Topical DNA vaccination with DNA/Lipid based complex. *Current drug delivery*, **3**(1), pp. 37-45.

CHOI, W.I., LEE, J.H., KIM, J.Y., KIM, J.C., KIM, Y.H. and TAE, G., 2012. Efficient skin permeation of soluble proteins via flexible and functional nano-carrier. *Journal of controlled release : official journal of the Controlled Release Society*, **157**(2), pp. 272-278.

CHOKSI, A.N., POONAWALLA, T. and WILKERSON, M.G., 2010. Nanoparticles: a closer look at their dermal effects. *Journal of drugs in dermatology : JDD*, **9**(5), pp. 475-481.

CHOURASIA, R. and JAIN, S.K., 2009. Drug targeting through pilosebaceous route. *Current Drug Targets*, **10**(10), pp. 950-967.

CHRISTENSEN, F.M., JOHNSTON, H.J., STONE, V., AITKEN, R.J., HANKIN, S., PETERS, S. and ASCHBERGER, K., 2011. Nano-TiO(2)--feasibility and challenges for human health risk assessment based on open literature. *Nanotoxicology*, **5**(2), pp. 110-124.

COHEN, D., SOROKA, Y., MA'OR, Z., ORON, M., PORTUGAL-COHEN, M., BREGEGERE, F.M., BERHANU, D., VALSAMI-JONES, E., HAI, N. and MILNER, Y., 2013. Evaluation of topically applied copper(II) oxide nanoparticle cytotoxicity in human skin organ culture. *Toxicology in vitro : an international journal published in association with BIBRA*, **27**(1), pp. 292-298.

COLONNA, C., CONTI, B., PERUGINI, P., PAVANETTO, F., MODENA, T., DORATI, R., IADAROLA, P. and GENTA, I., 2008. *Ex vivo* evaluation of prolidase loaded chitosan nanoparticles for the enzyme replacement therapy. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, **70**(1), pp. 58-65.

CONTADO, C. and PAGNONI, A., 2012. A new strategy for pressed powder eye shadow analysis: allergenic metal ion content and particle size distribution. *The Science of the total environment*, **432**, pp. 173-179.

CORTESI, R., RAVANI, L., MENEGATTI, E., DRECHSLER, M. and ESPOSITO, E., 2011. Colloidal Dispersions for the Delivery of Acyclovir: A Comparative Study. *Indian Journal of Pharmaceutical Sciences*, **73**(6), pp. 687-U136.

COULMAN, S.A., ANSTEY, A., GATELEY, C., MORRISSEY, A., MCLOUGHLIN, P., ALLENDER, C. and BIRCHALL, J.C., 2009. Microneedle mediated delivery of nanoparticles into human skin. *International journal of pharmaceutics*, **366**(1-2), pp. 190-200.

CROSERA, M., BOVENZI, M., MAINA, G., ADAMI, G., ZANETTE, C., FLORIO, C. and FILON LARESE, F., 2009. Nanoparticle dermal absorption and toxicity: a review of the literature. *International archives of occupational and environmental health*, **82**(9), pp. 1043-1055.

CROSS, S.E., INNES, B., ROBERTS, M.S., TSUZUKI, T., ROBERTSON, T.A. and MCCORMICK, P., 2007. Human skin penetration of sunscreen nanoparticles: in-vitro assessment of a novel micronized zinc oxide formulation. *Skin pharmacology and physiology*, **20**(3), pp. 148-154.

CURRY, A.C., CROW, M. and WAX, A., 2008. Molecular imaging of epidermal growth factor receptor in live cells with refractive index sensitivity using dark-field microspectroscopy and immunotargeted nanoparticles. *Journal of Biomedical Optics*, **13**(1), pp. 014022.

CURTIS, J., GREENBERG, M., KESTER, J., PHILLIPS, S. and KRIEGER, G., 2006. Nanotechnology and nanotoxicology: a primer for clinicians. *Toxicological reviews*, **25**(4), pp. 245-260.

D'ALBA, L., SARANATHAN, V., CLARKE, J.A., VINTHER, J.A., PRUM, R.O. and SHAWKEY, M.D., 2011. Colour-producing beta-keratin nanofibres in blue penguin (Eudyptula minor) feathers. *Biology letters*, **7**(4), pp. 543-546.

DANSCHER, G. and LOCHT, L.J., 2010. *In vivo* liberation of silver ions from metallic silver surfaces. *Histochemistry and cell biology*, **133**(3), pp. 359-366.

DARVIN, M.E., KONIG, K., KELLNER-HOEFER, M., BREUNIG, H.G., WERNCKE, W., MEINKE, M.C., PATZELT, A., STERRY, W. and LADEMANN, J., 2012. Safety assessment by multiphoton fluorescence/second harmonic generation/hyper-Rayleigh scattering tomography of ZnO nanoparticles used in cosmetic products. *Skin pharmacology and physiology*, **25**(4), pp. 219-226.

DATE, A.A., NAIK, B. and NAGARSENKER, M.S., 2006. Novel drug delivery systems: potential in improving topical delivery of antiacne agents. *Skin Pharmacol Physiol*, .

DE BRUIN, D.M., BREMMER, R.H., KODACH, V.M., DE KINKELDER, R., VAN MARLE, J., VAN LEEUWEN, T.G. and FABER, D.J., 2010. Optical phantoms of varying geometry based on thin building blocks with controlled optical properties. *Journal of Biomedical Optics*, **15**(2), pp. 025001.

DE GOMES, A., FAUSTINO, A., LUNARDI, C.N., LUNARDI, L.O. and DA MACHADO, A.E., 2006. Evaluation of nanoparticles loaded with benzopsoralen in rat peritoneal exudate cells. *Faseb Journal*, **20**(4), pp. A101-A101.

DE VARGAS, B.A., BIDONE, J., OLIVEIRA, L.K., KOESTER, L.S., BASSANI, V.L. and TEIXEIRA, H.F., 2012. Development of topical hydrogels containing genistein-loaded nanoemulsions. *Journal of biomedical nanotechnology*, **8**(2), pp. 330-336.

DEGIM, I.T., BURGESS, D.J. and PAPADIMITRAKOPOULOS, F., 2010. Carbon nanotubes for transdermal drug delivery. *Journal of microencapsulation*, **27**(8), pp. 669-681.

DELOUISE, L.A., 2012. Applications of nanotechnology in dermatology. *The Journal of investigative dermatology*, **132**(3 Pt 2), pp. 964-975.

DENARDO, S.J., DENARDO, G.L., MIERS, L.A., NATARAJAN, A., FOREMAN, A.R., GRUETTNER, C., ADAMSON, G.N. and IVKOV, R., 2005. Development of tumor targeting bioprobes ((111)In-chimeric L6 monoclonal antibody nanoparticles) for alternating magnetic field cancer therapy. *Clinical cancer research : an official journal of the American Association for Cancer Research*, **11**(19 Pt 2), pp. 7087s-7092s.

DESAI, P., PATLOLLA, R.R. and SINGH, M., 2010. Interaction of nanoparticles and cell-penetrating peptides with skin for transdermal drug delivery. *Molecular membrane biology*, **27**(7), pp. 247-259.

DESAI, P.R., SHAH, P.P., PATLOLLA, R.R. and SINGH, M., 2012. Dermal microdialysis technique to evaluate the trafficking of surface-modified lipid nanoparticles upon topical application. *Pharmaceutical research*, **29**(9), pp. 2587-2600.

DEY, S., BAKTHAVATCHALU, V., TSENG, M.T., WU, P., FLORENCE, R.L., GRULKE, E.A., YOKEL, R.A., DHAR, S.K., YANG, H.S., CHEN, Y. and ST CLAIR, D.K., 2008. Interactions between SIRT1 and AP-1 reveal a mechanistic insight into the growth promoting properties of alumina (Al_2O_3) nanoparticles in mouse skin epithelial cells. *Carcinogenesis*, .

DHAR, S., MURAWALA, P., SHIRAS, A., POKHARKAR, V. and PRASAD, B.L.V., 2012. Gellan gum capped silver nanoparticle dispersions and hydrogels: cytotoxicity and *in vitro* diffusion studies. *Nanoscale*, **4**(2), pp. 563-567.

DIDYCHUK, C.L., EPHRAT, P., CHAMSON-REIG, A., JACQUES, S.L. and CARSON, J.J.L., 2009. Depth of photothermal conversion of gold nanorods embedded in a tissue-like phantom. *Nanotechnology*, **20**(19), pp. 195102.

DINDA, A.K., PRASHANT, C.K., KUMAR, R., MAITRA, A. and SAMIM, M., 2008. Use of gold nanorods for laser-induced photothermal therapy. *Indian Journal of Pharmacology*, **40**, pp. 234-234.

DO NASCIMENTO, D.F., SILVA, A.C., MANSUR, C.R., PRESGRAVE RDE, F., ALVES, E.N., SILVA, R.S., RICCI-JUNIOR, E., DE FREITAS, Z.M. and DOS SANTOS, E.P., 2012. Characterization and evaluation of poly(epsilon-caprolactone) nanoparticles containing 2-ethylhexyl-p-methoxycinnamate, octocrylene, and benzophenone-3 in anti-solar preparations. *Journal of nanoscience and nanotechnology*, **12**(9), pp. 7155-7166.

DODD, D.E., ANGELL, M.A. and MATTIE, D.R., 2006. *Operational Toxicology Research.* .

DOMINGUEZ-DELGADO, C.L., RODRIGUEZ-CRUZ, I.M., ESCOBAR-CHAVEZ, J.J., CALDERON-LOJERO, I.O., QUINTANAR-GUERRERO, D. and GANEM, A., 2011. Preparation and characterization of triclosan nanoparticles intended to be used for the treatment of acne. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, **79**(1), pp. 102-107.

DONNELLY, R.F., MORROW, D.I., FAY, F., SCOTT, C.J., ABDELGHANY, S., SINGH, R.R., GARLAND, M.J. and WOOLFSON, A.D., 2010. Microneedle-mediated intradermal nanoparticle delivery: Potential for enhanced local administration of hydrophobic pre-formed photosensitisers. *Photodiagnosis and photodynamic therapy*, **7**(4), pp. 222-231.

DRAELOS, Z.D., 2012. Cosmetics, diet, and the future. *Dermatologic therapy*, **25**(3), pp. 267-272.

DRAGICEVIC-CURIC, N., SCHEGLMANN, D., ALBRECHT, V. and FAHR, A., 2009. Development of different temoporfin-loaded invasomes-novel nanocarriers of temoporfin: characterization, stability and *in vitro* skin penetration studies. *Colloids and surfaces.B, Biointerfaces*, **70**(2), pp. 198-206.

DURAND, L., HABRAN, N., HENSCHEL, V. and AMIGHI, K., 2010. Encapsulation of ethylhexyl methoxycinnamate, a light-sensitive UV filter, in lipid nanoparticles. *Journal of microencapsulation*, **27**(8), pp. 714-725.

DURR, N.J., LARSON, T., SMITH, D.K., KORGEL, B.A., SOKOLOV, K. and BEN-YAKAR, A., 2007. Two-photon luminescence imaging of cancer cells using molecularly targeted gold nanorods. *Nano Letters*, **7**(4), pp. 941-945.

ELDER, A., VIDYASAGAR, S. and DELOUISE, L., 2009. Physicochemical factors that affect metal and metal oxide nanoparticle passage across epithelial barriers. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*, .

ELMOSLEMANY, R.M., ABDALLAH, O.Y., EL-KHORDAGUI, L.K. and KHALAFALLAH, N.M., 2012. Propylene glycol liposomes as a topical delivery system for miconazole nitrate: comparison with conventional liposomes. *AAPS PharmSciTech*, **13**(2), pp. 723-731.

ELNAGGAR, Y.S., EL-MASSIK, M.A. and ABDALLAH, O.Y., 2011. Fabrication, appraisal, and transdermal permeation of sildenafil citrate-loaded nanostructured lipid carriers versus solid lipid nanoparticles. *International journal of nanomedicine*, **6**, pp. 3195-3205.

ELSHAFEEY, A.H., KAMEL, A.O. and FATHALLAH, M.M., 2009. Utility of nanosized microemulsion for transdermal delivery of tolterodine tartrate: ex-vivo permeation and in-vivo pharmacokinetic studies. *Pharmaceutical research*, **26**(11), pp. 2446-2453.

EL-TONI, A.M., YIN, S. and SATO, T., 2006. Synthesis and silica coating of calcia-doped ceria/mica nanocomposite by seeded polymerization technique. *Applied Surface Science*, **252**(14), pp. 5063-5070.

EL-TONI, A.M., YIN, S. and SATO, T., 2008. UV Shielding performance enhancement of CaO doped ceria by coupling with plate-like K(0.8)Li(0.27)Ti(1.73)O(4). *Journal of Materials Science*, **43**(7), pp. 2411-2417.

EL-TONI, A.M., YIN, S. and SATO, T., 2007. Synthesis and silica coating of calcia-doped ceria/plate-like titanate (K0.8Li0.27Ti1.73O4) nanocomposite by seeded polymerization technique. *Materials Chemistry and Physics*, **103**(2-3), pp. 345-350.

ERRICO, C., GONI-DE-CERIO, F., ALDERIGHI, M., FERRI, M., SUAREZ-MERINO, B., SOROKA, Y., FRUSIC-ZLOTKIN, M. and CHIELLINI, F., 2012. Retinyl palmitate-loaded poly(lactide-co-glycolide) nanoparticles for the topical treatment of skin diseases. *Journal of Bioactive and Compatible Polymers*, **27**(6), pp. 604-620.

ESKANDAR, N.G., SIMOVIC, S. and PRESTIDGE, C.A., 2010. Mechanistic insight into the dermal delivery from nanoparticle-coated submicron O/W emulsions. *Journal of pharmaceutical sciences*, **99**(2), pp. 890-904.

ESKANDAR, N.G., SIMOVIC, S. and PRESTIDGE, C.A., 2009. Nanoparticle coated emulsions as novel dermal delivery vehicles. *Current drug delivery*, **6**(4), pp. 367-373.

ESPOSITO, E., CORTESI, R., DRECHSLER, M., PACCAMICCIO, L., MARIANI, P., CONTADO, C., STELLIN, E., MENEGATTI, E., BONINA, F. and PUGLIA, C., 2005. Cubosome dispersions as delivery systems for percutaneous administration of indomethacin. *Pharmaceutical research*, **22**(12), pp. 2163-2173.

FAIYAZUDDIN, M., AKHTAR, N., AKHTER, J., SURI, S., SHAKEEL, F., SHAFIQ, S. and MUSTAFA, G., 2010. Production, characterization, *in vitro* and *ex vivo* studies of babchi oil-encapsulated nanostructured solid lipid carriers produced by a hot aqueous titration method. *Die Pharmazie*, **65**(5), pp. 348-355.

FANG, J.Y., FANG, C.L., LIU, C.H. and SU, Y.H., 2008. Lipid nanoparticles as vehicles for topical psoralen delivery: solid lipid nanoparticles (SLN) versus nanostructured lipid carriers (NLC). *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, **70**(2), pp. 633-640.

FARBOUD, E.S., NASROLLAHI, S.A. and TABBAKHI, Z., 2011. Novel formulation and evaluation of a Q10-loaded solid lipid nanoparticle cream: *in vitro* and *in vivo* studies. *International journal of nanomedicine*, **6**, pp. 611-617.

FASOLO, D., BASSANI, V.L. and TEIXEIRA, H.F., 2009. Development of topical nanoemulsions containing quercetin and 3-O-methylquercetin. *Die Pharmazie*, **64**(11), pp. 726-730.

FATHI-AZARBAYJANI, A., QUN, L., CHAN, Y.W. and CHAN, S.Y., 2010. Novel vitamin and gold-loaded nanofiber facial mask for topical delivery. *AAPS PharmSciTech*, **11**(3), pp. 1164-1170.

FIELD, J.A., LUNA-VELASCO, A., BOITANO, S.A., SHADMAN, F., RATNER, B.D., BARNES, C. and SIERRA-ALVAREZ, R., 2011. Cytotoxicity and physicochemical properties of hafnium oxide nanoparticles. *Chemosphere*, .

FILIPE, P., SILVA, J.N., SILVA, R., CIRNE DE CASTRO, J.L., MARQUES GOMES, M., ALVES, L.C., SANTUS, R. and PINHEIRO, T., 2009. SC is an effective barrier to TiO_2 and ZnO nanoparticle percutaneous absorption. *Skin pharmacology and physiology*, **22**(5), pp. 266-275.

FILIPOWICZ, A. and WOLOWIEC, S., 2011. Solubility and *in vitro* transdermal diffusion of riboflavin assisted by PAMAM dendrimers. *International journal of pharmaceutics*, **408**(1-2), pp. 152-156.

FILON, F.L., CROSERA, M., ADAMI, G., BOVENZI, M., ROSSI, F. and MAINA, G., 2011. Human skin penetration of gold nanoparticles through intact and damaged skin. *Nanotoxicology*, **5**(4), pp. 493-501.

FILON, F.L., D'AGOSTIN, F., CROSERA, M., ADAMI, G., ROSANI, R., ROMANO, C., BOVENZI, M. and MAINA, G., 2007. [*In vitro* percutaneous absorption of silver nanoparticles]. *Giornale italiano di medicina del lavoro ed ergonomia*, .

FILON, F.L., D'AGOSTIN, F., CROSERA, M., ADAMI, G., ROSANI, R., ROMANO, C., BOVENZI, M. and MAINA, G., 2007. *In vitro* percutaneous absorption of silver nanoparticles. *Giornale italiano di medicina del lavoro ed ergonomia*, **29**(3 Suppl), pp. 451-452.

FISHER, R.S. and HO, J., 2002. Potential new methods for antiepileptic drug delivery. *CNS drugs*, **16**(9), pp. 579-593.

FRAZAO, N.F., ALBUQUERQUE, E.L., FULCO, U.L., AZEVEDO, D.L., MENDONCA, G.L.F., LIMA-NETO, P., CAETANO, E.W.S., SANTANA, J.V. and FREIRE, V.N., 2012. Four-level levodopa adsorption on C-60 fullerene for transdermal and oral administration: a computational study. *Rsc Advances*, **2**(22), pp. 8306-8322.

FREESE, C., GIBSON, M.I., KLOK, H., UNGER, R.E. and KIRKPATRICK, C.J., 2012. Size- and Coating-Dependent Uptake of Polymer-Coated Gold Nanoparticles in Primary Human Dermal Microvascular Endothelial Cells. *Biomacromolecules*, **13**(5), pp. 1533-1543.

FRUSIC-ZLOTKIN, M., SOROKA, Y., TIVONY, R., LARUSH, L., VERKHOVSKY, L., BREGEGERE, F.M., NEUMAN, R., MAGDASSI, S. and MILNER, Y., 2012. Penetration and biological effects of topically applied cyclosporin A nanoparticles in a human skin organ culture inflammatory model. *Experimental dermatology*, **21**(12), pp. 938-943.

FULLER, L.C., 2005. Podoconiosis: endemic nonfilarial elephantiasis. *Current opinion in infectious diseases*, **18**(2), pp. 119-122.

FURUKAWA, F., DOI, Y., SUGURO, M., MORITA, O., KUWAHARA, H., MASUNAGA, T., HATAKEYAMA, Y. and MORI, F., 2011. Lack of skin carcinogenicity of topically applied titanium dioxide nanoparticles in the mouse. *Food and Chemical Toxicology*, **49**(4), pp. 744-749.

GANEM-QUINTANAR, A., SILVA-ALVAREZ, M., ALVAREZ-ROMAN, R., CASAS-ALANCASTER, N., CAZARES-DELGADILLO, J. and QUINTANAR-GUERRERO, D., 2006. Design and evaluation of a self-adhesive naproxen-loaded film prepared from a nanoparticle dispersion. *Journal of nanoscience and nanotechnology*, **6**(9-10), pp. 3235-3241.

GAO CAI-JIU, XU XIAO-HONG, MO XIU-MEI, LIU WEI and ZHOU XU, 2008. *Electrospinning and mechanical characterization of HA/GE nanofibers*.

GAO, J., WANG, H.L., SHREVE, A. and IYER, R., 2010. Fullerene derivatives induce premature senescence: a new toxicity paradigm or novel biomedical applications. *Toxicology and applied pharmacology*, .

GHOUCHI ESKANDAR, N., SIMOVIC, S. and PRESTIDGE, C.A., 2009. Nanoparticle coated submicron emulsions: sustained in-vitro release and improved dermal delivery of all-trans-retinol. *Pharmaceutical research*, **26**(7), pp. 1764-1775.

GOBIN, A.M., O'NEAL, D.P., WATKINS, D.M., HALAS, N.J., DREZEK, R.A. and WEST, J.L., 2005. Near infrared laser-tissue welding using nanoshells as an exogenous absorber. *Lasers in surgery and medicine*, **37**(2), pp. 123-129.

GOEL, A., AHMAD, F.J., SINGH, R.M. and SINGH, G.N., 2010. 3-Acetyl-11-keto-beta-boswellic acid loaded-polymeric nanomicelles for topical anti-inflammatory and anti-arthritic activity. *The Journal of pharmacy and pharmacology*, **62**(2), pp. 273-278.

GOKCE, E.H., KORKMAZ, E., DELLERA, E., SANDRI, G., BONFERONI, M.C. and OZER, O., 2012. Resveratrol-loaded solid lipid nanoparticles versus nanostructured lipid carriers: evaluation of antioxidant potential for dermal applications. *International journal of nanomedicine*, **7**, pp. 1841-1850.

GOLNESHAN, A.A. and LAHONIAN, M., 2011. The effect of magnetic nanoparticle dispersion on temperature distribution in a spherical tissue in magnetic fluid hyperthermia using the lattice Boltzmann method. *International Journal of Hyperthermia*, **27**(3), pp. 266-274.

GOMES, A.J., FAUSTINO, A.S., LUNARDI, C.N., LUNARDI, L.O. and MACHADO, A.E., 2007. Evaluation of nanoparticles loaded with benzopsoralen in rat peritoneal exudate cells. *International journal of pharmaceutics*, **332**(1-2), pp. 153-160.

GONTIER, E., YNSA, M., BIRO, T., HUNYADI, J., KISS, B., GASPAR, K., PINHEIRO, T., SILVA, J., FILIPE, P., STACHURA, J., DABROS, W., REINERT, T., BUTZ, T., MORETTO, P. and SURLEVE-BAZEILLE, J., 2008. Is there penetration of titania nanoparticles in sunscreens through skin? A comparative electron and ion microscopy study. *Nanotoxicology*, **2**(4), pp. 218-231.

GONZALEZ-MIRA, E., NIKOLIC, S., GARCIA, M.L., EGEA, M.A., SOUTO, E.B. and CALPENA, A.C., 2011. Potential use of nanostructured lipid carriers for topical delivery of flurbiprofen. *Journal of pharmaceutical sciences*, **100**(1), pp. 242-251.

GOPEE, N.V., ROBERTS, D.W., WEBB, P., COZART, C.R., SIITONEN, P.H., LATENDRESSE, J.R., WARBITTON, A.R., YU, W.W., COLVIN, V.L., WALKER, N.J. and HOWARD, P.C., 2009. Quantitative determination of skin penetration of PEG-coated CdSe quantum dots in dermabraded but not intact SKH-1 hairless mouse skin. *Toxicological sciences : an official journal of the Society of Toxicology*, **111**(1), pp. 37-48.

GOPEE, N.V., ROBERTS, D.W., WEBB, P., COZART, C.R., SIITONEN, P.H., WARBRITTON, A.R., YU, W.W., COLVIN, V.L., WALKER, N.J. and HOWARD, P.C., 2007. Migration of intradermally injected quantum dots to sentinel organs in mice. *Toxicological sciences : an official journal of the Society of Toxicology*, **98**(1), pp. 249-257.

GOUTAYER, M., DUFORT, S., JOSSERAND, V., ROYERE, A., HEINRICH, E., VINET, F., BIBETTE, J., COLL, J.L. and TEXIER, I., 2010. Tumor targeting of functionalized lipid nanoparticles: assessment by *in vivo* fluorescence imaging. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, **75**(2), pp. 137-147.

GRAF, C., MEINKE, M., GAO, Q., HADAM, S., RAABE, J., STERRY, W., BLUME-PEYTAVI, U., LADEMANN, J., RUHL, E. and VOGT, A., 2009. Qualitative detection of single submicron and nanoparticles in human skin by scanning transmission x-ray microscopy. *Journal of Biomedical Optics*, **14**(2), pp. 021015.

GRATIERI, T., SCHAEFER, U.F., JING, L., GAO, M., KOSTKA, K.H., LOPEZ, R.F. and SCHNEIDER, M., 2010. Penetration of quantum dot particles through human skin. *Journal of biomedical nanotechnology*, **6**(5), pp. 586-595.

GULBAKE, A., JAIN, A., KHARE, P. and JAIN, S.K., 2010. Solid lipid nanoparticles bearing oxybenzone: in-vitro and in-vivo evaluation. *Journal of microencapsulation*, **27**(3), pp. 226-233.

GULSON, B., MCCALL, M., KORSCH, M., GOMEZ, L., CASEY, P., OYTAM, Y., TAYLOR, A., MCCULLOCH, M., TROTTER, J., KINSLEY, L. and GREENOAK, G., 2010. Small amounts of zinc from zinc oxide particles in sunscreens applied outdoors are absorbed through human skin. *Toxicological sciences : an official journal of the Society of Toxicology*, **118**(1), pp. 140-149.

GULSON, B. and WONG, H., 2006. Stable isotopic tracing-a way forward for nanotechnology. *Environmental health perspectives*, **114**(10), pp. 1486-1488.

GULSON, B., WONG, H., KORSCH, M., GOMEZ, L., CASEY, P., MCCALL, M., MCCULLOCH, M., TROTTER, J., STAUBER, J. and GREENOAK, G., 2012. Comparison of dermal absorption of zinc from different sunscreen formulations and differing UV exposure based on stable isotope tracing. *The Science of the total environment*, **420**, pp. 313-318.

GULSON, B., WONG, H., MCCALL, M., CASEY, P., TROTTER, J., MCCULLOCH, M., GREENOAK, G. and STAUBER, J., 2008. Dermal absorption of ZnO nanoparticles in sunscreen using the stable isotope approach. *Toxicology letters*, **180**, pp. S222-S222.

GUNGOR, S. and OZSOY, Y., 2012. Systemic delivery of antihypertensive drugs via skin. *Therapeutic delivery*, **3**(9), pp. 1101-1116.

GUO, J.X., PING, Q.N. and WU, T., 2000. Transdermal delivery mechanisms of lecithin nanoparticles with cyclosporin A through mice skin. *Yao xue xue bao = Acta pharmaceutica Sinica*, **35**(10), pp. 782-785.

GUPTA, A.K. and CURTIS, A.S., 2004. Surface modified superparamagnetic nanoparticles for drug delivery: interaction studies with human fibroblasts in culture. *Journal of materials science.Materials in medicine*, **15**(4), pp. 493-496.

GUPTA, M. and VYAS, S.P., 2012. Development, characterization and *in vivo* assessment of effective lipidic nanoparticles for dermal delivery of fluconazole against cutaneous candidiasis. *Chemistry and physics of lipids*, **165**(4), pp. 454-461.

GUTERRES, S.S., ALVES, M.P. and POHLMANN, A.R., 2007. Polymeric nanoparticles, nanospheres and nanocapsules, for cutaneous applications. *Drug target insights*, **2**, pp. 147-157.

HAAG, S.F., CHEN, M., PETERS, D., KECK, C.M., TASKOPARAN, B., FAHR, A., TEUTLOFF, C., BITTL, R., LADEMANN, J., SCHAFER-KORTING, M. and MEINKE, M.C., 2011. Nanostructured lipid carriers as nitroxide depot system measured by electron paramagnetic resonance spectroscopy. *International journal of pharmaceutics*, **421**(2), pp. 364-369.

HAAG, S.F., FLEIGE, E., CHEN, M., FAHR, A., TEUTLOFF, C., BITTL, R., LADEMANN, J., SCHAFER-KORTING, M., HAAG, R. and MEINKE, M.C., 2011. Skin penetration enhancement of core-multishell nanotransporters and invasomes measured by electron paramagnetic resonance spectroscopy. *International journal of pharmaceutics*, **416**(1), pp. 223-228.

HABERLAND, A., SANTOS MAIA, C., JORES, K., DURRFELD, M., MEHNERT, W., SCHIMKE, I., CHRIST, B. and SCHAFER-KORTING, M., 2003. Albumin effects on drug absorption and metabolism in reconstructed epidermis and excised pig skin. *Altex*, **20**(1), pp. 3-9.

HAFNER, A., LOVRIC, J., PEPIC, I. and FILIPOVIC-GRCIC, J., 2011. Lecithin/chitosan nanoparticles for transdermal delivery of melatonin. *Journal of microencapsulation*, **28**(8), pp. 807-815.

HAN, F., LI, S., YIN, R., SHI, X. and JIA, Q., 2008. Investigation of nanostructured lipid carriers for transdermal delivery of flurbiprofen. *Drug development and industrial pharmacy*, **34**(4), pp. 453-458.

HAN, I.H., CHOI, S.U., NAM, D.Y., PARK, Y.M., KANG, M.J., KANG, K.H., KIM, Y.M., BAE, G., OH, I.Y., PARK, J.H., YE, J.S., CHOI, Y.B., KIM, D.K., LEE, J. and CHOI, Y.W., 2010. Identification and assessment of permeability enhancing vehicles for transdermal delivery of glucosamine hydrochloride. *Archives of Pharmacal Research*, **33**(2), pp. 293-299.

HANNO, I., ANSELMI, C. and BOUCHEMAL, K., 2012. Polyamide nanocapsules and nano-emulsions containing Parsol(R) MCX and Parsol(R) 1789: *in vitro* release, *ex vivo* skin penetration and photo-stability studies. *Pharmaceutical research*, **29**(2), pp. 559-573.

HANSEN, S.F., BAUN, A., MICHELSON, E.S., KAMPER, A., BORLING, P. and STUER-LAURIDSEN, F., 2009. NANOMATERIALS IN CONSUMER PRODUCTS Categorization and Exposure Assessment. *Nanomaterials: Risks and Benefits*, , pp. 359-367.

HANSEN, S. and LEHR, C., 2012. Nanoparticles for transcutaneous vaccination. *Microbial Biotechnology*, **5**(2), pp. 156-167.

HAROUN, A.A., GAMAL-ELDEEN, A. and HARDING, D.R.K., 2009. Preparation, characterization and *in vitro* biological study of biomimetic three-dimensional gelatin-montmorillonite/cellulose scaffold for tissue engineering. *Journal of Materials Science-Materials in Medicine*, **20**(12), pp. 2527-2540.

HART, J., RIVAL, D., TERRY, N., BONNET, S., BUFFEVANT, C., PERRIER, E. and ENGELHARD-LYON, 2006. Nano-sized hybrid capsules based on cyclodextrin esters: a totally new fluidic organization for penetration enhancement of cosmetic active compounds. *Journal of cosmetic science*, **57**(2), pp. 185-186.

HASANOVIC, A., ZEHL, M., REZNICEK, G. and VALENTA, C., 2009. Chitosan-tripolyphosphate nanoparticles as a possible skin drug delivery system for aciclovir with enhanced stability. *The Journal of pharmacy and pharmacology*, **61**(12), pp. 1609-1616.

HE, Q., YIN, S. and SATO, T., 2004. Synthesis and photochemical properties of zinc-aluminum layered double hydroxide/organic UV ray absorbing molecule/silica nanocomposites. *Journal of Physics and Chemistry of Solids*, **65**(2-3), pp. 395-402.

HEBER, G.K., MARKOVIC, B. and HAYES, A., 2006. An immunohistological study of anhydrous topical ascorbic acid compositions on *ex vivo* human skin. *Journal of cosmetic dermatology*, **5**(2), pp. 150-156.

HEDBERG, Y., MIDANDER, K. and WALLINDER, I.O., 2010. Particles, sweat, and tears: a comparative study on bioaccessibility of ferrochromium alloy and stainless steel particles, the pure metals and their metal oxides, in simulated skin and eye contact. *Integrated environmental assessment and management*, **6**(3), pp. 456-468.

HIGAKI, M., KAMEYAMA, M., UDAGAWA, M., UENO, Y., YAMAGUCHI, Y., IGARASHI, R., ISHIHARA, T. and MIZUSHIMA, Y., 2006. Transdermal delivery of CaCO3-nanoparticles containing insulin. *Diabetes technology & therapeutics*, **8**(3), pp. 369-374.

HIRAI, T., YOSHIKAWA, T., NABESHI, H., YOSHIDA, T., AKASE, T., YOSHIOKA, Y., ITOH, N. and TSUTSUMI, Y., 2012. Dermal absorption of amorphous nanosilica particles after topical exposure for three days. *Die Pharmazie*, **67**(8), pp. 742-743.

HIRAI, T., YOSHIKAWA, T., NABESHI, H., YOSHIDA, T., TOCHIGI, S., ICHIHASHI, K., UJI, M., AKASE, T., NAGANO, K., ABE, Y., KAMADA, H., ITOH, N., TSUNODA, S., YOSHIOKA, Y. and TSUTSUMI, Y., 2012. Amorphous silica nanoparticles size-dependently aggravate atopic dermatitis-like skin lesions following an intradermal injection. *Part Fibre Toxicol*, .

HOELLER, S., SPERGER, A. and VALENTA, C., 2009. Lecithin based nanoemulsions: A comparative study of the influence of non-ionic surfactants and the cationic phytosphingosine on physicochemical behaviour and skin permeation. *International journal of pharmaceutics*, **370**(1-2), pp. 181-186.

HOLAN, V., CHUDICKOVA, M., TROSAN, P., SVOBODOVA, E., KRULOVA, M., KUBINOVA, S., SYKOVA, E., SIRC, J., MICHALEK, J., JUKLICKOVA, M., MUNZAROVA, M. and ZAJICOVA, A., 2011. Cyclosporine A-loaded and stem cell-seeded electrospun nanofibers for cell-based therapy and local immunosuppression. *Journal of controlled release : official journal of the Controlled Release Society*, **156**(3), pp. 406-412.

HOU, S., WANG, J. and MARTIN, C.R., 2005. Template-synthesized DNA nanotubes. *Journal of the American Chemical Society*, .

HSIA, H.C., NAIR, M.R., MINTZ, R.C. and CORBETT, S.A., 2011. The fiber diameter of synthetic bioresorbable extracellular matrix influences human fibroblast morphology and fibronectin matrix assembly. *Plastic and Reconstructive Surgery*, **127**(6), pp. 2312-2320.

HSU, S.H., CHANG, Y.B., TSAI, C.L., FU, K.Y., WANG, S.H. and TSENG, H.J., 2011. Characterization and biocompatibility of chitosan nanocomposites. *Colloids and surfaces. B, Biointerfaces*, .

HU, X., COOK, S., WANG, P., HWANG, H.M., LIU, X. and WILLIAMS, Q.L., 2010. *In vitro* evaluation of cytotoxicity of engineered carbon nanotubes in selected human cell lines. *The Science of the total environment*, **408**(8), pp. 1812-1817.

HWANG, B.J., KUMAR, S.M.S., CHEN, C., MONALISA, CHENG, M., LIU, D. and LEE, J., 2007. An investigation of structure-catalytic activity relationship for Pt-Co/C bimetallic nanoparticles toward the oxygen reduction reaction. *Journal of Physical Chemistry C*, **111**(42), pp. 15267-15276.

IKEDA, Y., HIGASHI, K., MORIBE, K. and YAMAMOTO, K., 2012. Enhanced skin permeation of piroxicam and pranoprofen induced from nanoparticles dispersed in propylene glycol aqueous solution. *Journal of Drug Delivery Science and Technology*, **22**(2), pp. 131-137.

ISABEL VAZQUEZ, M., PELAEZ, L., BENAVENTE, J., MANUEL LOPEZ-ROMERO, J., RICO, R., HIERREZUELO, J., GUILLEN, E. and ROSA LOPEZ-RAMIREZ, M., 2011. Functionalized Lipid Nanoparticles-Cellophane Hybrid Films for Molecular Delivery: Preparation, Physicochemical Characterization, and Stability. *Journal of pharmaceutical sciences*, **100**(11), pp. 4815-4822.

ITO, S., ITOGA, K., YAMATO, M., AKAMATSU, H. and OKANO, T., 2010. The co-application effects of fullerene and ascorbic acid on UV-B irradiated mouse skin. *Toxicology*, **267**(1-3), pp. 27-38.

ITO, Y., OHASHI, Y., SHIROYAMA, K., SUGIOKA, N. and TAKADA, K., 2008. Self-dissolving micropiles for the percutaneous absorption of recombinant human growth hormone in rats. *Biological & pharmaceutical bulletin*, **31**(8), pp. 1631-1633.

IVKOV, R., DENARDO, S.J., MEIRS, L.A., NATARAJAN, A. and DENARDO, G.L., 2007. Development of antibody directed nanoparticles for cancer therapy - art. no. 64400I. *Thermal Treatment of Tissue: Energy Delivery and Assessment IV*, **6440**, pp. I4400-I4400.

JAIN, S., JAIN, S., KHARE, P., GULBAKE, A., BANSAL, D. and JAIN, S.K., 2010. Design and development of solid lipid nanoparticles for topical delivery of an anti-fungal agent. *Drug delivery*, **17**(6), pp. 443-451.

JAIN, S.K., CHOURASIA, M.K., MASURIHA, R., SONI, V., JAIN, A., JAIN, N.K. and GUPTA, Y., 2005. Solid lipid nanoparticles bearing flurbiprofen for transdermal delivery. *Drug delivery*, **12**(4), pp. 207-215.

JANTARAT, C., TANGTHONG, N., SONGKRO, S., MARTIN, G.P. and SUEDEE, R., 2008. S-propranolol imprinted polymer nanoparticle-on-microsphere composite porous cellulose membrane for the enantioselectively controlled delivery of racemic propranolol. *International journal of pharmaceutics*, **349**(1-2), pp. 212-225.

JENNING, V., GYSLER, A., SCHAFER-KORTING, M. and GOHLA, S.H., 2000. Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V,* **49**(3), pp. 211-218.

JENSEN, L.B., MAGNUSSSON, E., GUNNARSSON, L., VERMEHREN, C., NIELSEN, H.M. and PETERSSON, K., 2010. Corticosteroid solubility and lipid polarity control release from solid lipid nanoparticles. *International journal of pharmaceutics,* **390**(1), pp. 53-60.

JENSEN, L.B., PETERSSON, K. and NIELSEN, H.M., 2011. *In vitro* penetration properties of solid lipid nanoparticles in intact and barrier-impaired skin. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V,* **79**(1), pp. 68-75.

JEON, O., LIM, H.W., LEE, M., SONG, S.J. and KIM, B.S., 2007. Poly(L-lactide-co-glycolide) nanospheres conjugated with a nuclear localization signal for delivery of plasmid DNA. *Journal of drug targeting,* **15**(3), pp. 190-198.

JEONG, S.H., KIM, J.H., YI, S.M., LEE, J.P., KIM, J.H., SOHN, K.H., PARK, K.L., KIM, M.K. and SON, S.W., 2010. Assessment of penetration of quantum dots through *in vitro* and *in vivo* human skin using the human skin equivalent model and the tape stripping method. *Biochemical and biophysical research communications,* **394**(3), pp. 612-615.

JONAITIS, T.S., CARD, J.W. and MAGNUSON, B., 2010. Concerns regarding nano-sized titanium dioxide dermal penetration and toxicity study. *Toxicology letters,* **192**(2), pp. 268-269.

JONES, C.F. and GRAINGER, D.W., 2009. *In vitro* assessments of nanomaterial toxicity. *Advanced Drug Delivery Reviews,* .

JOO, H.H., LEE, H.Y. and KIM, J., 2009. Stability, Release Property and Skin Penetration of Stearic Acid Nanoparticles. *Molecular Crystals and Liquid Crystals,* **508**, pp. 137-149.

JOS, A., PICHARDO, S., PUERTO, M., SANCHEZ, E., GRILO, A. and CAMEAN, A.M., 2009. Cytotoxicity of carboxylic acid functionalized single wall carbon nanotubes on the human intestinal cell line Caco-2. *Toxicology in vitro : an international journal published in association with BIBRA,* **23**(8), pp. 1491-1496.

JOSHI, M. and PATRAVALE, V., 2008. Nanostructured lipid carrier (NLC) based gel of celecoxib. *International journal of pharmaceutics,* **346**(1-2), pp. 124-132.

JUNG, S., PATZELT, A., OTBERG, N., THIEDE, G., STERRY, W. and LADEMANN, J., 2009. Strategy of topical vaccination with nanoparticles. *Journal of Biomedical Optics,* **14**(2), pp. 021001.

KALARIYA, M., PADHI, B.K., CHOUGULE, M. and MISRA, A., 2005. Clobetasol propionate solid lipid nanoparticles cream for effective treatment of eczema: formulation and clinical implications. *Indian journal of experimental biology,* **43**(3), pp. 233-240.

KALE, S.N., ARORA, S., BHAYANI, K.R., PAKNIKAR, K.M., JANI, M., WAGH, U.V., KULKARNI, S.D. and OGALE, S.B., 2006. Cerium doping and stoichiometry control for biomedical use of La0.7Sr0.3MnO3 nanoparticles: microwave absorption and cytotoxicity study. *Nanomedicine : nanotechnology, biology, and medicine,* **2**(4), pp. 217-221.

KAMIYAMA, M., SOEDA, T., NAGAJIMA, S. and TANAKA, K., 2012. Development and application of high-strength polyester nanofibers. *Polymer Journal,* **44**(10), pp. 987-994.

KANG, K., JEONG, N., LEE, C. and PYO, H., 2009. Preparation and characterization of SLNs (W/O/W type) contained lipoic acid PEG ester by variation lipid. *Journal of Industrial and Engineering Chemistry,* **15**(4), pp. 529-536.

KATO, S., AOSHIMA, H., SAITOH, Y. and MIWA, N., 2009. Biological safety of LipoFullerene composed of squalane and fullerene-C_{60} upon mutagenesis, photocytotoxicity, and permeability into the human skin tissue. *Basic & clinical pharmacology & toxicology,* **104**(6), pp. 483-487.

KATO, S., KIKUCHI, R., AOSHIMA, H., SAITOH, Y. and MIWA, N., 2010. Defensive effects of fullerene-C_{60}/liposome complex against UVA-induced intracellular reactive oxygen species generation and cell death in human skin keratinocytes HaCaT, associated with intracellular uptake and extracellular excretion of fullerene-C_{60}. *Journal of photochemistry and photobiology.B, Biology,* **98**(2), pp. 144-151.

KAUR, I.P. and AGRAWAL, R., 2007. Nanotechnology: a new paradigm in cosmeceuticals. *Recent patents on drug delivery & formulation,* **1**(2), pp. 171-182.

KAUR, I.P. and KAKKAR, S., 2010. Topical delivery of antifungal agents. *Expert opinion on drug delivery*, **7**(11), pp. 1303-1327.

KAWAI, K., LARSON, B.J., ISHISE, H., CARRE, A.L., NISHIMOTO, S., LONGAKER, M. and LORENZ, H.P., 2011. Calcium-based nanoparticles accelerate skin wound healing. *PloS one*, **6**(11), pp. e27106.

KAWAI, M., HIGUCHI, H., TAKEDA, M., KOBAYASHI, Y. and OHUCHI, N., 2009. Dynamics of different-sized solid-state nanocrystals as tracers for a drug-delivery system in the interstitium of a human tumor xenograft. *Breast cancer research : BCR*, **11**(4), pp. R43.

K□CHLER, S., ABDEL-MOTTALEB, M., LAMPRECHT, A., RADOWSKI, M.R., HAAG, R. and SCH„FER-KORTING, M., 2009. Influence of nanocarrier type and size on skin delivery of hydrophilic agents. *International journal of pharmaceutics*, .

K□CHLER, S., HERRMANN, W., PANEK-MINKIN, G., BLASCHKE, T., ZOSCHKE, C., KRAMER, K.D., BITTL, R. and SCH„FER-KORTING, M., 2010. SLN for topical application in skin diseases--characterization of drug-carrier and carrier-target interactions. *International journal of pharmaceutics*, .

K□CHLER, S., RADOWSKI, M.R., BLASCHKE, T., DATHE, M., PLENDL, J., HAAG, R., SCH„FER-KORTING, M. and KRAMER, K.D., 2009. Nanoparticles for skin penetration enhancement--a comparison of a dendritic core-multishell-nanotransporter and solid lipid nanoparticles. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, .

KEZIC, S. and NIELSEN, J.B., 2009. Absorption of chemicals through compromised skin. *International archives of occupational and environmental health*, **82**(6), pp. 677-688.

KHANDAVILLI, S. and PANCHAGNULA, R., 2007. Nanoemulsions as versatile formulations for paclitaxel delivery: peroral and dermal delivery studies in rats. *The Journal of investigative dermatology*, **127**(1), pp. 154-162.

KHUDA-BUKHSH, A.R., BHATTACHARYYA, S.S., PAUL, S. and BOUJEDAINI, N., 2010. Polymeric nanoparticle encapsulation of a naturally occurring plant scopoletin and its effects on human melanoma cell A375. *Zhong xi yi jie he xue bao = Journal of Chinese integrative medicine*, **8**(9), pp. 853-862.

KIM, J., SHIM, J., KIM, Y.J., CHAR, K., SUH, K.D. and KIM, J.W., 2010. The design of polymer-based nanocarriers for effective transdermal delivery. *Macromolecular bioscience*, **10**(10), pp. 1171-1176.

KIM, J.K., LEE, J.S., JUNG, H.J., CHO, J.H., HEO, J.I. and CHANG, Y.H., 2007. Preparation and properties of collagen/modified hyaluronic acid hydrogel for biomedical application. *Journal of nanoscience and nanotechnology*, **7**(11), pp. 3852-3856.

KIM, S.T., JANG, D.J., KIM, J.H., PARK, J.Y., LIM, J.S., LEE, S.Y., LEE, K.M., LIM, S.J. and KIM, C.K., 2009. Topical administration of cyclosporin A in a solid lipid nanoparticle formulation. *Die Pharmazie*, **64**(8), pp. 510-514.

KIMURA, E., KAWANO, Y., TODO, H., IKARASHI, Y. and SUGIBAYASHI, K., 2012. Measurement of skin permeation/penetration of nanoparticles for their safety evaluation. *Biological & pharmaceutical bulletin*, **35**(9), pp. 1476-1486.

KIMURA, E., TODO, H. and SUGIBAYASHI, K., 2012. Skin penetration and safety of nanoparticles. *Yakugaku zasshi : Journal of the Pharmaceutical Society of Japan*, **132**(3), pp. 319-324.

KISIN, E.R., MURRAY, A.R., KEANE, M.J., SHI, X.C., SCHWEGLER-BERRY, D., GORELIK, O., AREPALLI, S., CASTRANOVA, V., WALLACE, W.E., KAGAN, V.E. and SHVEDOVA, A.A., 2007. Single-walled carbon nanotubes: geno- and cytotoxic effects in lung fibroblast V79 cells. *Journal of toxicology and environmental health.Part A*, **70**(24), pp. 2071-2079.

KISS, B., BIRO, T., CZIFRA, G., TOTH, B.I., KERTESZ, Z., SZIKSZAI, Z., KISS, A.Z., JUHASZ, I., ZOUBOULIS, C.C. and HUNYADI, J., 2008. Investigation of micronized titanium dioxide penetration in human skin xenografts and its effect on cellular functions of human skin-derived cells. *Experimental dermatology*, **17**(8), pp. 659-667.

KLANG, V., HABERFELD, S., HARTL, A. and VALENTA, C., 2012. Effect of gamma-cyclodextrin on the *in vitro* skin permeation of a steroidal drug from nanoemulsions: impact of experimental setup. *International journal of pharmaceutics*, **423**(2), pp. 535-542.

KLANG, V., MATSKO, N., RAUPACH, K., EL-HAGIN, N. and VALENTA, C., 2011. Development of sucrose stearate-based nanoemulsions and optimisation through gamma-cyclodextrin. *European journal of*

pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V, **79**(1), pp. 58-67.

KLANG, V., MATSKO, N., ZIMMERMANN, A.M., VOJNIKOVIC, E. and VALENTA, C., 2010. Enhancement of stability and skin permeation by sucrose stearate and cyclodextrins in progesterone nanoemulsions. *International journal of pharmaceutics,* **393**(1-2), pp. 152-160.

KOBIERSKI, S., OFORI-KWAKYE, K., MULLER, R.H. and KECK, C.M., 2009. Resveratrol nanosuspensions for dermal application--production, characterization, and physical stability. *Die Pharmazie,* **64**(11), pp. 741-747.

KOGURE, K., 2007. Development of a novel artificial gene delivery system multifunctional envelope-type nano device for gene therapy. *Yakugaku zasshi : Journal of the Pharmaceutical Society of Japan,* **127**(10), pp. 1685-1691.

KOHLI, A.K. and ALPAR, H.O., 2004. Potential use of nanoparticles for transcutaneous vaccine delivery: effect of particle size and charge. *International journal of pharmaceutics,* **275**(1-2), pp. 13-17.

KONAN-KOUAKOU, Y.N., BOCH, R., GURNY, R. and ALLEMANN, E., 2005. *In vitro* and *in vivo* activities of verteporfin-loaded nanoparticles. *Journal of controlled release : official journal of the Controlled Release Society,* **103**(1), pp. 83-91.

KONIG, K., EHLERS, A., STRACKE, F. and RIEMANN, I., 2006. *In vivo* drug screening in human skin using femtosecond laser multiphoton tomography. *Skin pharmacology and physiology,* **19**(2), pp. 78-88.

KORTING, H.C. and SCHAFER-KORTING, M., 2010. Carriers in the topical treatment of skin disease. *Handbook of Experimental Pharmacology,* **(197):435-68. doi**(197), pp. 435-468.

KOZINA, A.M., GENINA, E.A., TERENTYUK, G.S., TERENTYUK, A.G., BASHKATOV, A.N., TUCHIN, V.V. and KHLEBTSOV, B.N., 2012. The development of skin immersion clearing method for increasing of laser exposure efficiency on subcutaneous objects. *Biophotonics: Photonic Solutions for Better Health Care Iii,* **8427**, pp. 842726.

KRISHNAN, G., EDWARDS, J., CHEN, Y. and BENSON, H.A., 2010. Enhanced skin permeation of naltrexone by pulsed electromagnetic fields in human skin *in vitro*. *Journal of pharmaceutical sciences,* **99**(6), pp. 2724-2731.

KRISTL, J., TESKAC, K. and GRABNAR, P.A., 2010. Current view on nanosized solid lipid carriers for drug delivery to the skin. *Journal of biomedical nanotechnology,* **6**(5), pp. 529-542.

KRSTAJIC, N., SMITH, L.E., MATCHER, S.J., CHILDS, D.T.D., BONESI, M., GREENWOOD, P.D.L., HUGUES, M., KENNEDY, K., HOPKINSON, M., GROOM, K.M., MACNEIL, S., HOGG, R.A. and SMALLWOOD, R., 2010. Quantum Dot Superluminescent Diodes for Optical Coherence Tomography: Skin Imaging. *Ieee Journal of Selected Topics in Quantum Electronics,* **16**(4), pp. 748-754.

KRZEMINSKA, S. and IRZMANSKA, E., 2011. Exposure to mineral oils at worksites and novel solutions for polymer protective materials in selected personal protective equipment. *Medycyna pracy,* **62**(4), pp. 435-443.

KRZIC, M., SENTJURC, M. and KRISTL, J., 2001. Improved skin oxygenation after benzyl nicotinate application in different carriers as measured by EPR oximetry *in vivo*. *Journal of controlled release : official journal of the Controlled Release Society,* **70**(1-2), pp. 203-211.

KUCHLER, S., HERRMANN, W., PANEK-MINKIN, G., BLASCHKE, T., ZOSCHKE, C., KRAMER, K.D., BITTL, R. and SCHAFER-KORTING, M., 2010. SLN for topical application in skin diseases--characterization of drug-carrier and carrier-target interactions. *International journal of pharmaceutics,* **390**(2), pp. 225-233.

KUCHLER, S., RADOWSKI, M.R., BLASCHKE, T., DATHE, M., PLENDL, J., HAAG, R., SCHAFER-KORTING, M. and KRAMER, K.D., 2009. Nanoparticles for skin penetration enhancement--a comparison of a dendritic core-multishell-nanotransporter and solid lipid nanoparticles. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V,* **71**(2), pp. 243-250.

KUMAR, A., AGARWAL, S.P., AHUJA, A., ALI, J., CHOUDHRY, R. and BABOOTA, S., 2011. Preparation, characterization, and *in vitro* antimicrobial assessment of nanocarrier based formulation of nadifloxacin for acne treatment. *Die Pharmazie,* **66**(2), pp. 111-114.

KUMAR, D., AQIL, M., RIZWAN, M., SULTANA, Y. and ALI, M., 2009. Investigation of a nanoemulsion as vehicle for transdermal delivery of amlodipine. *Die Pharmazie*, **64**(2), pp. 80-85.

KUNTSCHE, J., BUNJES, H., FAHR, A., PAPPINEN, S., RONKKO, S., SUHONEN, M. and URTTI, A., 2008. Interaction of lipid nanoparticles with human epidermis and an organotypic cell culture model. *International journal of pharmaceutics*, **354**(1-2), pp. 180-195.

KUO, T.R., WU, C.L., HSU, C.T., LO, W., CHIANG, S.J., LIN, S.J., DONG, C.Y. and CHEN, C.C., 2009. Chemical enhancer induced changes in the mechanisms of transdermal delivery of zinc oxide nanoparticles. *Biomaterials*, **30**(16), pp. 3002-3008.

KWOK, K.W., AUFFAN, M., BADIREDDY, A.R., NELSON, C.M., WIESNER, M.R., CHILKOTI, A., LIU, J., MARINAKOS, S.M. and HINTON, D.E., 2012. Uptake of silver nanoparticles and toxicity to early life stages of Japanese medaka (Oryzias latipes): effect of coating materials. *Aquatic Toxicology (Amsterdam, Netherlands)*, **120-121**, pp. 59-66.

KWON, M.C., CHOI, W.Y., SEO, Y.C., KIM, J.S., YOON, C.S., LIM, H.W., KIM, H.S., AHN, J. and LEE, H.Y., 2012. Enhancement of the skin-protective activities of Centella asiatica L. Urban by a nano-encapsulation process. *Journal of Biotechnology*, **157**(1), pp. 100-106.

KWON, T.K. and KIM, J.C., 2010. *In vitro* skin permeation of monoolein nanoparticles containing hydroxypropyl beta-cyclodextrin/minoxidil complex. *International journal of pharmaceutics*, **392**(1-2), pp. 268-273.

L"W, K., KNOBLOCH, T., WAGNER, S., WIEHE, A., ENGEL, A., LANGER, K. and VON BRIESEN, H., 2011. Comparison of intracellular accumulation and cytotoxicity of free mTHPC and mTHPC-loaded PLGA nanoparticles in human colon carcinoma cells. *Nanotechnology*, .

LABOUTA, H.I., EL-KHORDAGUI, L.K., KRAUS, T. and SCHNEIDER, M., 2011. Mechanism and determinants of nanoparticle penetration through human skin. *Nanoscale*, **3**(12), pp. 4989-4999.

LABOUTA, H.I., EL-KHORDAGUI, L.K. and SCHNEIDER, M., 2012. Could chemical enhancement of gold nanoparticle penetration be extrapolated from established approaches for drug permeation? *Skin pharmacology and physiology*, **25**(4), pp. 208-218.

LABOUTA, H.I., HAMPEL, M., THUDE, S., REUTLINGER, K., KOSTKA, K.H. and SCHNEIDER, M., 2012. Depth profiling of gold nanoparticles and characterization of point spread functions in reconstructed and human skin using multiphoton microscopy. *Journal of biophotonics*, **5**(1), pp. 85-96.

LABOUTA, H.I., KRAUS, T., EL-KHORDAGUI, L.K. and SCHNEIDER, M., 2011. Combined multiphoton imaging-pixel analysis for semiquantitation of skin penetration of gold nanoparticles. *International journal of pharmaceutics*, **413**(1-2), pp. 279-282.

LABOUTA, H.I., LIU, D.C., LIN, L.L., BUTLER, M.K., GRICE, J.E., RAPHAEL, A.P., KRAUS, T., EL-KHORDAGUI, L.K., SOYER, H.P., ROBERTS, M.S., SCHNEIDER, M. and PROW, T.W., 2011. Gold nanoparticle penetration and reduced metabolism in human skin by toluene. *Pharmaceutical research*, **28**(11), pp. 2931-2944.

LABOUTA, H.I. and SCHNEIDER, M., 2013. Interaction of inorganic nanoparticles with the skin barrier: current status and critical review. *Nanomedicine : nanotechnology, biology, and medicine*, **9**(1), pp. 39-54.

LABOUTA, H.I., THUDE, S. and SCHNEIDER, M., 2013. Setup for investigating gold nanoparticle penetration through reconstructed skin and comparison to published human skin data. *Journal of Biomedical Optics*, **18**(6), pp. 061218.

LADEMANN, J., KNORR, F., RICHTER, H., BLUME-PEYTAVI, U., VOGT, A., ANTONIOU, C., STERRY, W. and PATZELT, A., 2008. Hair follicles--an efficient storage and penetration pathway for topically applied substances. Summary of recent results obtained at the Center of Experimental and Applied Cutaneous Physiology, Charite -Universitatsmedizin Berlin, Germany. *Skin pharmacology and physiology*, **21**(3), pp. 150-155.

LADEMANN, J., MEINKE, M., STERRY, W. and PATZELT, A., 2009. How safe are nanoparticles? *Hautarzt*, **60**(4), pp. 305-309.

LADEMANN, J., PATZELT, A., SCHANZER, S., RICHTER, H., GROSS, I., MENTING, K.H., FRAZIER, L., STERRY, W. and ANTONIOU, C., 2011. Decontamination of the skin with absorbing materials. *Skin pharmacology and physiology*, **24**(2), pp. 87-92.

LADEMANN, J., RICHTER, H., SCHAEFER, U.F., BLUME-PEYTAVI, U., TEICHMANN, A., OTBERG, N. and STERRY, W., 2006. Hair follicles - a long-term reservoir for drug delivery. *Skin pharmacology and physiology*, **19**(4), pp. 232-236.

LADEMANN, J., RICHTER, H., SCHANZER, S., KNORR, F., MEINKE, M., STERRY, W. and PATZELT, A., 2011. Penetration and storage of particles in human skin: perspectives and safety aspects. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, **77**(3), pp. 465-468.

LADEMANN, J., RICHTER, H., TEICHMANN, A., OTBERG, N., BLUME-PEYTAVI, U., LUENGO, J., WEISS, B., SCHAEFER, U.F., LEHR, C.M., WEPF, R. and STERRY, W., 2007. Nanoparticles--an efficient carrier for drug delivery into the hair follicles. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, **66**(2), pp. 159-164.

LANDSIEDEL, R., FABIAN, E., MA-HOCK, L., VAN RAVENZWAAY, B., WOHLLEBEN, W., WIENCH, K. and OESCH, F., 2012. Toxico-/biokinetics of nanomaterials. *Archives of Toxicology*, **86**(7), pp. 1021-1060.

LANE, M.E., 2011. Nanoparticles and the skin--applications and limitations. *Journal of microencapsulation*, **28**(8), pp. 709-716.

LARESE FILON, F., CROSERA, M., TIMEUS, E., ADAMI, G., BOVENZI, M., PONTI, J. and MAINA, G., 2013. Human skin penetration of cobalt nanoparticles through intact and damaged skin. *Toxicology in vitro : an international journal published in association with BIBRA*, **27**(1), pp. 121-127.

LARESE, F.F., D'AGOSTIN, F., CROSERA, M., ADAMI, G., RENZI, N., BOVENZI, M. and MAINA, G., 2009. Human skin penetration of silver nanoparticles through intact and damaged skin. *Toxicology*, **255**(1-2), pp. 33-37.

LARIOS-RODRIGUEZ, E., RANGEL-AYON, C., CASTILLO, S.J., ZAVALA, G. and HERRERA-URBINA, R., 2011. Bio-synthesis of gold nanoparticles by human epithelial cells, *in vivo*. *Nanotechnology*, **22**(35), pp. 355601-4484/22/35/355601. Epub 2011 Aug 5.

LEAVENS, T.L., MONTEIRO-RIVIERE, N.A., INMAN, A.O., BROOKS, J.D., OLDENBURG, S.J. and RIVIERE, J.E., 2012. *In vitro* biodistribution of silver nanoparticles in isolated perfused porcine skin flaps. *Journal of applied toxicology : JAT*, **32**(11), pp. 913-919.

LEAVENS, T.L., XIA, X.R., LEE, H.A., MONTEIRO-RIVIERE, N.A., BROOKS, J.D. and RIVIERE, J.E., 2010. Evaluation of perfused porcine skin as a model system to quantitate tissue distribution of fullerene nanoparticles. *Toxicology letters*, **197**(1), pp. 1-6.

LEE, H.A., IMRAN, M., MONTEIRO-RIVIERE, N.A., COLVIN, V.L., YU, W.W. and RIVIERE, J.E., 2007. Biodistribution of quantum dot nanoparticles in perfused skin: evidence of coating dependency and periodicity in arterial extraction. *Nano letters*, **7**(9), pp. 2865-2870.

LEE, H.Y., JEONG, Y.I. and CHOI, K.C., 2011. Hair dye-incorporated poly-gamma-glutamic acid/glycol chitosan nanoparticles based on ion-complex formation. *International journal of nanomedicine*, **6**, pp. 2879-2888.

LEE, J.H., SHIN, Y.C., JIN, O.S., LEE, E.J., HAN, D., KANG, S.H., HONG, S.W., AHN, J.Y. and KIM, S.H., 2012. Cytotoxicity evaluations of pristine graphene and carbon nanotubes in fibroblastic cells. *Journal of the Korean Physical Society*, **61**(6), pp. 873-877.

LEE, O., JEONG, S.H., SHIN, W.U., LEE, G., OH, C. and SON, S.W., 2013. Influence of surface charge of gold nanorods on skin penetration. *Skin research and technology : official journal of International Society for Bioengineering and the Skin (ISBS) [and] International Society for Digital Imaging of Skin (ISDIS) [and] International Society for Skin Imaging (ISSI)*, .

LEE, P.W., PENG, S.F., SU, C.J., MI, F.L., CHEN, H.L., WEI, M.C., LIN, H.J. and SUNG, H.W., 2008. The use of biodegradable polymeric nanoparticles in combination with a low-pressure gene gun for transdermal DNA delivery. *Biomaterials*, **29**(6), pp. 742-751.

LEE, S.E., CHOI, K.J., MENON, G.K., KIM, H.J., CHOI, E.H., AHN, S.K. and LEE, S.H., 2010. Penetration pathways induced by low-frequency sonophoresis with physical and chemical enhancers: iron oxide nanoparticles versus lanthanum nitrates. *The Journal of investigative dermatology*, **130**(4), pp. 1063-1072.

LEE, W.R., SHEN, S.C., AL-SUWAYEH, S.A., YANG, H.H., LI, Y.C. and FANG, J.Y., 2012. Skin Permeation of Small-Molecule Drugs, Macromolecules, and Nanoparticles Mediated by a Fractional Carbon Dioxide Laser: The Role of Hair Follicles. *Pharmaceutical research*, .

LERTSUTTHIWONG, P. and ROJSITTHISAK, P., 2011. Chitosan-alginate nanocapsules for encapsulation of turmeric oil. *Die Pharmazie*, **66**(12), pp. 911-915.

LEUNG, K., 2004. Poly(ethylene glycol)-coated gold nanocages bioconjugated with [Nle4,d-Phe7]-alpha-melanotropin-stimulating hormone. *Molecular Imaging and Contrast Agent Database (MICAD)*. Bethesda (MD): .

LEUNG, K., 2004. Quantum dot 800-prostate-specific membrane antigen antibody J591. *Molecular Imaging and Contrast Agent Database (MICAD)*. Bethesda (MD): .

LI, B. and GE, Z.Q., 2012. Nanostructured lipid carriers improve skin permeation and chemical stability of idebenone. *AAPS PharmSciTech*, **13**(1), pp. 276-283.

LI, N., PENG, L.H., CHEN, X., NAKAGAWA, S. and GAO, J.Q., 2011. Effective transcutaneous immunization by antigen-loaded flexible liposome *in vivo*. *International journal of nanomedicine*, **6**, pp. 3241-3250.

LI, S., SUN, B., LI, X. and YUAN, X., 2008. Characterization of electrospun core/shell poly(vinyl pyrrolidone)/poly(L-lactide-co-epsilon-caprolactone) fibrous membranes and their cytocompatibility *in vitro*. *Journal of biomaterials science.Polymer edition*, **19**(2), pp. 245-258.

LI, X., ZHOU, H., YANG, L., DU, G., PAI-PANANDIKER, A.S., HUANG, X. and YAN, B., 2011. Enhancement of cell recognition *in vitro* by dual-ligand cancer targeting gold nanoparticles. *Biomaterials*, **32**(10), pp. 2540-2545.

LIAO, C.M., CHIANG, Y.H. and CHIO, C.P., 2009. Assessing the airborne titanium dioxide nanoparticle-related exposure hazard at workplace. *Journal of hazardous materials*, **162**(1), pp. 57-65.

LIN, C.H., FANG, C.L., AL-SUWAYEH, S.A., YANG, S.Y. and FANG, J.Y., 2011. *In vitro* and *in vivo* percutaneous absorption of seleno-L-methionine, an antioxidant agent, and other selenium species. *Acta Pharmacologica Sinica*, **32**(9), pp. 1181-1190.

LIN, L.L., GRICE, J.E., BUTLER, M.K., ZVYAGIN, A.V., BECKER, W., ROBERTSON, T.A., SOYER, H.P., ROBERTS, M.S. and PROW, T.W., 2011. Time-correlated single photon counting for simultaneous monitoring of zinc oxide nanoparticles and NAD(P)H in intact and barrier-disrupted volunteer skin. *Pharmaceutical research*, **28**(11), pp. 2920-2930.

LIN, Y.K., AL-SUWAYEH, S.A., LEU, Y.L., SHEN, F.M. and FANG, J.Y., 2012. Squalene-Containing Nanostructured Lipid Carriers Promote Percutaneous Absorption and Hair Follicle Targeting of Diphencyprone for Treating Alopecia Areata. *Pharmaceutical research*, .

LIN, Y.K., HUANG, Z.R., ZHUO, R.Z. and FANG, J.Y., 2010. Combination of calcipotriol and methotrexate in nanostructured lipid carriers for topical delivery. *International journal of nanomedicine*, **5**, pp. 117-128.

LING, M.P., LIN, W.C., LIU, C.C., HUANG, Y.S., CHUEH, M.J. and SHIH, T.S., 2012. Risk management strategy to increase the safety of workers in the nanomaterials industry. *Journal of hazardous materials*, **229-230**, pp. 83-93.

LING, T.Y., WANG, J. and PUI, D.Y.H., 2012. Numerical modeling of nanoparticle penetration through personal protective garments. *Separation and Purification Technology*, **98**, pp. 230-239.

LIU, D., GE, Y., TANG, Y., YUAN, Y., ZHANG, Q., LI, R. and XU, Q., 2010. Solid lipid nanoparticles for transdermal delivery of diclofenac sodium: preparation, characterization and *in vitro* studies. *Journal of microencapsulation*, **27**(8), pp. 726-734.

LIU, D.C., RAPHAEL, A.P., SUNDH, D., GRICE, J.E., SOYER, H.P., ROBERTS, M.S. and PROW, T.W., 2012. The Human SC Prevents Small Gold Nanoparticle Penetration and Their Potential Toxic Metabolic Consequences. *Journal of Nanomaterials*, , pp. 721706.

LIU, J., HU, W., CHEN, H., NI, Q., XU, H. and YANG, X., 2007. Isotretinoin-loaded solid lipid nanoparticles with skin targeting for topical delivery. *International journal of pharmaceutics*, **328**(2), pp. 191-195.

LIU, J., WANG, Z., LIU, F.D., KANE, A.B. and HURT, R.H., 2012. Chemical transformations of nanosilver in biological environments. *ACS nano*, **6**(11), pp. 9887-9899.

LIU, S., XU, L., ZHANG, T., REN, G. and YANG, Z., 2010. Oxidative stress and apoptosis induced by nanosized titanium dioxide in PC12 cells. *Toxicology*, **267**(1-3), pp. 172-177.

LIU, T., ZHEN, X., CHENG, H., LI, W. and HAO, B., 2009. Preparation and transdermal diffusion of flexible nanoliposomes of ginkgolide B. *Zhongguo zhong yao za zhi = Zhongguo zhongyao zazhi = China journal of Chinese materia medica*, **34**(17), pp. 2181-2184.

LIU, W., CHAURAND, P., DI GIORGIO, C., DE MEO, M., THILL, A., AUFFAN, M., MASION, A., BORSCHNECK, D., CHASPOUL, F., GALLICE, P., BOTTA, A., BOTTERO, J.Y. and ROSE, J., 2012. Influence of the Length of Imogolite-Like Nanotubes on Their Cytotoxicity and Genotoxicity toward Human Dermal Cells. *Chemical research in toxicology*, **25**(11), pp. 2513-2522.

LIU, W., HU, M., LIU, W., XUE, C., XU, H. and YANG, X., 2008. Investigation of the carbopol gel of solid lipid nanoparticles for the transdermal iontophoretic delivery of triamcinolone acetonide acetate. *International journal of pharmaceutics*, **364**(1), pp. 135-141.

LIU, X., TAO, H., YANG, K., ZHANG, S., LEE, S.T. and LIU, Z., 2011. Optimization of surface chemistry on single-walled carbon nanotubes for *in vivo* photothermal ablation of tumors. *Biomaterials*, **32**(1), pp. 144-151.

LOIRA-PASTORIZA, C., SAPIN-MINET, A., DIAB, R., GROSSIORD, J.L. and MAINCENT, P., 2012. Low molecular weight heparin gels, based on nanoparticles, for topical delivery. *International journal of pharmaceutics*, **426**(1-2), pp. 256-262.

LOMBARDI BORGIA, S., REGEHLY, M., SIVARAMAKRISHNAN, R., MEHNERT, W., KORTING, H.C., DANKER, K., RODER, B., KRAMER, K.D. and SCHAFER-KORTING, M., 2005. Lipid nanoparticles for skin penetration enhancement-correlation to drug localization within the particle matrix as determined by fluorescence and parelectric spectroscopy. *Journal of controlled release : official journal of the Controlled Release Society*, **110**(1), pp. 151-163.

LOPES, L.B., FERREIRA, D.A., DE PAULA, D., GARCIA, M.T., THOMAZINI, J.A., FANTINI, M.C. and BENTLEY, M.V., 2006. Reverse hexagonal phase nanodispersion of monoolein and oleic acid for topical delivery of peptides: *in vitro* and *in vivo* skin penetration of cyclosporin A. *Pharmaceutical research*, **23**(6), pp. 1332-1342.

LORENSON, L., DESANTIS, A., MANNATH, T., MIRZA, F. and WEEKS, B.S., 2010. The antimicrobial and phagocytic defense mechanisms of the terrestrial gastropod Lehmannia nyctelia against Escherichia coli and Staphylococcus aureus. *Medical Science Monitor*, **16**(11), pp. BR339-BR346.

LORENZ, C., TIEDE, K., TEAR, S., BOXALL, A., VON GOETZ, N. and HUNGERBUEHLER, K., 2010. Imaging and Characterization of Engineered Nanoparticles in Sunscreens by Electron Microscopy, Under Wet and Dry Conditions. *International Journal of Occupational and Environmental Health*, **16**(4), pp. 406-428.

LU, W., ZHANG, G., ZHANG, R., FLORES, L.G.,2ND, HUANG, Q., GELOVANI, J.G. and LI, C., 2010. Tumor site-specific silencing of NF-kappaB p65 by targeted hollow gold nanosphere-mediated photothermal transfection. *Cancer research*, **70**(8), pp. 3177-3188.

LUDLOW, K., 2007. One size fits all? Australian regulation of nanoparticle exposure in the workplace. *Journal of law and medicine*, **15**(1), pp. 136-152.

LUENGO, J., WEISS, B., SCHNEIDER, M., EHLERS, A., STRACKE, F., KONIG, K., KOSTKA, K.H., LEHR, C.M. and SCHAEFER, U.F., 2006. Influence of nanoencapsulation on human skin transport of flufenamic acid. *Skin pharmacology and physiology*, **19**(4), pp. 190-197.

LUPPI, B., CERCHIARA, T., BIGUCCI, F., BASILE, R. and ZECCHI, V., 2004. Polymeric nanoparticles composed of fatty acids and polyvinylalcohol for topical application of sunscreens. *The Journal of pharmacy and pharmacology*, **56**(3), pp. 407-411.

LV, Q., YU, A., XI, Y., LI, H., SONG, Z., CUI, J., CAO, F. and ZHAI, G., 2009. Development and evaluation of penciclovir-loaded solid lipid nanoparticles for topical delivery. *International journal of pharmaceutics*, **372**(1-2), pp. 191-198.

MAA, Y.F., AMERI, M., SHU, C., ZULEGER, C.L., CHE, J., OSORIO, J.E., PAYNE, L.G. and CHEN, D., 2007. Hepatitis-B surface antigen (HBsAg) powder formulation: process and stability assessment. *Current drug delivery*, **4**(1), pp. 57-67.

MADSEN, H.B., ARBOE-ANDERSEN, H.M., ROZLOSNIK, N., MADSEN, F., IFVERSEN, P., KASIMOVA, M.R. and NIELSEN, H.M., 2010. Investigation of the interaction between modified ISCOMs and SC lipid model systems. *Biochimica et biophysica acta*, .

MADSEN, H.B., IFVERSEN, P., MADSEN, F., BRODIN, B., HAUSSER, I. and NIELSEN, H.M., 2009. *In vitro* cutaneous application of ISCOMs on human skin enhances delivery of hydrophobic model compounds through the SC. *The AAPS journal*, **11**(4), pp. 728-739.

MAHDI, E.S., NOOR, A.M., SAKEENA, M.H., ABDULLAH, G.Z., ABDULKARIM, M.F. and SATTAR, M.A., 2011. Formulation and *in vitro* release evaluation of newly synthesized palm kernel oil esters-based nanoemulsion delivery system for 30% ethanolic dried extract derived from local Phyllanthus urinaria for skin antiaging. *International journal of nanomedicine*, **6**, pp. 2499-2512.

MAHE, B., VOGT, A., LIARD, C., DUFFY, D., ABADIE, V., BONDUELLE, O., BOISSONNAS, A., STERRY, W., VERRIER, B., BLUME-PEYTAVI, U. and COMBADIERE, B., 2009. Nanoparticle-based targeting of vaccine compounds to skin antigen-presenting cells by hair follicles and their transport in mice. *The Journal of investigative dermatology*, **129**(5), pp. 1156-1164.

MAIA, C.S., MEHNERT, W. and SCHAFER-KORTING, M., 2000. Solid lipid nanoparticles as drug carriers for topical glucocorticoids. *International journal of pharmaceutics*, **196**(2), pp. 165-167.

MAIA, C.S., MEHNERT, W., SCHALLER, M., KORTING, H.C., GYSLER, A., HABERLAND, A. and SCHAFER-KORTING, M., 2002. Drug targeting by solid lipid nanoparticles for dermal use. *Journal of drug targeting*, **10**(6), pp. 489-495.

MAMOT, C., RITSCHARD, R., WICKI, A., STEHLE, G., DIETERLE, T., BUBENDORF, L., HILKER, C., DEUSTER, S., HERRMANN, R. and ROCHLITZ, C., 2012. Tolerability, safety, pharmacokinetics, and efficacy of doxorubicin-loaded anti-EGFR immunoliposomes in advanced solid tumours: a phase 1 dose-escalation study. *The lancet oncology*, **13**(12), pp. 1234-1241.

MANDAWGADE, S.D. and PATRAVALE, V.B., 2008. Development of SLNs from natural lipids: application to topical delivery of tretinoin. *International journal of pharmaceutics*, **363**(1-2), pp. 132-138.

MANN, E.E., THOMPSON, L.C., SHANNAHAN, J.H. and WINGARD, C.J., 2012. Changes in cardiopulmonary function induced by nanoparticles. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*, .

MANOLOVA, V., FLACE, A., BAUER, M., SCHWARZ, K., SAUDAN, P. and BACHMANN, M.F., 2008. Nanoparticles target distinct dendritic cell populations according to their size. *European journal of immunology*, **38**(5), pp. 1404-1413.

MARCATO, P.D., CAVERZAN, J., ROSSI-BERGMANN, B., PINTO, E.F., MACHADO, D., SILVA, R.A., JUSTO, G.Z., FERREIRA, C.V. and DURAN, N., 2011. Nanostructured polymer and lipid carriers for sunscreen. Biological effects and skin permeation. *Journal of nanoscience and nanotechnology*, **11**(3), pp. 1880-1886.

MARIE-ALEXANDRINE, B., STEPHANIE, B. and YVES, C., 2011. Nanoparticles through the skin: managing conflicting results of inorganic and organic particles in cosmetics and pharmaceutics. *Wiley interdisciplinary reviews.Nanomedicine and nanobiotechnology*, .

MARK, D., 2007. Occupational exposure to nanoparticles and nanotubes. *Issues in Environmental Science and Technology*, **24**, pp. 50-80.

MATSUI, Y., SAKAI, N., TSUDA, A., TERADA, Y., TAKAOKA, M., FUJIMAKI, H. and UCHIYAMA, I., 2009. Tracking the pathway of diesel exhaust particles from the nose to the brain by X-ray florescence analysis. *Spectrochimica Acta Part B-Atomic Spectroscopy*, **64**(8), pp. 796-801.

MATTHEOLABAKIS, G., LAGOUMINTZIS, G., PANAGI, Z., PAPADIMITRIOU, E., PARTIDOS, C.D. and AVGOUSTAKIS, K., 2010. Transcutaneous delivery of a nanoencapsulated antigen: induction of immune responses. *International journal of pharmaceutics*, **385**(1-2), pp. 187-193.

MAURER-JONES, M.A., LIN, Y. and HAYNES, C.L., 2010. Functional Assessment of Metal Oxide Nanoparticle Toxicity in Immune Cells. *Acs Nano*, **4**(6), pp. 3363-3373.

MCGUIRE, M.J., SYKES, K.F., SAMLI, K.N., TIMARES, L., BARRY, M.A., STEMKE-HALE, K., TAGLIAFERRI, F., LOGAN, M., JANSA, K., TAKASHIMA, A., BROWN, K.C. and JOHNSTON, S.A., 2004. A library-selected, Langerhans cell-targeting peptide enhances an immune response. *DNA and cell biology*, **23**(11), pp. 742-752.

MCLEISH, J.A., CHICO, T.J., TAYLOR, H.B., TUCKER, C., DONALDSON, K. and BROWN, S.B., 2010. Skin exposure to micro- and nano-particles can cause haemostasis in zebrafish larvae. *Thrombosis and haemostasis*, **103**(4), pp. 797-807.

MEI, Z., CHEN, H., WENG, T., YANG, Y. and YANG, X., 2003. Solid lipid nanoparticle and microemulsion for topical delivery of triptolide. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, **56**(2), pp. 189-196.

MEI, Z., WU, Q., HU, S., LI, X. and YANG, X., 2005. Triptolide loaded solid lipid nanoparticle hydrogel for topical application. *Drug development and industrial pharmacy*, **31**(2), pp. 161-168.

MENON, J.U., KONA, S., WADAJKAR, A.S., DESAI, F., VADLA, A. and NGUYEN, K.T., 2012. Effects of surfactants on the properties of PLGA nanoparticles. *Journal of biomedical materials research.Part A*, **100**(8), pp. 1998-2005.

MICHEL, D., CHITANDA, J.M., BALOGH, R., YANG, P., SINGH, J., DAS, U., EL-ANEED, A., DIMMOCK, J., VERRALL, R. and BADEA, I., 2012. Design and evaluation of cyclodextrin-based delivery systems to incorporate poorly soluble curcumin analogs for the treatment of melanoma. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, .

MICHINAKA, Y. and MITRAGOTRI, S., 2011. Delivery of polymeric particles into skin using needle-free liquid jet injectors. *Journal of controlled release : official journal of the Controlled Release Society*, **153**(3), pp. 249-254.

MIGDAL, C., RAHAL, R., RUBOD, A., CALLEJON, S., COLOMB, E., ATRUX-TALLAU, N., HAFTEK, M., VINCENT, C., SERRES, M. and DANIELE, S., 2010. Internalisation of hybrid titanium dioxide/para-amino benzoic acid nanoparticles in human dendritic cells did not induce toxicity and changes in their functions. *Toxicology letters*, **199**(1), pp. 34-42.

MILEWSKI, M., BROGDEN, N.K. and STINCHCOMB, A.L., 2010. Current aspects of formulation efforts and pore lifetime related to microneedle treatment of skin. *Expert opinion on drug delivery*, **7**(5), pp. 617-629.

MIN, S., GAO, X., HAN, C., CHEN, Y., YANG, M., ZHU, L., ZHANG, H., LIU, L. and YAO, J., 2012. Preparation of a silk fibroin spongy wound dressing and its therapeutic efficiency in skin defects. *Journal of biomaterials science.Polymer edition*, **23**(1-4), pp. 97-110.

MIQUEL-JEANJEAN, C., CREPEL, F., RAUFAST, V., PAYRE, B., DATAS, L., BESSOU-TOUYA, S. and DUPLAN, H., 2012. Penetration study of formulated nanosized titanium dioxide in models of damaged and sun-irradiated skins. *Photochemistry and photobiology*, **88**(6), pp. 1513-1521.

MIRONAVA, T., HADJIARGYROU, M., SIMON, M., JURUKOVSKI, V. and RAFAILOVICH, M.H., 2010. Gold nanoparticles cellular toxicity and recovery: effect of size, concentration and exposure time. *Nanotoxicology*, **4**(1), pp. 120-137.

MIRONAVA, T., HADJIARGYROU, M., SIMON, M. and RAFAILOVICH, M.H., 2012. The effects of UV emission from compact fluorescent light exposure on human dermal fibroblasts and keratinocytes *in vitro*. *Photochemistry and photobiology*, **88**(6), pp. 1497-1506.

MITRI, K., SHEGOKAR, R., GOHLA, S., ANSELMI, C. and MULLER, R.H., 2011. Lipid nanocarriers for dermal delivery of lutein: preparation, characterization, stability and performance. *International journal of pharmaceutics*, **414**(1-2), pp. 267-275.

MITRI, K., SHEGOKAR, R., GOHLA, S., ANSELMI, C. and MULLER, R.H., 2011. Lutein nanocrystals as antioxidant formulation for oral and dermal delivery. *International journal of pharmaceutics*, **420**(1), pp. 141-146.

MIYAYAMA, T., ARAI, Y. and HIRANO, S., 2012. Environmental exposure to silver and its health effects. *Nihon eiseigaku zasshi.Japanese journal of hygiene*, **67**(3), pp. 383-389.

MIYAZAKI, H., KINOSHITA, M., SAITO, A., FUJIE, T., KABATA, K., HARA, E., ONO, S., TAKEOKA, S. and SAITOH, D., 2012. An ultrathin poly(L-lactic acid) nanosheet as a burn wound dressing for protection against bacterial infection. *Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society*, .

MIZUSHIMA, Y., IKOMA, T., TANAKA, J., HOSHI, K., ISHIHARA, T., OGAWA, Y. and UENO, A., 2006. Injectable porous hydroxyapatite microparticles as a new carrier for protein and lipophilic drugs. *Journal of controlled release : official journal of the Controlled Release Society*, **110**(2), pp. 260-265.

M□NSTER, U., NAKAMURA, C., HABERLAND, A., JORES, K., MEHNERT, W., RUMMEL, S., SCHALLER, M., KORTING, H.C., ZOUBOULIS, C., BLUME-PEYTAVI, U. and SCH„FER-KORTING, M., 2005. RU 58841-myristate--prodrug development for topical treatment of acne and androgenetic alopecia. *Die Pharmazie*, .

MODDARESI, M., BROWN, M.B., TAMBURIC, S. and JONES, S.A., 2010. Tocopheryl acetate disposition in porcine and human skin when administered using lipid nanocarriers. *The Journal of pharmacy and pharmacology*, **62**(6), pp. 762-769.

MODDARESI, M., BROWN, M.B., ZHAO, Y., TAMBURIC, S. and JONES, S.A., 2010. The role of vehicle-nanoparticle interactions in topical drug delivery. *International journal of pharmaceutics*, **400**(1-2), pp. 176-182.

MOGHIMI, H.R., VARSHOCHIAN, R., KOBARFARD, F. and ERFAN, M., 2010. Reduction of percutaneous absorption of toxic chemicals by dendrimers. *Cutaneous and ocular toxicology*, **29**(1), pp. 34-40.

MONTEIRO-RIVIERE, N. and CUNNINGHAM, M., 2006. Screening Methods For Assessing Skin Toxicity Of Nanomaterials. 2006, pp. 5-6.

MONTEIRO-RIVIERE, N.A., 2006. Assessing Nanomaterial Interactions In Skin. 2006, pp. 6.

MONTEIRO-RIVIERE, N.A. and INMAN, A.O., 2006. Challenges for assessing carbon nanomaterial toxicity to the skin. *Carbon*, **44**(6), pp. 1070-1078.

MONTEIRO-RIVIERE, N.A., WIENCH, K., LANDSIEDEL, R., SCHULTE, S., INMAN, A.O. and RIVIERE, J.E., 2011. Safety evaluation of sunscreen formulations containing titanium dioxide and zinc oxide nanoparticles in UVB sunburned skin: an *in vitro* and *in vivo* study. *Toxicological sciences : an official journal of the Society of Toxicology*, **123**(1), pp. 264-280.

MONTEIRO-RIVIERE, N.A., YANG, J., INMAN, A.O., RYMAN-RASMUSSEN, J., BARRON, A.R. and RIVIERE, J.E., 2006. Skin Penetration Of Fullerene Substituted Amino Acids And Their Interactions With Human Epidermal Keratinocytes. 2006, pp. 168.

MONTEIRO-RIVIERE, N.A. and ZHANG, L.W., 2009. Assessment of Quantum Dot Penetration into Skin in Different Species Under Different Mechanical Actions. *Nanomaterials: Risks and Benefits*, , pp. 43-52.

MONTEIRO-RIVIERE, N.A. and RIVIERE, J.E., 2009. Interaction of nanomaterials with skin: Aspects of absorption and biodistribution. *Nanotoxicology*, **3**(3), pp. 188-193.

MONTENEGRO, L., SINICO, C., CASTANGIA, I., CARBONE, C. and PUGLISI, G., 2012. Idebenone-loaded solid lipid nanoparticles for drug delivery to the skin: *in vitro* evaluation. *International journal of pharmaceutics*, **434**(1-2), pp. 169-174.

MORABITO, K., SHAPLEY, N.C., STEELEY, K.G. and TRIPATHI, A., 2011. Review of sunscreen and the emergence of non-conventional absorbers and their applications in ultraviolet protection. *International Journal of Cosmetic Science*, **33**(5), pp. 385-390.

MORGANTI, P. and MORGANTI, G., 2008. Chitin nanofibrils for advanced cosmeceuticals. *Clinics in dermatology*, **26**(4), pp. 334-340.

MORGEN, M., LU, G.W., DU, D., STEHLE, R., LEMBKE, F., CERVANTES, J., CIOTTI, S., HASKELL, R., SMITHEY, D., HALEY, K. and FAN, C., 2011. Targeted delivery of a poorly water-soluble compound to hair follicles using polymeric nanoparticle suspensions. *International journal of pharmaceutics*, **416**(1), pp. 314-322.

MORTENSEN, L.J., GLAZOWSKI, C.E., ZAVISLAN, J.M. and DELOUISE, L.A., 2011. Near-IR fluorescence and reflectance confocal microscopy for imaging of quantum dots in mammalian skin. *Biomedical optics express*, **2**(6), pp. 1610-1625.

MORTENSEN, L.J., JATANA, S., GELEIN, R., DEBENEDETTO, A., DE MESY BENTLEY, K.L., BECK, L., ELDER, A. and DELOUISE, L., 2012. Quantification of quantum dot murine skin penetration with UVR barrier impairment. *Nanotoxicology*, .

MORTENSEN, L.J., OBERDORSTER, G., PENTLAND, A.P. and DELOUISE, L.A., 2008. *In vivo* skin penetration of quantum dot nanoparticles in the murine model: the effect of UVR. *Nano letters*, **8**(9), pp. 2779-2787.

MORTENSEN, L.J., RAVICHANDRAN, S. and DELOUISE, L.A., 2012. The impact of UVB exposure and differentiation state of primary keratinocytes on their interaction with quantum dots. *Nanotoxicology*, .

MORTENSEN, L.J., RAVICHANDRAN, S., ZHENG, H. and DELOUISE, L.A., 2010. Progress and challenges in quantifying skin permeability to nanoparticles using a quantum dot model. *Journal of biomedical nanotechnology*, **6**(5), pp. 596-604.

MORTENSEN, L., ZHENG, H., FAULKNOR, R., DE BENEDETTO, A., BECK, L. and DELOUISE, L.A., 2009. Increased *in vivo* skin penetration of quantum dots with UVR and *in vitro* quantum dot cytotoxicity. *Colloidal Quantum Dots for Biomedical Applications Iv*, **7189**, pp. 718919.

MULLER, R.H., RADTKE, M. and WISSING, S.A., 2002. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Advanced Drug Delivery Reviews*, **54 Suppl 1**, pp. S131-55.

MUNSTER, U., NAKAMURA, C., HABERLAND, A., JORES, K., MEHNERT, W., RUMMEL, S., SCHALLER, M., KORTING, H.C., ZOUBOULIS, C., BLUME-PEYTAVI, U. and SCHAFER-KORTING, M., 2005. RU 58841-myristate--prodrug development for topical treatment of acne and androgenetic alopecia. *Die Pharmazie*, **60**(1), pp. 8-12.

MURRAY, A.R., KISIN, E., LEONARD, S.S., YOUNG, S.H., KOMMINENI, C., KAGAN, V.E., CASTRANOVA, V. and SHVEDOVA, A.A., 2009. Oxidative stress and inflammatory response in dermal toxicity of single-walled carbon nanotubes. *Toxicology*, **257**(3), pp. 161-171.

MUTALIK, S., NAYAK, U.Y., KALRA, R., KUMAR, A., KULKARNI, R.V. and PAREKH, H.S., 2012. Sonophoresis-mediated permeation and retention of peptide dendrimers across human epidermis. *Skin research and technology : official journal of International Society for Bioengineering and the Skin (ISBS) [and] International Society for Digital Imaging of Skin (ISDIS) [and] International Society for Skin Imaging (ISSI)*, **18**(1), pp. 101-107.

NABESHI, H., YOSHIKAWA, T., IMAZAWA, T., TSUNODA, S. and TSUTSUMI, Y., 2010. Safety assessment of nanomaterials using toxicokinetics and toxicoproteome analysis. *Yakugaku zasshi : Journal of the Pharmaceutical Society of Japan*, **130**(4), pp. 465-470.

NABESHI, H., YOSHIKAWA, T., MATSUYAMA, K., NAKAZATO, Y., ARIMORI, A., ISOBE, M., TOCHIGI, S., KONDOH, S., HIRAI, T., AKASE, T., YAMASHITA, T., YAMASHITA, K., YOSHIDA, T., NAGANO, K., ABE, Y., YOSHIOKA, Y., KAMADA, H., IMAZAWA, T., ITOH, N., TSUNODA, S. and TSUTSUMI, Y., 2010. Size-dependent cytotoxic effects of amorphous silica nanoparticles on Langerhans cells. *Die Pharmazie*, **65**(3), pp. 199-201.

NABESHI, H., YOSHIKAWA, T., MATSUYAMA, K., NAKAZATO, Y., MATSUO, K., ARIMORI, A., ISOBE, M., TOCHIGI, S., KONDOH, S., HIRAI, T., AKASE, T., YAMASHITA, T., YAMASHITA, K., YOSHIDA, T., NAGANO, K., ABE, Y., YOSHIOKA, Y., KAMADA, H., IMAZAWA, T., ITOH, N., NAKAGAWA, S., MAYUMI, T., TSUNODA, S. and TSUTSUMI, Y., 2011. Systemic distribution, nuclear entry and cytotoxicity of amorphous nanosilica following topical application. *Biomaterials*, **32**(11), pp. 2713-2724.

NADWORNY, P.L., LANDRY, B.K., WANG, J., TREDGET, E.E. and BURRELL, R.E., 2010. Does nanocrystalline silver have a transferable effect? *Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society*, **18**(2), pp. 254-265.

NAGANO, K., 2011. Biodistribution of nanosilica particles in pregnant mice and the potential risk on the reproductive development. *Yakugaku zasshi : Journal of the Pharmaceutical Society of Japan*, **131**(2), pp. 225-228.

NAGARAJAN, S. and ZHANG, Y., 2011. Upconversion fluorescent nanoparticles as a potential tool for in-depth imaging. *Nanotechnology*, **22**(39), pp. 395101-4484/22/39/395101. Epub 2011 Sep 2.

NAHA, P.C., BHATTACHARYA, K., TENUTA, T., DAWSON, K.A., LYNCH, I., GRACIA, A., LYNG, F.M. and BYRNE, H.J., 2010. Intracellular localisation, geno- and cytotoxic response of polyN-isopropylacrylamide (PNIPAM) nanoparticles to human keratinocyte (HaCaT) and colon cells (SW 480). *Toxicology letters*, **198**(2), pp. 134-143.

NAM, Y.S., KIM, J.W., PARK, J., SHIM, J., LEE, J.S. and HAN, S.H., 2012. Tocopheryl acetate nanoemulsions stabilized with lipid-polymer hybrid emulsifiers for effective skin delivery. *Colloids and surfaces.B, Biointerfaces*, **94**, pp. 51-57.

NASIR, A., 2009. Nanotechnology in vaccine development: a step forward. *The Journal of investigative dermatology*, **129**(5), pp. 1055-1059.

NASTERLACK, M., ZOBER, A. and OBERLINNER, C., 2008. Considerations on occupational medical surveillance in employees handling nanoparticles. *International archives of occupational and environmental health*, **81**(6), pp. 721-726.

NEDOSEKIN, D.A., SHASHKOV, E.V., GALANZHA, E.I., HENNINGS, L. and ZHAROV, V.P., 2010. Photothermal multispectral image cytometry for quantitative histology of nanoparticles and micrometastasis in intact, stained and selectively burned tissues. *Cytometry.Part A : the journal of the International Society for Analytical Cytology*, **77**(11), pp. 1049-1058.

NEIMARK, A.V., RUETSCH, S., KORNEV, K.G. and RAVIKOVITCH, P.I., 2003. Hierarchical pore structure and wetting properties of single-wall carbon nanotube fibers. *Nano Letters*, **3**(3), pp. 419-423.

NELSON, M.A., DOMANN, F.E., BOWDEN, G.T., HOOSER, S.B., FERNANDO, Q. and CARTER, D.E., 1993. Effects of acute and subchronic exposure of topically applied fullerene extracts on the mouse skin. *Toxicology and industrial health*, **9**(4), pp. 623-630.

NEWMAN, M.D., STOTLAND, M. and ELLIS, J.I., 2009. The safety of nanosized particles in titanium dioxide- and zinc oxide-based sunscreens. *Journal of the American Academy of Dermatology*, **61**(4), pp. 685-692.

NICKLAS, M., SCHATTON, W., HEINEMANN, S., HANKE, T. and KREUTER, J., 2009. Preparation and characterization of marine sponge collagen nanoparticles and employment for the transdermal delivery of 17 beta-estradiol-hemihydrate. *Drug development and industrial pharmacy*, **35**(9), pp. 1035-1042.

NIELSEN, G.D., ROURSGAARD, M., JENSEN, K.A., POULSEN, S.S. and LARSEN, S.T., 2008. *In vivo* biology and toxicology of fullerenes and their derivatives. *Basic & clinical pharmacology & toxicology*, **103**(3), pp. 197-208.

NOHYNEK, G.J., ANTIGNAC, E., RE, T. and TOUTAIN, H., 2010. Safety assessment of personal care products/cosmetics and their ingredients. *Toxicology and applied pharmacology*, **243**(2), pp. 239-259.

NOHYNEK, G.J. and DUFOUR, E.K., 2012. Nano-sized cosmetic formulations or solid nanoparticles in sunscreens: a risk to human health? *Archives of Toxicology*, **86**(7), pp. 1063-1075.

NOHYNEK, G.J., DUFOUR, E.K. and ROBERTS, M.S., 2008. Nanotechnology, cosmetics and the skin: is there a health risk? *Skin pharmacology and physiology*, **21**(3), pp. 136-149.

NOHYNEK, G.J., LADEMANN, J., RIBAUD, C. and ROBERTS, M.S., 2007. Grey goo on the skin? Nanotechnology, cosmetic and sunscreen safety. *Critical reviews in toxicology*, **37**(3), pp. 251-277.

NOSE, K., PISSUWAN, D., GOTO, M., KATAYAMA, Y. and NIIDOME, T., 2012. Gold nanorods in an oil-base formulation for transdermal treatment of type 1 diabetes in mice. *Nanoscale*, **4**(12), pp. 3776-3780.

OBEIDAT, W.M., SCHWABE, K., MULLER, R.H. and KECK, C.M., 2010. Preservation of nanostructured lipid carriers (NLC). *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, **76**(1), pp. 56-67.

OBERD"RSTER, G., MAYNARD, A., DONALDSON, K., CASTRANOVA, V., FITZPATRICK, J., AUSMAN, K., CARTER, J., KARN, B., KREYLING, W., LAI, D., OLIN, S., MONTEIRO-RIVIERE, N., WARHEIT, D., YANG, H., ILSI RESEARCH FOUNDATION/RISK SCIENCE INSTITUTE NANOMATERIAL,TOXICITY and SCREENING WORKING, G., 2005. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. *Part Fibre Toxicol*, .

OBERDORSTER, G., OBERDORSTER, E. and OBERDORSTER, J., 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environmental health perspectives*, **113**(7), pp. 823-839.

OFFORD, E.A., GAUTIER, J.C., AVANTI, O., SCALETTA, C., RUNGE, F., KRAMER, K. and APPLEGATE, L.A., 2002. Photoprotective potential of lycopene, beta-carotene, vitamin E, vitamin C and carnosic acid in UVA-irradiated human skin fibroblasts. *Free radical biology & medicine*, **32**(12), pp. 1293-1303.

OH, J.H., PARK, H.H., DO, K.Y., HAN, M., HYUN, D.H., KIM, C.G., KIM, C.H., LEE, S.S., HWANG, S.J., SHIN, S.C. and CHO, C.W., 2008. Influence of the delivery systems using a microneedle array on the permeation of a hydrophilic molecule, calcein. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, **69**(3), pp. 1040-1045.

OLAFSSON, R., BAUER, D.R., MONTILLA, L.G. and WITTE, R.S., 2010. Real-time, contrast enhanced photoacoustic imaging of cancer in a mouse window chamber. *Optics express*, **18**(18), pp. 18625-18632.

OLSZEWSKI, W.L. and TARNOK, A., 2008. Photoacoustic Listening of Cells in Lymphatics: Research Art or Novel Clinical Noninvasive Lymph Test. *Cytometry Part a*, **73A**(12), pp. 1111-1113.

O'NEILL, B.E., VO, H., ANGSTADT, M., LI, K.P., QUINN, T. and FRENKEL, V., 2009. Pulsed high intensity focused ultrasound mediated nanoparticle delivery: mechanisms and efficacy in murine muscle. *Ultrasound in medicine & biology*, **35**(3), pp. 416-424.

OSTIGUY, C., LAPOINTE, G., M,NARD, L., CLOUTIER, Y., TROTTIER, M., BOUTIN, M., ANTOUN, M. and NORMAND, C., 2006. [Nanoparticles: Current knowledge about occupational health and safety risks and prevention measures].

OZBAS-TURAN, S., AKBUGA, J. and SEZER, A.D., 2010. Topical application of antisense oligonucleotide-loaded chitosan nanoparticles to rats. *Oligonucleotides*, **20**(3), pp. 147-153.

PADOIS, K., CANTIENI, C., BERTHOLLE, V., BARDEL, C., PIROT, F. and FALSON, F., 2011. Solid lipid nanoparticles suspension versus commercial solutions for dermal delivery of minoxidil. *International journal of pharmaceutics*, **416**(1), pp. 300-304.

PALMA, S., MANZO, R., LO NOSTRO, P. and ALLEMANDI, D., 2007. Nanostructures from alkyl vitamin C derivatives (ASCn): properties and potential platform for drug delivery. *International journal of pharmaceutics*, **345**(1-2), pp. 26-34.

PAN, Z., LEE, W., SLUTSKY, L., CLARK, R.A., PERNODET, N. and RAFAILOVICH, M.H., 2009. Adverse effects of titanium dioxide nanoparticles on human dermal fibroblasts and how to protect cells. *Small (Weinheim an der Bergstrasse, Germany)*, **5**(4), pp. 511-520.

PAPAKOSTAS, D., RANCAN, F., STERRY, W., BLUME-PEYTAVI, U. and VOGT, A., 2011. Nanoparticles in dermatology. *Archives of Dermatological Research*, **303**(8), pp. 533-550.

PARDAVI-HORVATH, M., MAKEEVA, G.S. and GOLOVANOV, O.A., 2011. Spin-Wave Resonances Affected by Skin-Effect in Conducting Magnetic Nanowire Arrays at Terahertz Frequencies. *IEEE Transactions on Magnetics*, **47**(2), pp. 313-316.

PARDEIKE, J., HOMMOSS, A. and MULLER, R.H., 2009. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. *International journal of pharmaceutics*, **366**(1-2), pp. 170-184.

PARDEIKE, J., SCHMIDT, C., VOLZ, I. and MULLER, R.H., 2011. Nanostructured lipid carriers as delivery system for the phopholipase A2 inhibitors PX-18 and PX-13 for dermal application. *Die Pharmazie*, **66**(5), pp. 357-361.

PARK, J., ESTRADA, A., SCHWARTZ, J.A., DIAGARADJANE, P., KRISHNAN, S., DUNN, A.K. and TUNNELL, J.W., 2010. Intra-organ Biodistribution of Gold Nanoparticles Using Intrinsic Two-photon Induced Photoluminescence. *Lasers in surgery and medicine*, **42**(7), pp. 630-639.

PARK, J.S., YANG, H.N., WOO, D.G., JEON, S.Y. and PARK, K.H., 2013. Multilineage differentiation of human-derived dermal fibroblasts transfected with genes coated on PLGA nanoparticles plus growth factors. *Biomaterials*, **34**(2), pp. 582-597.

PARK, K., 2012. No penetration of nanoparticles through intact skin. *Journal of controlled release : official journal of the Controlled Release Society*, **162**(1), pp. 258.

PASSERINI, N., GAVINI, E., ALBERTINI, B., RASSU, G., DI SABATINO, M., SANNA, V., GIUNCHEDI, P. and RODRIGUEZ, L., 2009. Evaluation of solid lipid microparticles produced by spray congealing for topical application of econazole nitrate. *The Journal of pharmacy and pharmacology*, **61**(5), pp. 559-567.

PATHAK, P. and NAGARSENKER, M., 2009. Formulation and evaluation of lidocaine lipid nanosystems for dermal delivery. *AAPS PharmSciTech*, **10**(3), pp. 985-992.

PATLOLLA, A., KNIGHTEN, B. and TCHOUNWOU, P., 2010. Multi-walled carbon nanotubes induce cytotoxicity, genotoxicity and apoptosis in normal human dermal fibroblast cells. *Ethnicity & disease*, .

PATLOLLA, A., PATLOLLA, B. and TCHOUNWOU, P., 2010. Evaluation of cell viability, DNA damage, and cell death in normal human dermal fibroblast cells induced by functionalized multiwalled carbon nanotube. *Molecular and cellular biochemistry*, .

PAWAR, K.R. and BABU, R.J., 2010. Polymeric and Lipid-Based Materials for Topical Nanoparticle Delivery Systems. *Critical reviews in therapeutic drug carrier systems*, **27**(5), pp. 419-459.

PETROVA, H., LIN, C.H., DE LIEJER, S., HU, M., MCLELLAN, J.M., SIEKKINEN, A.R., WILEY, B.J., MARQUEZ, M., XIA, Y., SADER, J.E. and HARTLAND, G.V., 2007. Time-resolved spectroscopy of

silver nanocubes: observation and assignment of coherently excited vibrational modes. *The Journal of chemical physics*, **126**(9), pp. 094709.

PIAO, H., KAMIYA, N., HIRATA, A., FUJII, T. and GOTO, M., 2008. A novel solid-in-oil nanosuspension for transdermal delivery of diclofenac sodium. *Pharmaceutical research*, **25**(4), pp. 896-901.

PITAKSUTEEPONG, T., SOMSIRI, A. and WARANUCH, N., 2007. Targeted transfollicular delivery of artocarpin extract from Artocarpus incisus by means of microparticles. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, **67**(3), pp. 639-645.

POLAT, B.E., BLANKSCHTEIN, D. and LANGER, R., 2010. Low-frequency sonophoresis: application to the transdermal delivery of macromolecules and hydrophilic drugs. *Expert opinion on drug delivery*, **7**(12), pp. 1415-1432.

PONTI, J., COLOGNATO, R., RAUSCHER, H., GIORIA, S., BROGGI, F., FRANCHINI, F., PASCUAL, C., GIUDETTI, G. and ROSSI, F., 2010. Colony Forming Efficiency and microscopy analysis of multi-wall carbon nanotubes cell interaction. *Toxicology letters*, .

POPLE, P.V. and SINGH, K.K., 2012. Targeting tacrolimus to deeper layers of skin with improved safety for treatment of atopic dermatitis-Part II: *in vivo* assessment of dermatopharmacokinetics, biodistribution and efficacy. *International journal of pharmaceutics*, **434**(1-2), pp. 70-79.

POPLE, P.V. and SINGH, K.K., 2011. Development and evaluation of colloidal modified nanolipid carrier: application to topical delivery of tacrolimus. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, **79**(1), pp. 82-94.

POPLE, P.V. and SINGH, K.K., 2010. Targeting tacrolimus to deeper layers of skin with improved safety for treatment of atopic dermatitis. *International journal of pharmaceutics*, **398**(1-2), pp. 165-178.

POPLE, P.V. and SINGH, K.K., 2006. Development and evaluation of topical formulation containing solid lipid nanoparticles of vitamin A. *AAPS PharmSciTech*, **7**(4), pp. 91.

POPOV, A.P., PRIEZZHEV, A.V., LADEMANN, J. and MYLLYLA, R., 2006. Advantages of NIR radiation use for optical determination of skin horny layer thickness with embedded TiO_2 nanoparticles during tape stripping procedure. *Laser Physics*, **16**(5), pp. 751-757.

POPOV, A.P., ZVYAGIN, A.V., LADEMANN, J., ROBERTS, M.S., SANCHEZ, W., PRIEZZHEV, A.V. and MYLLYLA, R., 2010. Designing inorganic light-protective skin nanotechnology products. *Journal of biomedical nanotechnology*, **6**(5), pp. 432-451.

PORNPATTANANANGKUL, D., OLSON, S., ARYAL, S., SARTOR, M., HUANG, C., VECCHIO, K. and ZHANG, L., 2010. Stimuli-Responsive Liposome Fusion Mediated by Gold Nanoparticles. *Acs Nano*, **4**(4), pp. 1935-1942.

PRAUSNITZ, M.R., 2004. Microneedles for transdermal drug delivery. *Advanced Drug Delivery Reviews*, **56**(5), pp. 581-587.

PREVEL, B., LERME, J., GAUDRY, M., COTTANCIN, E., PELLARIN, M., TREILLEUX, M., MELINON, P., PEREZ, A., VIALLE, J.L. and BROYER, M., 2001. Optical properties of nanostructured thin films containing noble metal clusters: Au-N, (Au0.5Ag0.5)(N) and Ag-N. *Scripta Materialia*, **44**(8-9), pp. 1235-1238.

PROW, T.W., GRICE, J.E., LIN, L.L., FAYE, R., BUTLER, M., BECKER, W., WURM, E.M., YOONG, C., ROBERTSON, T.A., SOYER, H.P. and ROBERTS, M.S., 2011. Nanoparticles and microparticles for skin drug delivery. *Advanced Drug Delivery Reviews*, **63**(6), pp. 470-491.

PROW, T.W., 2012. Multiphoton microscopy applications in nanodermatology. *Wiley Interdisciplinary Reviews-Nanomedicine and Nanobiotechnology*, **4**(6), pp. 680-690.

PROW, T.W., MONTEIRO-RIVIERE, N.A., INMAN, A.O., GRICE, J.E., CHEN, X., ZHAO, X., SANCHEZ, W.H., GIERDEN, A., KENDALL, M.A.F., ZVYAGIN, A.V., ERDMANN, D., RIVIERE, J.E. and ROBERTS, M.S., 2012. Quantum dot penetration into viable human skin. *Nanotoxicology*, **6**(2), pp. 173-185.

PUGLIA, C., BLASI, P., RIZZA, L., SCHOUBBEN, A., BONINA, F., ROSSI, C. and RICCI, M., 2008. Lipid nanoparticles for prolonged topical delivery: an *in vitro* and *in vivo* investigation. *International journal of pharmaceutics*, **357**(1-2), pp. 295-304.

PUGLIA, C. and BONINA, F., 2012. Lipid nanoparticles as novel delivery systems for cosmetics and dermal pharmaceuticals. *Expert opinion on drug delivery*, **9**(4), pp. 429-441.

PUGLIA, C., BONINA, F., CASTELLI, F., MICIELI, D. and SARPIETRO, M.G., 2009. Evaluation of percutaneous absorption of the repellent diethyltoluamide and the sunscreen ethylhexyl p-methoxycinnamate-loaded solid lipid nanoparticles: an in-vitro study. *The Journal of pharmacy and pharmacology*, **61**(8), pp. 1013-1019.

PUGLIA, C., BONINA, F., RIZZA, L., CORTESI, R., MERLOTTI, E., DRECHSLER, M., MARIANI, P., CONTADO, C., RAVANI, L. and ESPOSITO, E., 2010. Evaluation of percutaneous absorption of naproxen from different liposomal formulations. *Journal of pharmaceutical sciences*, **99**(6), pp. 2819-2829.

PUGLIA, C., RIZZA, L., DRECHSLER, M. and BONINA, F., 2010. Nanoemulsions as vehicles for topical administration of glycyrrhetic acid: characterization and *in vitro* and *in vivo* evaluation. *Drug delivery*, **17**(3), pp. 123-129.

PUGLIA, C., SARPIETRO, M.G., BONINA, F., CASTELLI, F., ZAMMATARO, M. and CHIECHIO, S., 2011. Development, characterization, and *in vitro* and *in vivo* evaluation of benzocaine- and lidocaine-loaded nanostructrured lipid carriers. *Journal of pharmaceutical sciences*, **100**(5), pp. 1892-1899.

QIN, X.Q., YUAN, Y., LIU, C.S., WANG, Q.Y., SHEN, X. and YANG, B.C., 2007. Preparation of liposomal brucine and its pharmaceutical/pharmacodynamic characterization. *Acta Pharmacologica Sinica*, **28**(11), pp. 1851-1858.

QU, Y. and L□, X., 2009. Aqueous synthesis of gold nanoparticles and their cytotoxicity in human dermal fibroblasts-fetal. *Biomed Mater*, .

RAJAGOPALAN, S., 2004. From Nanoparticles to Novel Protective Garments.

RANCAN, F., GAO, Q., GRAF, C., TROPPENS, S., HADAM, S., HACKBARTH, S., KEMBUAN, C., BLUME-PEYTAVI, U., RUHL, E., LADEMANN, J. and VOGT, A., 2012. Skin penetration and cellular uptake of amorphous silica nanoparticles with variable size, surface functionalization, and colloidal stability. *ACS nano*, **6**(8), pp. 6829-6842.

RANCAN, F., PAPAKOSTAS, D., HADAM, S., HACKBARTH, S., DELAIR, T., PRIMARD, C., VERRIER, B., STERRY, W., BLUME-PEYTAVI, U. and VOGT, A., 2009. Investigation of polylactic acid (PLA) nanoparticles as drug delivery systems for local dermatotherapy. *Pharmaceutical research*, **26**(8), pp. 2027-2036.

RAO, B., MASLOV, K., DANIELLI, A., CHEN, R., SHUNG, K.K., ZHOU, Q. and WANG, L.V., 2011. Real-time four-dimensional optical-resolution photoacoustic microscopy with Au nanoparticle-assisted subdiffraction-limit resolution. *Optics Letters*, **36**(7), pp. 1137-1139.

RAPHAEL, A.P., PROW, T.W., CRICHTON, M.L., CHEN, X., FERNANDO, G.J. and KENDALL, M.A., 2010. Targeted, needle-free vaccinations in skin using multilayered, densely-packed dissolving microprojection arrays. *Small (Weinheim an der Bergstrasse, Germany)*, **6**(16), pp. 1785-1793.

RASTOGI, R., ANAND, S. and KOUL, V., 2010. Electroporation of polymeric nanoparticles: an alternative technique for transdermal delivery of insulin. *Drug development and industrial pharmacy*, **36**(11), pp. 1303-1311.

RASTOGI, R., ANAND, S. and KOUL, V., 2009. Flexible polymerosomes--an alternative vehicle for topical delivery. *Colloids and surfaces.B, Biointerfaces*, **72**(1), pp. 161-166.

RATTANARUENGSRIKUL, V., PIMPHA, N. and SUPAPHOL, P., 2012. *In vitro* efficacy and toxicology evaluation of silver nanoparticle-loaded gelatin hydrogel pads as antibacterial wound dressings. *Journal of Applied Polymer Science*, **124**(2), pp. 1668-1682.

RAVICHANDRAN, S., MORTENSEN, L.J. and DELUISE, L.A., 2011. Quantification of human skin barrier function and susceptibility to quantum dot skin penetration. *Nanotoxicology*, **5**(4), pp. 675-686.

RIANGJANAPATEE, P. and OKONOGI, S., 2012. Effect of surfactant on lycopene-loaded nanostructured lipid carriers. *Drug discoveries & therapeutics*, **6**(3), pp. 163-168.

RICHARDS, J.M., SHAW, C.A., LANG, N.N., WILLIAMS, M.C., SEMPLE, S.I., MACGILLIVRAY, T.J., GRAY, C., CRAWFORD, J.H., ALAM, S.R., ATKINSON, A.P., FORREST, E.K., BIENEK, C., MILLS, N.L., BURDESS, A., DHALIWAL, K., SIMPSON, A.J., WALLACE, W.A., HILL, A.T., RODDIE, P.H., MCKILLOP, G., CONNOLLY, T.A., FEUERSTEIN, G.Z., BARCLAY, G.R., TURNER, M.L. and NEWBY, D.E., 2012. *In vivo* mononuclear cell tracking using superparamagnetic particles of iron oxide: feasibility and safety in humans. *Circulation.Cardiovascular imaging*, **5**(4), pp. 509-517.

RIVERA, H.L., JARA, F.R. and CUNNINGHAM, V., 2011. Modelling and characterization of Photothermal effects assisted with Gold Nanorods in ex-vivo samples and in a murine model. *Energy-Based Treatment of Tissue and Assessment Vi*, **7901**, pp. 79010D.

RIVIERE, J.E., LEAVENS, T.L., BROOKS, J.D. and MONTEIRO-RIVIERE, N.A., 2012. Acute vascular effects of nanoparticle infusion in isolated perfused skin. *Nanomedicine : nanotechnology, biology, and medicine*, **8**(4), pp. 428-431.

RIZWAN, M., AQIL, M., TALEGAONKAR, S., AZEEM, A., SULTANA, Y. and ALI, A., 2009. Enhanced transdermal drug delivery techniques: an extensive review of patents. *Recent patents on drug delivery & formulation*, **3**(2), pp. 105-124.

ROBERTS, D.W., GOPEE, N.V., WARBRITTON, A.R., YU, W.W., COLVIN, V.L., WALKER, N.J. and HOWARD, P.C., 2006. Polymer Standards For Quantitation Of Quantum Dots In Tissue. 2006, pp. 168.

ROBERTS, M.S., DANCIK, Y., PROW, T.W., THORLING, C.A., LIN, L.L., GRICE, J.E., ROBERTSON, T.A., KONIG, K. and BECKER, W., 2011. Non-invasive imaging of skin physiology and percutaneous penetration using fluorescence spectral and lifetime imaging with multiphoton and confocal microscopy. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, **77**(3), pp. 469-488.

ROBERTS, M.S., ROBERTS, M.J., ROBERTSON, T.A., SANCHEZ, W., THORLING, C., ZOU, Y., ZHAO, X., BECKER, W. and ZVYAGIN, A.V., 2008. *In vitro* and *in vivo* imaging of xenobiotic transport in human skin and in the rat liver. *Journal of biophotonics*, **1**(6), pp. 478-493.

ROBERTS, S.M., 2006. Assessing Dermal Penetration Of Nanomaterials By Light And Electron Microscopy. 2006, pp. 6.

ROBERTSON, T.A., SANCHEZ, W.Y. and ROBERTS, M.S., 2010. Are commercially available nanoparticles safe when applied to the skin? *Journal of biomedical nanotechnology*, **6**(5), pp. 452-468.

ROUSE, J.G., HASLAUER, C.M., LOBOA, E.G. and MONTEIRO-RIVIERE, N.A., 2008. Cyclic tensile strain increases interactions between human epidermal keratinocytes and quantum dot nanoparticles. *Toxicology in vitro : an international journal published in association with BIBRA*, **22**(2), pp. 491-497.

ROUSE, J.G., YANG, J., BARRON, A.R. and MONTEIRO-RIVIERE, N.A., 2006. Fullerene-based amino acid nanoparticle interactions with human epidermal keratinocytes. *Toxicology in vitro : an international journal published in association with BIBRA*, **20**(8), pp. 1313-1320.

ROUSE, J.G., YANG, J., RYMAN-RASMUSSEN, J.P., BARRON, A.R. and MONTEIRO-RIVIERE, N.A., 2007. Effects of mechanical flexion on the penetration of fullerene amino acid-derivatized peptide nanoparticles through skin. *Nano letters*, **7**(1), pp. 155-160.

RYMAN-RASMUSSEN, J.P., RIVIERE, J.E. and MONTEIRO-RIVIERE, N.A., 2007. Surface coatings determine cytotoxicity and irritation potential of quantum dot nanoparticles in epidermal keratinocytes. *The Journal of investigative dermatology*, **127**(1), pp. 143-153.

RYMAN-RASMUSSEN, J.P., RIVIERE, J.E. and MONTEIRO-RIVIERE, N.A., 2006. Penetration of intact skin by quantum dots with diverse physicochemical properties. *Toxicological sciences : an official journal of the Society of Toxicology*, .

RYMAN-RASMUSSEN, J.P., RIVIERE, J.E. and MONTEIRO-RIVIERE, N.A., 2006. Skin Penetration, Cellular Uptake, Cytotoxicity, And Inflammatory Potential Of Quantum Dots. 2006, pp. 168.

SAATHOFF, J.G., INMAN, A.O., XIA, X.R., RIVIERE, J.E. and MONTEIRO-RIVIERE, N.A., 2011. *In vitro* toxicity assessment of three hydroxylated fullerenes in human skin cells. *Toxicology in vitro : an international journal published in association with BIBRA*, **25**(8), pp. 2105-2112.

SADRIEH, N., WOKOVICH, A.M., GOPEE, N.V., ZHENG, J., HAINES, D., PARMITER, D., SIITONEN, P.H., COZART, C.R., PATRI, A.K., MCNEIL, S.E., HOWARD, P.C., DOUB, W.H. and BUHSE, L.F., 2010. Lack of significant dermal penetration of titanium dioxide from sunscreen formulations containing nano- and submicron-size TiO_2 particles. *Toxicological sciences : an official journal of the Society of Toxicology*, **115**(1), pp. 156-166.

SAHLE, F.F., METZ, H., WOHLRAB, J. and NEUBERT, R.H., 2012. Polyglycerol fatty acid ester surfactant-based microemulsions for targeted delivery of ceramide AP into the SC: formulation, characterisation, *in vitro* release and penetration investigation. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, **82**(1), pp. 139-150.

SAINO, V., MONTI, D., BURGALASSI, S., TAMPUCCI, S., PALMA, S., ALLEMANDI, D. and CHETONI, P., 2010. Optimization of skin permeation and distribution of ibuprofen by using nanostructures (coagels) based on alkyl vitamin C derivatives. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V,* **76**(3), pp. 443-449.

SAKEENA, M.H., ELRASHID, S.M., MUTHANNA, F.A., GHASSAN, Z.A., KANAKAL, M.M., LAILA, L., MUNAVVAR, A.S. and AZMIN, M.N., 2010. Effect of limonene on permeation enhancement of ketoprofen in palm oil esters nanoemulsion. *Journal of oleo science,* **59**(7), pp. 395-400.

SAMAH, N.A., WILLIAMS, N. and HEARD, C.M., 2010. Nanogel particulates located within diffusion cell receptor phases following topical application demonstrates uptake into and migration across skin. *International journal of pharmaceutics,* **401**(1-2), pp. 72-78.

SAMBERG, M.E., OLDENBURG, S.J. and MONTEIRO-RIVIERE, N.A., 2010. Evaluation of silver nanoparticle toxicity in skin *in vivo* and keratinocytes *in vitro*. *Environmental health perspectives,* **118**(3), pp. 407-413.

SANDRI, G., BONFERONI, M.C., GOKCE, E.H., FERRARI, F., ROSSI, S., PATRINI, M. and CARAMELLA, C., 2010. Chitosan-associated SLN: *in vitro* and *ex vivo* characterization of cyclosporine A loaded ophthalmic systems. *Journal of microencapsulation,* **27**(8), pp. 735-746.

SANKAR, V., PRAVEEN, C., PRASANTH, K.G., SRINIVAS, C.R. and RUCKMANN, K., 2009. Formulation and evaluation of a proniosome hydrocortisone gel in comparison with a commercial cream. *Die Pharmazie,* **64**(11), pp. 731-734.

SANNA, V., GAVINI, E., COSSU, M., RASSU, G. and GIUNCHEDI, P., 2007. Solid lipid nanoparticles (SLN) as carriers for the topical delivery of econazole nitrate: in-vitro characterization, ex-vivo and in-vivo studies. *The Journal of pharmacy and pharmacology,* **59**(8), pp. 1057-1064.

SANNA, V., MARIANI, A., CARIA, G. and SECHI, M., 2009. Synthesis and evaluation of different fatty acid esters formulated into Precirol ATO-Based lipid nanoparticles as vehicles for topical delivery. *Chemical & pharmaceutical bulletin,* .

SANTAMARIA, A.B. and RACHMAN, N.J., 2005. Risk Assessment And Safety Evaluation Of Nanomaterials In Consumer Products. 2005, pp. 133.

SANTANDER-ORTEGA, M.J., STAUNER, T., LORETZ, B., ORTEGA-VINUESA, J.L., BASTOS-GONZALEZ, D., WENZ, G., SCHAEFER, U.F. and LEHR, C.M., 2010. Nanoparticles made from novel starch derivatives for transdermal drug delivery. *Journal of controlled release : official journal of the Controlled Release Society,* **141**(1), pp. 85-92.

SANTOS, C.M., DE OLIVEIRA, R.B., ARANTES, V.T., CALDEIRA, L.R., DE OLIVEIRA, M.C., EGITO, E.S. and FERREIRA, L.A., 2012. Amphotericin B-loaded nanocarriers for topical treatment of cutaneous leishmaniasis: development, characterization, and *in vitro* skin permeation studies. *Journal of biomedical nanotechnology,* **8**(2), pp. 322-329.

SARKAR, S., GURJARPADHYE, A.A., RYLANDER, C.G. and RYLANDER, M.N., 2011. Optical properties of breast tumor phantoms containing carbon nanotubes and nanohorns. *Journal of Biomedical Optics,* **16**(5), pp. 051304.

SARLO, K., BLACKBURN, K.L., CLARK, E.D., GROTHAUS, J., CHANEY, J., NEU, S., FLOOD, J., ABBOTT, D., BOHNE, C., CASEY, K., FRYER, C. and KUHN, M., 2009. Tissue distribution of 20 nm, 100 nm and 1000 nm fluorescent polystyrene latex nanospheres following acute systemic or acute and repeat airway exposure in the rat. *Toxicology,* **263**(2-3), pp. 117-126.

SASAKI, K., ASANUMA, K., JOHKURA, K., KASUGA, T., OKOUCHI, Y., OGIWARA, N., KUBOTA, S., TENG, R., CUI, L. and ZHAO, X., 2006. Ultrastructural analysis of TiO_2 nanotubes with photodecomposition of water into O_2 and H_2 implanted in the nude mouse. *Annals of Anatomy = Anatomischer Anzeiger : Official Organ of the Anatomische Gesellschaft,* **188**(2), pp. 137-142.

SCALIA, S., FRANCESCHINIS, E., BERTELLI, D. and IANNUCCELLI, V., 2012. Comparative Evaluation of the Effect of Permeation Enhancers, Lipid Nanoparticles and Colloidal Silica on *in vivo* Human Skin Penetration of Quercetin. *Skin pharmacology and physiology,* **26**(2), pp. 57-67.

SCH„FER-KORTING, M., H"LTJE, M., KORTING, H.C. and H"LTJE, H.D., 2010. Innovative agents for actinic keratosis and nanocarriers enhancing skin penetration. *Skin Pharmacol Physiol,* .

SCH„FER-KORTING, M., MEHNERT, W. and KORTING, H.C., 2007. Lipid nanoparticles for improved topical application of drugs for skin diseases. *Advanced Drug Delivery Reviews*, .

SCHAFER-KORTING, M., HOLTJE, M., KORTING, H.C. and HOLTJE, H.D., 2010. Innovative agents for actinic keratosis and nanocarriers enhancing skin penetration. *Skin pharmacology and physiology*, **23**(1), pp. 6-14.

SCHAFER-KORTING, M., MEHNERT, W. and KORTING, H.C., 2007. Lipid nanoparticles for improved topical application of drugs for skin diseases. *Advanced Drug Delivery Reviews*, **59**(6), pp. 427-443.

SCHIFFTER, H., CONDLIFFE, J. and VONHOFF, S., 2010. Spray-freeze-drying of nanosuspensions: the manufacture of insulin particles for needle-free ballistic powder delivery. *J R Soc Interface*, .

SCHILLING, K., BRADFORD, B., CASTELLI, D., DUFOUR, E., NASH, J.F., PAPE, W., SCHULTE, S., TOOLEY, I., VAN DEN BOSCH, J. and SCHELLAUF, F., 2010. Human safety review of "nano" titanium dioxide and zinc oxide. *Photochemical & photobiological sciences: Official journal of the European Photochemistry Association and the European Society for Photobiology*, **9**(4), pp. 495-509.

SCHMIDT, M., MARTIN, S.F., FREUDENBERG, M.A. and GOEBELER, M., 2011. Animal models for nickel allergy. *Nature nanotechnology*, **6**(9), pp. 533; author reply 533.

SCHNEIDER, M., STRACKE, F., HANSEN, S. and SCHAEFER, U.F., 2009. Nanoparticles and their interactions with the dermal barrier. *Dermato-endocrinology*, **1**(4), pp. 197-206.

SCHROETER, A., ENGELBRECHT, T., NEUBERT, R.H. and GOEBEL, A.S., 2010. New nanosized technologies for dermal and transdermal drug delivery. A review. *Journal of biomedical nanotechnology*, **6**(5), pp. 511-528.

SCHWARZ, J.C., KLANG, V., KARALL, S., MAHRHAUSER, D., RESCH, G.P. and VALENTA, C., 2012. Optimisation of multiple W/O/W nanoemulsions for dermal delivery of aciclovir. *International journal of pharmaceutics*, **435**(1), pp. 69-75.

SCHWARZ, J.C., WEIXELBAUM, A., PAGITSCH, E., LOW, M., RESCH, G.P. and VALENTA, C., 2012. Nanocarriers for dermal drug delivery: influence of preparation method, carrier type and rheological properties. *International journal of pharmaceutics*, **437**(1-2), pp. 83-88.

SCHWERTE, T., 2010. Skin epithelium of zebrafish may work as an airway epithelia analogue model to evaluate systemic effects of micro- and nano-particles. *Thrombosis and haemostasis*, **103**(4), pp. 692-693.

SCOTT, B.R., 2012. Are some neurons hypersensitive to metallic nanoparticles? *Dose-response : a publication of International Hormesis Society*, **10**(1), pp. 37-57.

SCOWN, T.M., GOODHEAD, R.M., JOHNSTON, B.D., MOGER, J., BAALOUSHA, M., LEAD, J.R., VAN AERLE, R., IGUCHI, T. and TYLER, C.R., 2010. Assessment of cultured fish hepatocytes for studying cellular uptake and (eco)toxicity of nanoparticles. *Environmental Chemistry*, **7**(1), pp. 36-49.

SENYIGIT, T., SONVICO, F., BARBIERI, S., OZER, O., SANTI, P. and COLOMBO, P., 2010. Lecithin/chitosan nanoparticles of clobetasol-17-propionate capable of accumulation in pig skin. *Journal of controlled release : official journal of the Controlled Release Society*, **142**(3), pp. 368-373.

SENZUI, M., TAMURA, T., MIURA, K., IKARASHI, Y., WATANABE, Y. and FUJII, M., 2010. Study on penetration of titanium dioxide (TiO(2)) nanoparticles into intact and damaged skin *in vitro*. *The Journal of toxicological sciences*, **35**(1), pp. 107-113.

SEO, J.H., JEON, W.I., DEMBERELDORJ, U., LEE, S.Y. and JOO, S.W., 2011. Cytotoxicity of serum protein-adsorbed visible-light photocatalytic Ag/AgBr/TiO_2 nanoparticles. *Journal of hazardous materials*, **198**, pp. 347-355.

SERPONE, N., SALINARO, A.E., HORIKOSHI, S. and HIDAKA, H., 2006. Beneficial effects of photo-inactive titanium dioxide specimens on plasmid DNA, human cells and yeast cells exposed to UVA/UVB simulated sunlight. *Journal of Photochemistry and Photobiology A-Chemistry*, **179**(1-2), pp. 200-212.

SETO, J.E., POLAT, B.E., LOPEZ, R.F., BLANKSCHTEIN, D. and LANGER, R., 2010. Effects of ultrasound and sodium lauryl sulfate on the transdermal delivery of hydrophilic permeants: Comparative *in vitro* studies with full-thickness and split-thickness pig and human skin. *Journal of controlled release : official journal of the Controlled Release Society*, **145**(1), pp. 26-32.

SHAH, K.A., DATE, A.A., JOSHI, M.D. and PATRAVALE, V.B., 2007. Solid lipid nanoparticles (SLN) of tretinoin: potential in topical delivery. *International journal of pharmaceutics*, **345**(1-2), pp. 163-171.

SHAH, P.P., DESAI, P.R., CHANNER, D. and SINGH, M., 2012. Enhanced skin permeation using polyarginine modified nanostructured lipid carriers. *Journal of controlled release : official journal of the Controlled Release Society*, .

SHAH, P.P., DESAI, P.R. and SINGH, M., 2012. Effect of oleic acid modified polymeric bilayered nanoparticles on percutaneous delivery of spantide II and ketoprofen. *Journal of controlled release : official journal of the Controlled Release Society*, **158**(2), pp. 336-345.

SHAKEEL, F., BABOOTA, S., AHUJA, A., ALI, J., AQIL, M. and SHAFIQ, S., 2007. Nanoemulsions as vehicles for transdermal delivery of aceclofenac. *AAPS PharmSciTech*, **8**(4), pp. E104.

SHAKEEL, F., BABOOTA, S., AHUJA, A., ALI, J. and SHAFIQ, S., 2009. Enhanced anti-inflammatory effects of celecoxib from a transdermally applied nanoemulsion. *Die Pharmazie*, **64**(4), pp. 258-259.

SHAKEEL, F., BABOOTA, S., AHUJA, A., ALI, J. and SHAFIQ, S., 2008. Celecoxib nanoemulsion: skin permeation mechanism and bioavailability assessment. *Journal of drug targeting*, **16**(10), pp. 733-740.

SHAKEEL, F., BABOOTA, S., AHUJA, A., ALL, J. and SHAFIQ, S., 2008. Skin permeation mechanism of aceclofenac using novel nanoemulsion formulation. *Die Pharmazie*, **63**(8), pp. 580-584.

SHAKEEL, F. and RAMADAN, W., 2010. Transdermal delivery of anticancer drug caffeine from water-in-oil nanoemulsions. *Colloids and surfaces.B, Biointerfaces*, **75**(1), pp. 356-362.

SHAKEEL, F., RAMADAN, W. and AHMED, M.A., 2009. Investigation of true nanoemulsions for transdermal potential of indomethacin: characterization, rheological characteristics, and *ex vivo* skin permeation studies. *Journal of drug targeting*, **17**(6), pp. 435-441.

SHARIFI, S., BEHZADI, S., LAURENT, S., FORREST, M.L., STROEVE, P. and MAHMOUDI, M., 2012. Toxicity of nanomaterials. *Chemical Society Reviews*, **41**(6), pp. 2323-2343.

SHARMA, R. and PATHAK, K., 2011. Polymeric nanosponges as an alternative carrier for improved retention of econazole nitrate onto the skin through topical hydrogel formulation. *Pharmaceutical development and technology*, **16**(4), pp. 367-376.

SHARMA, S.K., DAI, T., KHARKWAL, G.B., HUANG, Y.Y., HUANG, L., DE ARCE, V.J., TEGOS, G.P. and HAMBLIN, M.R., 2011. Drug discovery of antimicrobial photosensitizers using animal models. *Current pharmaceutical design*, **17**(13), pp. 1303-1319.

SHARMA, V., SINGH, S.K., ANDERSON, D., TOBIN, D.J. and DHAWAN, A., 2011. Zinc oxide nanoparticle induced genotoxicity in primary human epidermal keratinocytes. *Journal of nanoscience and nanotechnology*, **11**(5), pp. 3782-3788.

SHEIHET, L., CHANDRA, P., BATHEJA, P., DEVORE, D., KOHN, J. and MICHNIAK, B., 2008. Tyrosine-derived nanospheres for enhanced topical skin penetration. *International journal of pharmaceutics*, **350**(1-2), pp. 312-319.

SHI, L., SHAN, J., JU, Y., AIKENS, P. and PRUD'HOMME, R.K., 2012. Nanoparticles as delivery vehicles for sunscreen agents. *Colloids and Surfaces A-Physicochemical and Engineering Aspects*, **396**, pp. 122-129.

SHIM, J., SEOK KANG, H., PARK, W.S., HAN, S.H., KIM, J. and CHANG, I.S., 2004. Transdermal delivery of mixnoxidil with block copolymer nanoparticles. *Journal of controlled release : official journal of the Controlled Release Society*, **97**(3), pp. 477-484.

SHIRALI, A.C., LOOK, M., DU, W., KASSIS, E., STOUT-DELGADO, H.W., FAHMY, T.M. and GOLDSTEIN, D.R., 2011. Nanoparticle delivery of mycophenolic acid upregulates PD-L1 on dendritic cells to prolong murine allograft survival. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, **11**(12), pp. 2582-2592.

SHUKLA, R.K., SHARMA, V., PANDEY, A.K., SINGH, S., SULTANA, S. and DHAWAN, A., 2011. ROS-mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells. *Toxicology in vitro : an international journal published in association with BIBRA*, **25**(1), pp. 231-241.

SILVA, A.P., NUNES, B.R., DE OLIVEIRA, M.C., KOESTER, L.S., MAYORGA, P., BASSANI, V.L. and TEIXEIRA, H.F., 2009. Development of topical nanoemulsions containing the isoflavone genistein. *Die Pharmazie*, **64**(1), pp. 32-35.

SIMOVIC, S., GHOUCHI-ESKANDAR, N. and PRESTIDGE, C.A., 2011. Pickering emulsions for dermal delivery. *Journal of Drug Delivery Science and Technology*, **21**(1), pp. 123-133.

SIMOVIC, S., BARNES, T.J., TAN, A. and PRESTIDGE, C.A., 2012. Assembling nanoparticle coatings to improve the drug delivery performance of lipid based colloids. *Nanoscale,* **4**(4), pp. 1220-1230.

SINGH, P. and NANDA, A., 2012. Nanotechnology in cosmetics: a boon or bane? *Toxicological and Environmental Chemistry,* **94**(8), pp. 1467-1479.

SINGH, R. and SINGH, D., 2012. Chitin membranes containing silver nanoparticles for wound dressing application. *International wound journal,* .

SINGH, Y., GAO, D., GU, Z., LI, S., STEIN, S. and SINKO, P.J., 2012. Noninvasive detection of passively targeted poly(ethylene glycol) nanocarriers in tumors. *Molecular pharmaceutics,* **9**(1), pp. 144-155.

SINGKA, G.S., SAMAH, N.A., ZULFAKAR, M.H., YURDASIPER, A. and HEARD, C.M., 2010. Enhanced topical delivery and anti-inflammatory activity of methotrexate from an activated nanogel. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V,* **76**(2), pp. 275-281.

SIQUEIRA, N.M., CONTRI, R.V., PAESE, K., BECK, R.C., POHLMANN, A.R. and GUTERRES, S.S., 2011. Innovative sunscreen formulation based on benzophenone-3-loaded chitosan-coated polymeric nanocapsules. *Skin pharmacology and physiology,* **24**(3), pp. 166-174.

SIVARAMAKRISHNAN, R., NAKAMURA, C., MEHNERT, W., KORTING, H.C., KRAMER, K.D. and SCHAFER-KORTING, M., 2004. Glucocorticoid entrapment into lipid carriers--characterisation by parelectric spectroscopy and influence on dermal uptake. *Journal of controlled release : official journal of the Controlled Release Society,* **97**(3), pp. 493-502.

SKIDAN, I.N., BOBRUSKIN, A.I., GULIAEV, A.E., SHALKHARBAEVA, S.D., SEVERIN, S.E., STEINNIGER, S., KREUTER, J. and GEL'PERINA, S.E., 2001. Optimization of "Photosens" pharmakokinetics by biodegradable nanospheres. *Antibiotiki i khimioterapiia = Antibiotics and chemoterapy [sic] / Ministerstvo meditsinskoi i mikrobiologicheskoi promyshlennosti SSSR,* **46**(4), pp. 6-10.

SKOCAJ, M., FILIPIC, M., PETKOVIC, J. and NOVAK, S., 2011. Titanium dioxide in our everyday life; is it safe? *Radiology and oncology,* **45**(4), pp. 227-247.

SLASTNIKOVA, T.A., ROSENKRANZ, A.A., GULAK, P.V., SCHIFFELERS, R.M., LUPANOVA, T.N., KHRAMTSOV, Y.V., ZALUTSKY, M.R. and SOBOLEV, A.S., 2012. Modular nanotransporters: a multipurpose *in vivo* working platform for targeted drug delivery. *International journal of nanomedicine,* **7**, pp. 467-482.

SMIJS, T.G. and BOUWSTRA, J.A., 2010. Focus on skin as a possible port of entry for solid nanoparticles and the toxicological impact. *Journal of biomedical nanotechnology,* **6**(5), pp. 469-484.

SONAVANE, G., TOMODA, K., SANO, A., OHSHIMA, H., TERADA, H. and MAKINO, K., 2008. *In vitro* permeation of gold nanoparticles through rat skin and rat intestine: effect of particle size. *Colloids and surfaces.B, Biointerfaces,* **65**(1), pp. 1-10.

SONG, K.H., KIM, C., COBLEY, C.M., XIA, Y. and WANG, L.V., 2009. Near-infrared gold nanocages as a new class of tracers for photoacoustic sentinel lymph node mapping on a rat model. *Nano Lett,* .

SONG, Y., LI, X. and DU, X., 2009. Exposure to nanoparticles is related to pleural effusion, pulmonary fibrosis and granuloma. *European Respiratory Journal,* **34**(3), pp. 559-567.

SONG, Z., ANISSIMOV, Y.G., ZHAO, J., NECHAEV, A.V., NADORT, A., JIN, D., PROW, T.W., ROBERTS, M.S. and ZVYAGIN, A.V., 2013. Background free imaging of upconversion nanoparticle distribution in human skin. *Journal of Biomedical Optics,* **18**(6), pp. 061215.

SONG, Z., KELF, T.A., SANCHEZ, W.H., ROBERTS, M.S., RICKA, J., FRENZ, M. and ZVYAGIN, A.V., 2011. Characterization of optical properties of ZnO nanoparticles for quantitative imaging of transdermal transport. *Biomedical optics express,* **2**(12), pp. 3321-3333.

SOUTO, E.B. and M□LLER, R.H., 2010. Lipid nanoparticles: effect on bioavailability and pharmacokinetic changes. *Handbook of Experimental Pharmacology,* .

SOUTO, E.B., WISSING, S.A., BARBOSA, C.M. and MULLER, R.H., 2004. Comparative study between the viscoelastic behaviors of different lipid nanoparticle formulations. *Journal of cosmetic science,* **55**(5), pp. 463-471.

SOUTO, E.B. and DOKTOROVOVA, S., 2009. Solid Lipid Nanoparticle Formulations: Pharmacokinetic and Biopharmaceutical Aspects in Drug Delivery. *Methods in Enzymology; Liposomes, Pt F,* **464**, pp. 105-129.

SPAGNUL, A., BOUVIER-CAPELY, C., PHAN, G., REBIERE, F. and FATTAL, E., 2010. A new formulation containing calixarene molecules as an emergency treatment of uranium skin contamination. *Health physics*, **99**(3), pp. 430-434.

STECOVA, J., MEHNERT, W., BLASCHKE, T., KLEUSER, B., SIVARAMAKRISHNAN, R., ZOUBOULIS, C.C., SELTMANN, H., KORTING, H.C., KRAMER, K.D. and SCHAFER-KORTING, M., 2007. Cyproterone acetate loading to lipid nanoparticles for topical acne treatment: particle characterisation and skin uptake. *Pharmaceutical research*, **24**(5), pp. 991-1000.

STERN, S.T. and MCNEIL, S.E., 2008. Nanotechnology safety concerns revisited. *Toxicological sciences : an official journal of the Society of Toxicology*, **101**(1), pp. 4-21.

STRACKE, F., WEISS, B., LEHR, C.M., K"NIG, K., SCHAEFER, U.F. and SCHNEIDER, M., 2006. Multiphoton microscopy for the investigation of dermal penetration of nanoparticle-borne drugs. *The Journal of investigative dermatology*, .

SUBBIAH, R.P., LEE, H., VEERAPANDIAN, M., SADHASIVAM, S., SEO, S. and YUN, K., 2011. Structural and biological evaluation of a multifunctional SWCNT-AgNPs-DNA/PVA bio-nanofilm. *Analytical and Bioanalytical Chemistry*, **400**(2), pp. 547-560.

SUGAWARA, Y., KUROKI, H., TAMAKI, T., OHASHI, H., ITO, T. and YAMAGUCHI, T., 2012. Conversion of a molecular signal into a visual color based on the permeation of nanoparticles through a biomolecule-recognition gating membrane. *Analytical Methods*, **4**(9), pp. 2635-2637.

SUREKHA, P., KISHORE, A.S., SRINIVAS, A., SELVAM, G., GOPARAJU, A., REDDY, P.N. and MURTHY, P.B., 2012. Repeated dose dermal toxicity study of nano zinc oxide with Sprague-Dawley rats. *Cutaneous and ocular toxicology*, **31**(1), pp. 26-32.

SUTTHANUT, K., LU, X., JAY, M. and SRIPANIDKULCHAI, B., 2009. Solid lipid nanoparticles for topical administration of Kaempferia parviflora extracts. *Journal of biomedical nanotechnology*, **5**(2), pp. 224-232.

SZABO, N.J., WASDO, S.C., BRUBAKER, G., JENKINS, C.M. and ROBERTS, S.M., 2005. Dissolution Rates Of Nanoparticulate Metallic Species In Artificial Human Sweat Under Physiologically Relevant Conditions. 2005, pp. 142.

SZIKSZAI, Z., KERTESZ, Z., BODNAR, E., BORBIRO, I., ANGYAL, A., CSEDREKI, L., FURU, E., SZOBOSZLAI, Z., KISS, A.Z. and HUNYADI, J., 2011. Nuclear microprobe investigation of the penetration of ultrafine zinc oxide into human skin affected by atopic dermatitis. *Nuclear Instruments & Methods in Physics Research Section B-Beam Interactions with Materials and Atoms*, **269**(20), pp. 2278-2280.

SZIKSZAI, Z., KERTESZ, Z., BODNAR, E., MAJOR, I., BORBIRO, I., KISS, A.Z. and HUNYADI, J., 2010. Nuclear microprobe investigation of the penetration of ultrafine zinc oxide into intact and tape-stripped human skin. *Nuclear Instruments & Methods in Physics Research Section B-Beam Interactions with Materials and Atoms*, **268**(11-12), pp. 2160-2163.

TABATA, Y., MURAKAMI, Y. and IKADA, Y., 1997. Photodynamic effect of polyethylene glycol-modified fullerene on tumor. *Japanese journal of cancer research : Gann*, **88**(11), pp. 1108-1116.

TAN MH, COMMENS CA, BURNETT L, SNITCH PJ. 1996. A pilot study on the percutaneous absorption of microfine titanium dioxide from sunscreens. *Australas J Dermatol*., **37**(4):185-7.

TAHARA, Y., HONDA, S., KAMIYA, N., PIAO, H., HIRATA, A., HAYAKAWA, E., FUJII, T. and GOTO, M., 2008. A solid-in-oil nanodispersion for transcutaneous protein delivery. *Journal of controlled release : official journal of the Controlled Release Society*, **131**(1), pp. 14-18.

TAIRA, S., MORITAKE, S., HATANAKA, T., ICHIYANAGI, Y. and SETOU, M., 2009. Functionalized magnetic nanoparticles as an *in vivo* delivery system. *Methods in molecular biology (Clifton, N.J.)*, **544**, pp. 571-587.

TAJIMA, T., UENO, S., YABU, N. and SUKIGARA, S., 2011. Fabrication and Characterization of Poly-gamma-glutamic Acid Nanofiber with the Difunctional Epoxy cross-linker. *Sen-i Gakkaishi*, **67**(8), pp. 169-175.

TAKEMOTO, S., YAMAOKA, K., NISHIKAWA, M., YANO, Y. and TAKAKURA, Y., 2010. Bootstrap method-based estimation of the minimum sample number for obtaining pharmacokinetic parameters in preclinical experiments. *Journal of pharmaceutical sciences*, **99**(4), pp. 2176-2184.

TAN, Q., LIU, W., GUO, C. and ZHAI, G., 2011. Preparation and evaluation of quercetin-loaded lecithin-chitosan nanoparticles for topical delivery. *International journal of nanomedicine,* **6**, pp. 1621-1630.

TAVEIRA, S.F., ARAUJO, L.M., DE SANTANA, D.C., NOMIZO, A., DE FREITAS, L.A. and LOPEZ, R.F., 2012. Development of cationic solid lipid nanoparticles with factorial design-based studies for topical administration of doxorubicin. *Journal of biomedical nanotechnology,* **8**(2), pp. 219-228.

TAYLOR, M.B. and GUTIERREZ, M.J., 2008. Absorption, bioavailability, and partner transfer of estradiol from a topical emulsion. *Pharmacotherapy,* **28**(6), pp. 712-718.

TEERANACHAIDEEKUL, V., BOONME, P., SOUTO, E.B., MULLER, R.H. and JUNYAPRASERT, V.B., 2008. Influence of oil content on physicochemical properties and skin distribution of Nile red-loaded NLC. *Journal of controlled release : official journal of the Controlled Release Society,* **128**(2), pp. 134-141.

TEERANACHAIDEEKUL, V., SOUTO, E.B., M□LLER, R.H. and JUNYAPRASERT, V.B., 2008. Physicochemical characterization and *in vitro* release studies of ascorbyl palmitate-loaded semi-solid nanostructured lipid carriers (NLC gels). *Journal of microencapsulation,* .

TEICHMANN, A., OSSADNIK, M., RICHTER, H., STERRY, W. and LADEMANN, J., 2006. Semiquantitative determination of the penetration of a fluorescent hydrogel formulation into the hair follicle with and without follicular closure by microparticles by means of differential stripping. *Skin pharmacology and physiology,* **19**(2), pp. 101-105.

TEIXEIRA, Z., DREISS, C.A., LAWRENCE, M.J., HEENAN, R.K., MACHADO, D., JUSTO, G.Z., GUTERRES, S.S. and DURAN, N., 2012. Retinyl palmitate polymeric nanocapsules as carriers of bioactives. *Journal of colloid and interface science,* **382**(1), pp. 36-47.

TEIXEIRA, Z., ZANCHETTA, B., MELO, B.A., OLIVEIRA, L.L., SANTANA, M.H., PAREDES-GAMERO, E.J., JUSTO, G.Z., NADER, H.B., GUTERRES, S.S. and DURAN, N., 2010. Retinyl palmitate flexible polymeric nanocapsules: characterization and permeation studies. *Colloids and surfaces.B, Biointerfaces,* **81**(1), pp. 374-380.

TERENTYUK, G.S., GENINA, E.A., BASHKATOV, A.N., RYZHOVA, M.V., TSYGANOVA, N.A., CHUMAKOV, D.S., KHLEBTSOV, B.N., SAZONOV, A.A., DOLOTOV, L.E., TUCHIN, V.V., KHLEBTSOV, N.G. and INOZEMTSEVA, O.A., 2012. Use of fractional laser microablation and ultrasound to facilitate the delivery of gold nanoparticles into skin *in vivo*. *Quantum Electronics,* **42**(6), pp. 471-477.

TESKAC, K. and KRISTL, J., 2010. The evidence for solid lipid nanoparticles mediated cell uptake of resveratrol. *International journal of pharmaceutics,* **390**(1), pp. 61-69.

TIANO, L., ARMENI, T., VENDITTI, E., BARUCCA, G., MINCARELLI, L. and DAMIANI, E., 2010. Modified TiO(2) particles differentially affect human skin fibroblasts exposed to UVA light. *Free radical biology & medicine,* **49**(3), pp. 408-415.

TINKLE, S.S., 2004. Skin Exposure To Particles: Penetration Is Dependent On Particle Size. 2004, pp. 381.

TINKLE, S.S., ANTONINI, J.M., RICH, B.A., ROBERTS, J.R., SALMEN, R., DEPREE, K. and ADKINS, E.J., 2003. Skin as a route of exposure and sensitization in chronic beryllium disease. *Environmental health perspectives,* **111**(9), pp. 1202-1208.

TODO, H., KIMURA, E., YASUNO, H., TOKUDOME, Y., HASHIMOTO, F., IKARASHI, Y. and SUGIBAYASHI, K., 2010. Permeation pathway of macromolecules and nanospheres through skin. *Biological & pharmaceutical bulletin,* **33**(8), pp. 1394-1399.

TOMODA, K., TERASHIMA, H., SUZUKI, K., INAGI, T., TERADA, H. and MAKINO, K., 2011. Enhanced transdermal delivery of indomethacin-loaded PLGA nanoparticles by iontophoresis. *Colloids and Surfaces B-Biointerfaces,* **88**(2), pp. 706-710.

TOMODA, K., WATANABE, A., SUZUKI, K., INAGI, T., TERADA, H. and MAKINO, K., 2012. Enhanced transdermal permeability of estradiol using combination of PLGA nanoparticles system and iontophoresis. *Colloids and Surfaces B-Biointerfaces,* **97**, pp. 84-89.

TONG, J., ZIMMERMAN, M.C., LI, S., YI, X., LUXENHOFER, R., JORDAN, R. and KABANOV, A.V., 2011. Neuronal uptake and intracellular superoxide scavenging of a fullerene (C-60)-poly(2-oxazoline)s nanoformulation. *Biomaterials,* **32**(14), pp. 3654-3665.

TONG, Y.C., YU, T.Y., CHANG, S.F. and LIAW, J., 2012. Nanopolymeric micelle effect on the transdermal permeability, the bioavailability and gene expression of plasmid. *Molecular pharmaceutics*, **9**(1), pp. 111-120.

TRAN, D.T. and SALMON, R., 2011. Potential photocarcinogenic effects of nanoparticle sunscreens. *Australasian Journal of Dermatology*, **52**(1), pp. 1-6.

TRAN, M.A., GOWDA, R., SHARMA, A., PARK, E.J., ADAIR, J., KESTER, M., SMITH, N.B. and ROBERTSON, G.P., 2008. Targeting V600EB-Raf and Akt3 using nanoliposomal-small interfering RNA inhibits cutaneous melanocytic lesion development. *Cancer research*, **68**(18), pp. 7638-7649.

TRAN, M.A., WATTS, R.J. and ROBERTSON, G.P., 2009. Use of liposomes as drug delivery vehicles for treatment of melanoma. *Pigment cell & melanoma research*, **22**(4), pp. 388-399.

TRAPASSO, E., COSCO, D., CELIA, C., FRESTA, M. and PAOLINO, D., 2009. Retinoids: new use by innovative drug-delivery systems. *Expert opinion on drug delivery*, **6**(5), pp. 465-483.

TSUJI, J.S., 2005. Emerging Issues In Risk Assessment And Risk Perception Of Nanomaterials. 2005, pp. 132.

TSUJI, J.S., MAYNARD, A.D., HOWARD, P.C., JAMES, J.T., LAM, C.W., WARHEIT, D.B. and SANTAMARIA, A.B., 2006. Research strategies for safety evaluation of nanomaterials, part IV: risk assessment of nanoparticles. *Toxicological sciences : an official journal of the Society of Toxicology*, **89**(1), pp. 42-50.

TSUNODA, S., 2011. Transdermal penetration and biodistribution of nanomaterials and their acute toxicity *in vivo*. *Yakugaku zasshi : Journal of the Pharmaceutical Society of Japan*, **131**(2), pp. 203-207.

TYNER, K.M., WOKOVICH, A.M., GODAR, D.E., DOUB, W.H. and SADRIEH, N., 2011. The state of nano-sized titanium dioxide (TiO_2) may affect sunscreen performance. *International Journal of Cosmetic Science*, **33**(3), pp. 234-244.

ULMER, M., PATZELT, A., VERGOU, T., LADEMANN, J., RICHTER, H., KRAMER, A., MUELLER, G., STERRY, W. and LANGE-ASSCHENFELDT, B., 2012. *In vitro* investigation of the follicular penetration of porcine ear skin using a nanoparticle-emulsion containing the antiseptic polihexanide. *Laser Physics Letters*, **9**(5), pp. 381-386.

UNER, M., WISSING, S.A., YENER, G. and MULLER, R.H., 2005. Skin moisturizing effect and skin penetration of ascorbyl palmitate entrapped in solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) incorporated into hydrogel. *Die Pharmazie*, **60**(10), pp. 751-755.

UNNITHAN, J., REHMAN, M.U., AHMAD, F.J. and SAMIM, M., 2011. Aqueous synthesis and concentration-dependent dermal toxicity of TiO_2 nanoparticles in Wistar rats. *Biological trace element research*, **143**(3), pp. 1682-1694.

UPADHYAY, P., 2006. Enhanced transdermal-immunization with diptheria-toxoid using local hyperthermia. *Vaccine*, **24**(27-28), pp. 5593-5598.

VALIZADEH, H., MOHAMMADI, G., EHYAEI, R., MILANI, M., AZHDARZADEH, M., ZAKERI-MILANI, P. and LOTFIPOUR, F., 2012. Antibacterial activity of clarithromycin loaded PLGA nanoparticles. *Die Pharmazie*, **67**(1), pp. 63-68.

VAN DER MERWE, D., TAWDE, S., PICKRELL, J.A. and ERICKSON, L.E., 2009. Nanocrystalline titanium dioxide and magnesium oxide *in vitro* dermal absorption in human skin. *Cutaneous and ocular toxicology*, **28**(2), pp. 78-82.

VAN 'T KLOOSTER, G., HOEBEN, E., BORGHYS, H., LOOSZOVA, A., BOUCHE, M.P., VAN VELSEN, F. and BAERT, L., 2010. Pharmacokinetics and disposition of rilpivirine (TMC278) nanosuspension as a long-acting injectable antiretroviral formulation. *Antimicrobial Agents and Chemotherapy*, **54**(5), pp. 2042-2050.

VARGAS, E.A., DO VALE BARACHO, N.C., DE BRITO, J. and DE QUEIROZ, A.A., 2010. Hyperbranched polyglycerol electrospun nanofibers for wound dressing applications. *Acta biomaterialia*, **6**(3), pp. 1069-1078.

VARSHOSAZ, J., HAJHASHEMI, V. and SOLTANZADEH, S., 2011. Lipid nanocapsule-based gels for enhancement of transdermal delivery of ketorolac tromethamine. *Journal of drug delivery*, **2011**, pp. 571272.

VEGA-VILLA, K.R., TAKEMOTO, J.K., Y ¤EZ, J.A., REMSBERG, C.M., FORREST, M.L. and DAVIES, N.M., 2008. Clinical toxicities of nanocarrier systems. *Advanced Drug Delivery Reviews*, .

VEISEH, O., GUNN, J.W., KIEVIT, F.M., SUN, C., FANG, C., LEE, J.S. and ZHANG, M., 2009. Inhibition of tumor-cell invasion with chlorotoxin-bound superparamagnetic nanoparticles. *Small (Weinheim an der Bergstrasse, Germany)*, **5**(2), pp. 256-264.

VEMULA, P.K., ANDERSON, R.R. and KARP, J.M., 2011. Nanoparticles reduce nickel allergy by capturing metal ions. *Nature nanotechnology*, **6**(5), pp. 291-295.

VENUGANTI, V.V. and PERUMAL, O.P., 2009. Poly(amidoamine) dendrimers as skin penetration enhancers: Influence of charge, generation, and concentration. *Journal of pharmaceutical sciences*, **98**(7), pp. 2345-2356.

VENUGANTI, V.V. and PERUMAL, O.P., 2008. Effect of poly(amidoamine) (PAMAM) dendrimer on skin permeation of 5-fluorouracil. *International journal of pharmaceutics*, **361**(1-2), pp. 230-238.

VENUGANTI, V.V., SAHDEV, P., HILDRETH, M., GUAN, X. and PERUMAL, O., 2011. Structure-skin permeability relationship of dendrimers. *Pharmaceutical research*, **28**(9), pp. 2246-2260.

VERMA, P. and PATHAK, K., 2012. Nanosized ethanolic vesicles loaded with econazole nitrate for the treatment of deep fungal infections through topical gel formulation. *Nanomedicine : nanotechnology, biology, and medicine*, **8**(4), pp. 489-496.

VETTOR, M., BOURGEOIS, S., FESSI, H., PELLETIER, J., PERUGINI, P., PAVANETTO, F. and BOLZINGER, M.A., 2010. Skin absorption studies of octyl-methoxycinnamate loaded poly(D,L-lactide) nanoparticles: estimation of the UV filter distribution and release behaviour in skin layers. *Journal of microencapsulation*, **27**(3), pp. 253-262.

VILLALOBOS-HERNANDEZ, J.R. and MULLER-GOYMANN, C.C., 2006. Artificial sun protection: sunscreens and their carrier systems. *Current drug delivery*, **3**(4), pp. 405-415.

VLACHOU, E., CHIPP, E., SHALE, E., WILSON, Y.T., PAPINI, R. and MOIEMEN, N.S., 2007. The safety of nanocrystalline silver dressings on burns: a study of systemic silver absorption. *Burns : journal of the International Society for Burn Injuries*, **33**(8), pp. 979-985.

VOGT, A., COMBADIERE, B., HADAM, S., STIELER, K.M., LADEMANN, J., SCHAEFER, H., AUTRAN, B., STERRY, W. and BLUME-PEYTAVI, U., 2006. 40 nm, but not 750 or 1,500 nm, nanoparticles enter epidermal CD1a+ cells after transcutaneous application on human skin. *The Journal of investigative dermatology*, **126**(6), pp. 1316-1322.

WALKER, M., COCHRANE, C.A., BOWLER, P.G., PARSONS, D. and BRADSHAW, P., 2006. Silver deposition and tissue staining associated with wound dressings containing silver. *Ostomy/wound management*, **52**(1), pp. 42-4, 46-50.

WANG, C.Y. and MAIBACH, H.I., 2013. Immunologic contact urticaria - the human touch. *Cutaneous and Ocular Toxicology*, **32**(2), pp. 154-160.

WANG, J., YUAN, Y., LIU, C., ZHU, D., SHEN, X. and YANG, B., 2009. Preparation and pharmaceutical/pharmacodynamic evaluation of topical brucine-loaded liposomal hydrogel. *Journal of materials science.Materials in medicine*, **20**(10), pp. 2075-2084.

WANG, J.J., LIU, K.S., SUNG, K.C., TSAI, C.Y. and FANG, J.Y., 2009. Skin permeation of buprenorphine and its ester prodrugs from lipid nanoparticles: lipid emulsion, nanostructured lipid carriers and solid lipid nanoparticles. *Journal of microencapsulation*, **26**(8), pp. 734-747.

WANG, S., FALK, M.M., RASHAD, A., SAAD, M.M., MARQUES, A.C., ALMEIDA, R.M., MAREI, M.K. and JAIN, H., 2011. Evaluation of 3D nano-macro porous bioactive glass scaffold for hard tissue engineering. *Journal of materials science.Materials in medicine*, **22**(5), pp. 1195-1203.

WANG, S., HUNTER, L.A., ARSLAN, Z., WILKERSON, M.G. and WICKLIFFE, J.K., 2011. Chronic exposure to nanosized, anatase titanium dioxide is not cyto- or genotoxic to Chinese hamster ovary cells. *Environmental and molecular mutagenesis*, **52**(8), pp. 614-622.

WANG, S.Q. and TOOLEY, I.R., 2011. Photoprotection in the era of nanotechnology. *Seminars in cutaneous medicine and surgery*, **30**(4), pp. 210-213.

WANG, S., TAN, M., ZHONG, Z., CHEN, M. and WANG, Y., 2011. Nanotechnologies for Curcumin: An Ancient Puzzler Meets Modern Solutions. *Journal of Nanomaterials*, , pp. 723178.

WARHEIT, D.B., BORM, P.J., HENNES, C. and LADEMANN, J., 2007. Testing strategies to establish the safety of nanomaterials: conclusions of an ECETOC workshop. *Inhalation toxicology*, **19**(8), pp. 631-643.

WARHEIT, D.B. and EVERITT, J., 2004. Assessing The Biological And Environmental Risks Of Nanoparticulates. 2004, pp. 381.

WEISS-ANGELI, V., BOURGEOIS, S., PELLETIER, J., GUTERRES, S.S., FESSI, H. and BOLZINGER, M.A., 2010. Development of an original method to study drug release from polymeric nanocapsules in the skin. *The Journal of pharmacy and pharmacology*, **62**(1), pp. 35-45.

WEISSE, S., KALIMOUTTOU, S., LAHIANI-SKIBA, M., DJEDAINI-PILARD, F., PERLY, B. and SKIBA, M., 2009. Investigations on topically applied vitamin A loaded amphiphilic cyclodextrin nanocapsules. *Journal of nanoscience and nanotechnology*, **9**(1), pp. 640-645.

WELSHER, K., LIU, Z., SHERLOCK, S.P., ROBINSON, J.T., CHEN, Z., DARANCIANG, D. and DAI, H., 2009. A route to brightly fluorescent carbon nanotubes for near-infrared imaging in mice. *Nature nanotechnology*, **4**(11), pp. 773-780.

WENG, H., ZHOU, J., TANG, L. and HU, Z., 2004. Tissue responses to thermally-responsive hydrogel nanoparticles. *Journal of Biomaterials Science. Polymer Edition*, .

WESCHLER, C.J. and NAZAROFF, W.W., 2012. SVOC exposure indoors: fresh look at dermal pathways. *Indoor air*, **22**(5), pp. 356-377.

WIECHERS, J.W. and MUSEE, N., 2010. Engineered inorganic nanoparticles and cosmetics: facts, issues, knowledge gaps and challenges. *Journal of biomedical nanotechnology*, **6**(5), pp. 408-431.

WIESNER, M.R., LOWRY, G.V., ALVAREZ, P., DIONYSIOU, D. and BISWAS, P., 2006. Assessing the risks of manufactured nanomaterials. *Environmental science & technology*, **40**(14), pp. 4336-4345.

WISNER, E.R., KATZBERG, R.W., GRIFFEY, S.M., HALEY, P.J., JOHNSON, D.K. and VESSEY, A.R., 1996. Characterization of normal and cancerous lymph nodes on indirect computed tomography lymphographic studies after interstitial injection of iodinated nanoparticles. *Academic Radiology*, **3 Suppl 2**, pp. S257-60.

WISSING, S. and MULLER, R., 2002. The influence of the crystallinity of lipid nanoparticles on their occlusive properties. *International journal of pharmaceutics*, **242**(1-2), pp. 377-379.

WISSING, S.A. and MULLER, R.H., 2003. Cosmetic applications for solid lipid nanoparticles (SLN). *International journal of pharmaceutics*, **254**(1), pp. 65-68.

WISSING, S.A. and MULLER, R.H., 2002. Solid lipid nanoparticles as carrier for sunscreens: *in vitro* release and *in vivo* skin penetration. *Journal of controlled release : official journal of the Controlled Release Society*, **81**(3), pp. 225-233.

WISSING, S.A. and MULLER, R.H., 2001. Solid lipid nanoparticles (SLN)--a novel carrier for UV blockers. *Die Pharmazie*, **56**(10), pp. 783-786.

WOKOVICH, A., TYNER, K., DOUB, W., SADRIEH, N. and BUHSE, L.F., 2009. Particle size determination of sunscreens formulated with various forms of titanium dioxide. *Drug development and industrial pharmacy*, **35**(10), pp. 1180-1189.

WOLF, N.B., KUCHLER, S., RADOWSKI, M.R., BLASCHKE, T., KRAMER, K.D., WEINDL, G., KLEUSER, B., HAAG, R. and SCHAFER-KORTING, M., 2009. Influences of opioids and nanoparticles on *in vitro* wound healing models. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, **73**(1), pp. 34-42.

WU XIAOMEI, BRANFORD-WHITE, C.J. and ZHU LIMIN, 2009. *Electrospun cellulose acetate nanofiber mats of naproxen esters*.

WU, J., LIU, W., XUE, C., ZHOU, S., LAN, F., BI, L., XU, H., YANG, X. and ZENG, F.D., 2009. Toxicity and penetration of TiO_2 nanoparticles in hairless mice and porcine skin after subchronic dermal exposure. *Toxicology letters*, **191**(1), pp. 1-8.

WU, J., PAUDEL, K.S., STRASINGER, C., HAMMELL, D., STINCHCOMB, A.L. and HINDS, B.J., 2010. Programmable transdermal drug delivery of nicotine using carbon nanotube membranes. *Proceedings of the National Academy of Sciences of the United States of America*, **107**(26), pp. 11698-11702.

WU, X., GRIFFIN, P., PRICE, G.J. and GUY, R.H., 2009. Preparation and *in vitro* evaluation of topical formulations based on polystyrene-poly-2-hydroxyl methacrylate nanoparticles. *Molecular pharmaceutics*, **6**(5), pp. 1449-1456.

WU, X., LANDFESTER, K., MUSYANOVYCH, A. and GUY, R.H., 2010. Disposition of charged nanoparticles after their topical application to the skin. *Skin pharmacology and physiology*, **23**(3), pp. 117-123.

WU, X., PRICE, G.J. and GUY, R.H., 2009. Disposition of Nanoparticles and an Associated Lipophilic Permeant following Topical Application to the Skin. *Molecular Pharmaceutics*, **6**(5), pp. 1441-1448.

XIA, X.R., MONTEIRO-RIVIERE, N.A. and RIVIERE, J.E., 2010. Intrinsic biological property of colloidal fullerene nanoparticles (nC_{60}): lack of lethality after high dose exposure to human epidermal and bacterial cells. *Toxicology letters*, **197**(2), pp. 128-134.

XIA, X.R., MONTEIRO-RIVIERE, N.A. and RIVIERE, J.E., 2010. Skin penetration and kinetics of pristine fullerenes (C_{60}) topically exposed in industrial organic solvents. *Toxicology and applied pharmacology*, **242**(1), pp. 29-37.

XIA, X.R., MONTEIRO-RIVIERE, N.A. and RIVIERE, J.E., 2006. Trace analysis of fullerenes in biological samples by simplified liquid-liquid extraction and high-performance liquid chromatography. *Journal of chromatography.A*, **1129**(2), pp. 216-222.

XING, J., DENG, L., LI, J. and DONG, A., 2009. Amphiphilic poly graft copolymer nanoparticles as carriers for transdermal drug delivery. *Int J Nanomedicine*, .

XU, J., SAGAWA, Y., FUTAKUCHI, M., FUKAMACHI, K., ALEXANDER, D.B., FURUKAWA, F., IKARASHI, Y., UCHINO, T., NISHIMURA, T., MORITA, A., SUZUI, M. and TSUDA, H., 2011. Lack of promoting effect of titanium dioxide particles on ultraviolet B-initiated skin carcinogenesis in rats. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, **49**(6), pp. 1298-1302.

XU, L., PAN, J., CHEN, Q., YU, Q., CHEN, H., XU, H., QIU, Y. and YANG, X., 2008. *In vivo* evaluation of the safety of triptolide-loaded hydrogel-thickened microemulsion. *Food and Chemical Toxicology*, **46**(12), pp. 3792-3799.

XUE, C., WU, J., LAN, F., LIU, W., YANG, X., ZENG, F. and XU, H., 2010. Nano titanium dioxide induces the generation of ROS and potential damage in HaCaT cells under UVA irradiation. *Journal of nanoscience and nanotechnology*, **10**(12), pp. 8500-8507.

XUE, M., ZHAO, Y., LI, X.J., JIANG, Z.Z., ZHANG, L., LIU, S.H., LI, X.M., ZHANG, L.Y. and YANG, S.Y., 2012. Comparison of toxicokinetic and tissue distribution of triptolide-loaded solid lipid nanoparticles vs free triptolide in rats. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences*, **47**(4), pp. 713-717.

YAH, C.S., SIMATE, G.S. and IYUKE, S.E., 2012. Nanoparticles toxicity and their routes of exposures. *Pakistan journal of pharmaceutical sciences*, **25**(2), pp. 477-491.

YAMAGUCHI, Y., NAGASAWA, T., NAKAMURA, N., TAKENAGA, M., MIZOGUCHI, M., KAWAI, S., MIZUSHIMA, Y. and IGARASHI, R., 2005. Successful treatment of photo-damaged skin of nano-scale atRA particles using a novel transdermal delivery. *Journal of controlled release : official journal of the Controlled Release Society*, **104**(1), pp. 29-40.

YANG, Y., SUNOQROT, S., STOWELL, C., JI, J., LEE, C.W., KIM, J.W., KHAN, S.A. and HONG, S., 2012. Effect of size, surface charge, and hydrophobicity of poly(amidoamine) dendrimers on their skin penetration. *Biomacromolecules*, **13**(7), pp. 2154-2162.

YANG, Z., LIANG, G., MA, M., ABBAH, A.S., LU, W.W. and XU, B., 2007. D-glucosamine-based supramolecular hydrogels to improve wound healing. *Chem Commun (Camb)*, .

YOKEL, R.A. and MACPHAIL, R.C., 2011. Engineered nanomaterials: exposures, hazards, and risk prevention. *J Occup Med Toxicol*, .

YOSHIDA, T., YOSHIOKA, Y. and TSUTSUMI, Y., 2012. [The safety assessment of nanomaterials for development of nano-cosmetics]. *Yakugaku zasshi. Journal of the Pharmaceutical Society of Japan*, .

YOSHIOKA, Y., YOSHIKAWA, T. and TSUTSUMI, Y., 2010. Nano-safety science for assuring the safety of nanomaterials. *Nihon eiseigaku zasshi.Japanese journal of hygiene*, **65**(4), pp. 487-492.

YUAN, H., FALES, A.M. and VO-DINH, T., 2012. TAT peptide-functionalized gold nanostars: enhanced intracellular delivery and efficient NIR photothermal therapy using ultralow irradiance. *Journal of the American Chemical Society*, **134**(28), pp. 11358-11361.

YUE, S., SLIPCHENKO, M.N. and CHENG, J., 2011. Multimodal nonlinear optical microscopy. *Laser & Photonics Reviews*, **5**(4), pp. 496-512.

YUE, Y., ZHOU, H., LIU, G., LI, Y., YAN, Z. and DUAN, M., 2010. The advantages of a novel CoQ10 delivery system in skin photo-protection. *International journal of pharmaceutics*, **392**(1-2), pp. 57-63.

ZAGAYNOVA, E.V., SHIRMANOVA, M.V., KIRILLIN, M.Y., KHLEBTSOV, B.N., ORLOVA, A.G., BALALAEVA, I.V., SIROTKINA, M.A., BUGROVA, M.L., AGRBA, P.D. and KAMENSKY, V.A., 2008. Contrasting properties of gold nanoparticles for optical coherence tomography: phantom, *in vivo* studies and Monte Carlo simulation. *Physics in Medicine and Biology*, **53**(18), pp. 4995-5009.

ZAVAN, B., VINDIGNI, V., VEZZU, K., ZORZATO, G., LUNI, C., ABATANGELO, G., ELVASSORE, N. and CORTIVO, R., 2009. Hyaluronan based porous nano-particles enriched with growth factors for the treatment of ulcers: a placebo-controlled study. *Journal of materials science.Materials in medicine*, **20**(1), pp. 235-247.

ZHANG QING, LI RUI, ZHAI YONG-AI, LIU FENG-QI and GAO GE, 2010. Synthesis of Octylmethoxycinamate-silica Core-shell Nanoparticles with Self-templating Method. *Chemical Research in Chinese Universities*, **26**(3), pp. 339-343.

ZHANG, B., LUO, Y. and WANG, Q., 2011. Development of silver/α-lactalbumin nanocomposites: a new approach to reduce silver toxicity. *International journal of antimicrobial agents*, .

ZHANG, J., LIANG, Y., LI, N., LI, X., HU, R., XING, J., DENG, L., HU, F. and DONG, A., 2012. Thermosensitive hydrogel based on poly(ether-ester anhydride) nanoparticle as drug delivery system: preparation, characterization and biocompatibility. *Colloids and surfaces.B, Biointerfaces*, **96**, pp. 56-61.

ZHANG, J. and SMITH, E., 2011. Percutaneous permeation of betamethasone 17-valerate incorporated in lipid nanoparticles. *Journal of pharmaceutical sciences*, **100**(3), pp. 896-903.

ZHANG, L.W., AL-SUWAYEH, S.A., HUNG, C.F., CHEN, C.C. and FANG, J.Y., 2011. Oil components modulate the skin delivery of 5-aminolevulinic acid and its ester prodrug from oil-in-water and water-in-oil nanoemulsions. *Int J Nanomedicine*, .

ZHANG, L.W. and MONTEIRO-RIVIERE, N.A., 2013. Use of confocal microscopy for nanoparticle drug delivery through skin. *Journal of Biomedical Optics*, **18**(6), pp. 061214.

ZHANG, L.W. and MONTEIRO-RIVIERE, N.A., 2010. Lectins modulate multi-walled carbon nanotubes cellular uptake in human epidermal keratinocytes. *Toxicology in vitro : an international journal published in association with BIBRA*, **24**(2), pp. 546-551.

ZHANG, L.W. and MONTEIRO-RIVIERE, N.A., 2008. Assessment of quantum dot penetration into intact, tape-stripped, abraded and flexed rat skin. *Skin pharmacology and physiology*, **21**(3), pp. 166-180.

ZHANG, L.W., YU, W.W., COLVIN, V.L. and MONTEIRO-RIVIERE, N.A., 2008. Biological interactions of quantum dot nanoparticles in skin and in human epidermal keratinocytes. *Toxicology and applied pharmacology*, **228**(2), pp. 200-211.

ZHANG, L.W., BAEUMER, W. and MONTEIRO-RIVIERE, N.A., 2011. Cellular uptake mechanisms and toxicity of quantum dots in dendritic cells. *Nanomedicine*, **6**(5), pp. 777-791.

ZHANG, W., GAO, J., ZHU, Q., ZHANG, M., DING, X., WANG, X., HOU, X., FAN, W., DING, B., WU, X., WANG, X. and GAO, S., 2010. Penetration and distribution of PLGA nanoparticles in the human skin treated with microneedles. *International journal of pharmaceutics*, **402**(1-2), pp. 205-212.

ZHANG, W., KALIVE, M., CAPCO, D.G. and CHEN, Y., 2010. Adsorption of hematite nanoparticles onto Caco-2 cells and the cellular impairments: effect of particle size. *Nanotechnology*, **21**(35), pp. 355103-4484/21/35/355103. Epub 2010 Aug 9.

ZHANG, W., DING, B., TANG, R., DING, X., HOU, X., WANG, X., GU, S., LU, L., ZHANG, Y., GAO, S. and GAO, J., 2011. Combination of Microneedles with PLGA Nanoparticles as a Potential Strategy for Topical Drug Delivery. *Current Nanoscience*, **7**(4), pp. 545-551.

ZHANG, X., ZHANG, T., WANG, B., TANG, N., SUN, L. and LUO, Q., 2011. Preparation and characterization of human malignant melanoma ganglioside ScFv antibody conjugated quantum dot probe. *African Journal of Pharmacy and Pharmacology*, **5**(24), pp. 2689-2693.

ZHANG, Y., GAO, J., ZHENG, H., ZHANG, R. and HAN, Y., 2011. The preparation of 3,5-dihydroxy-4-isopropylstilbene nanoemulsion and *in vitro* release. *International journal of nanomedicine*, **6**, pp. 649-657.

ZHANG, Y.T., HUANG, Z.B., ZHANG, S.J., ZHAO, J.H., WANG, Z., LIU, Y. and FENG, N.P., 2012. *In vitro* cellular uptake of evodiamine and rutaecarpine using a microemulsion. *International journal of nanomedicine*, **7**, pp. 2465-2472.

ZHANG, Z., KONG, F., VARDHANABHUTI, B., MUSTAPHA, A. and LIN, M., 2012. Detection of engineered silver nanoparticle contamination in pears. *Journal of Agricultural and Food Chemistry*, **60**(43), pp. 10762-10767.

ZHAO, Q.H., ZHANG, Y., LIU, Y., WANG, H.L., SHEN, Y.Y., YANG, W.J. and WEN, L.P., 2010. Anticancer effect of realgar nanoparticles on mouse melanoma skin cancer *in vivo* via transdermal drug delivery. *Medical oncology (Northwood, London, England)*, **27**(2), pp. 203-212.

ZHAO, X., ZU, Y., ZU, S., WANG, D., ZHANG, Y. and ZU, B., 2010. Insulin nanoparticles for transdermal delivery: preparation and physicochemical characterization and *in vitro* evaluation. *Drug development and industrial pharmacy*, **36**(10), pp. 1177-1185.

ZHAO, Y., BROWN, M.B. and JONES, S.A., 2010. The effects of particle properties on nanoparticle drug retention and release in dynamic minoxidil foams. *International journal of pharmaceutics*, .

ZHAO, Y., BROWN, M.B. and JONES, S.A., 2010. The topical delivery of benzoyl peroxide using elegant dynamic hydrofluoroalkane foams. *Journal of pharmaceutical sciences*, **99**(3), pp. 1384-1398.

ZHAO, Y., MODDARESI, M., JONES, S.A. and BROWN, M.B., 2009. A dynamic topical hydrofluoroalkane foam to induce nanoparticle modification and drug release in situ. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, **72**(3), pp. 521-528.

ZHAO, Y., BROWN, M.B. and JONES, S.A., 2010. Pharmaceutical foams: are they the answer to the dilemma of topical nanoparticles? *Nanomedicine-Nanotechnology Biology and Medicine*, **6**(2), pp. 227-236.

ZHENG, D., GILJOHANN, D.A., CHEN, D.L., MASSICH, M.D., WANG, X., IORDANOV, H., MIRKIN, C.A. and PALLER, A.S., 2012. Topical delivery of siRNA-based spherical nucleic acid nanoparticle conjugates for gene regulation. *Proceedings of the National Academy of Sciences of the United States of America*, **109**(30), pp. 11975-11980.

ZHENG, W.W., ZHAO, L., WEI, Y.M., YE, Y. and XIAO, S.H., 2010. Preparation and the *in vitro* evaluation of nanoemulsion system for the transdermal delivery of granisetron hydrochloride. *Chemical & pharmaceutical bulletin*, **58**(8), pp. 1015-1019.

ZHOU GUO-QIANG, CHEN CHUN-YING, LI YU-FENG, LI WEI, GAO YU-XI and ZHAO YU-LIANG, 2008. Recent progress on the pro and cons of biological effects of nanomaterials. *Progress in Biochemistry and Biophysics*, **35**(9), pp. 998-1006.

ZHU, X.L., LI, G.F., ZENG, K. and CHENG, Z.L., 2010. Preparation of lidocaine nanoemulsion and its transdermal absorption by rat skin *ex vivo*. *Nan fang yi ke da xue xue bao = Journal of Southern Medical University*, **30**(3), pp. 451-454.

ZIPPIN, J.H. and FRIEDMAN, A., 2009. Nanotechnology in Cosmetics and Sunscreens: An Update. *Journal of Drugs in Dermatology*, **8**(10), pp. 955-958.

ZVYAGIN, A.V., ZHAO, X., GIERDEN, A., SANCHEZ, W., ROSS, J.A. and ROBERTS, M.S., 2008. Imaging of zinc oxide nanoparticle penetration in human skin *in vitro* and *in vivo*. *Journal of Biomedical Optics*, **13**(6), pp. 064031.

The following are additional references not included in the peer reviewed literature:

AITKEN, R.J., ASCHBERGER, K., BAUN, A., CHRISTENSEN, F., FERNANDES, T., HANSEN, S.F., HARTMANN, N.B., HUTCHISON, G., JOHNSTON, H., MICHELETTI, C., PETERS, S., ROSS, B., SOKULL-KLEUTTGEN, B., STARK, D., TRAN, L., 2009. ENRHES Final Report "Engineered Nanoparticles: A Review of Health and Environmental Safety" European Commission. EC Project Number 218433.

BUTZ, T., REINERT, T., PINHEIRO, T., MORETTO, P., PALLON, J., KISS, A.Z., STACHURA, J., DABROS, W., STACHURA, A., LEKKI, J., LEKKA., M., HUNYADI, J., BIRO, T., STICHERLING, M., VAN VAECK, L., VAN ROYEN, P., SURLEVE-BASEILLE, J., 2007. NANODERM Final Report "Quality of skin as a barrier to ultra-fine particles". European Commission. Project Number QLK4-CT-2002-02678

DANISH MINISTRY OF THE ENVIRONMENT, ENVIRONMENTAL PROTECTION AGENCY, 2011. Survey of the basic knowledge about exposure and potential environmental and health risks for selected nanomaterials. DEPA Environmental Project No. 1370 2011

DANISH MINISTRY OF THE ENVIRONMENT, ENVIRONMENTAL PROTECTION AGENCY,2012. Assessment of nanosilver in textiles on the Danish market. DEPA Environmental Project No. 1432 2012

Department for Environment, Food and Rural Affairs (DEFRA), 2006. Characterising the potential risks posed by engineered nanomaterials. Department for Environment, Food and Rural Affairs

EUROPEAN COMMISSION JOINT RESEARCH COUNCIL, 2007. Testing Strategies for the Prediction of Skin and Eye Irritation and Corrosion for Regulatory Purposes. European Commission. EC EUR 22881 EN

EUROPEAN COMMISSION, 2002. Opinion of the Scientific Committee on plants on Commission Draft Guidance on Dermal Absorption. European Commission. EC SANCO/222/2000-rev4

EUROPEAN COMMISSION. 2004. Guidance Document on Dermal Absorption. European Commission. EC SANCA/222/2000 rev.7

National Institute for Public Health and the Environment (RIVM), 2011. Risks of systemic effects after dermal exposure for workers, Part A: Proposed approaches for risk evaluation. National Institute for Public Health and the Environment (RIVM). RIVM Letter Report 320041001

National Institute for Public Health and the Environment (RIVM), 2011. Risks of systemic effects after dermal exposure for workers, Part B: Inventory of substances of which systemic health effects can be expected due to dermal exposure of workers. National Institute for Public Health and the Environment (RIVM). RIVM Letter Report 320041002

NOHYNEK, G.J., 2006. Nanoparticles and the skin- A health risk for the consumer? L'Oreal Global Safety Evaluation, Berlin.

OECD, 2004. Guidance document for the conduct of skin absorption studies.

OECD. Document Ref ENV/JM/MONO (2004)2.

OECD, 2008. Guidance notes for the estimation of dermal absorption values. OECD. DRAFT.

OECD, 2009. Preliminary review of OECD test guidelines for their applicability to manufactured nanomaterials. OECD. Document Ref ENV/MONO(2009)21

OECD, 2010. Explanatory background document to the OECD test guideline on *in vitro* skin irritation testing. OECD. Document Ref ENV/MONO(2010)36

OECD, 2010. Guidance manual for the testing of manufactured nanomaterials: OECD's sponsorship programme; first revision. OECD. Document Ref ENV/JM/MONO(2009)20/REV

OECD, 2011. Guidance notes on dermal absorption, OECD. Document Ref ENV/JM/MONO(2011)36

OECD, 2011. Peer review of the validation of the skin irritation test using labcyte EPI-Model 24. OECD. Document Ref ENV/JM/MONO(2011)44

OECD, 2012. Guidance on sample preparation and dosimetry for the safety testing of manufactured nanomaterials. OECD. Document Ref ENV/MONO(2012)40

SAFEWORK AUSTRALIA, 2006. A review of the potential occupational health and safety implications of nanotechnology. Safework Australia

Scientific Committee on Consumer Products (SCCP), 2006. Opinion on: Basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients. Scientific Committee on Consumer Products (SCCP). Document Ref SCCP/0970/06

Scientific Committee on Consumer Products (SCCP), Scientific Committee on Consumer Safety (SCCS) and Scientific Committee of Emerging and Newly Identified Health Risks (SCENIHR), 2012. Opinion on: Use of the Threshold of Toxicological Concern (TCC) approach for human safety assessment of chemical substances with focus on cosmetics and consumer products. Scientific Committee of Emerging and Newly Identified Health Risks (SCENIHR). Document Ref SCCP/1171/08

Scientific Committee on Consumer Safety (SCCS), 2012. Guidance on the safety assessment of nanomaterials in cosmetics. Scientific Committee on Consumer Safety (SCCS). Document Ref SCCS/1484/12

Scientific Committee on Consumer Safety (SCCS), 2012. The SCCS's notes of guidance for the testing of cosmetic substances and their safety evaluation. 8th Revision. Scientific Committee on Consumer Safety (SCCS) Document Ref SCCS/1501/12

STARONOVA, K., NIELSEN, J.B., ROURSGAARD, M., KNUDSEN, L.E., 2012. Transport of SiO_2 nanoparticles through human skin, Basic and Clinical Pharmacology & Toxicology, 111, pp. 142-144.

The National Institute for Occupational Safety and Health (NIOSH), Skin permeation calculator.[online] Atlanta, USA. Available from: http://www.cdc.gov/niosh/topics/skin/skinPermCalc.html

U.S ENVIRONMENTAL PROTECTION AGENCY, 1984. Review of Dermal Absorption. Environmental Protection Agency. Ref. EPA/600/8-84/033

U.S ENVIRONMENTAL PROTECTION AGENCY, 2010. State of the Science Literature Review: Everything Nanosilver and More. Environmental Protection Agency. EPA Ref EPA/600/R-10/084

U.S ENVIRONMENTAL PROTECTION AGENCY, 2010. State of the Science Literature Review: Nano Titanium Dioxide Environmental Matters. Environmental Protection Agency. EPA Ref EPA/ 600/R-10-089

WORLD HEALTH ORGANIZATION, 2006. Dermal Absorption. WHO, Environmental Health Criteria 235.

Appendix 2: Summary Table of Nanoparticle Physicochemical Properties and Dermal Penetration

Chemical Composition	Primary Particle Size [nm]	Shape	Surface chemistry/ functionalisation	Surface Charge	Other Physico-chemical Properties	Vehicle	Model	Species [H/P/R]	Level of Penetration* [NP/SC/SG/SS /SGV/SP/SR]	Total Appraisal Score	High Interest	Ref.	Data-base ID
Titanium dioxide	Mean: 26.4 ± 9.5 Range: 3-54	Polyhedral	-	-	Crystal structure: anatase	Oil/water	In vivo - direct application	R	SC	22		Adachi et al., 2010	3
Polystyrene (FluoSpheres®)	200 and 20	Spherical	Carboxylated, fluorescent	Negative and polar surface charge (unquantified)	-	-	Ex vivo - diffusion cell	P	SC	17		Alvarez-Roman et al., 2004	8
Cobalt-ferrite magnetic Nanoparticles	50	-	Coated with silica-containing fluorescent dye and then PEG-coated	Uncharged	Labelling: RITC	-	In vivo – sub-cutaneous injection	R	NA	17		Baik et al., 2008	12
Iron (III) oxide (γ-Fe2O3)	5.9 ± 2.5	-	Trimethylammonium hydroxide (TMAOH)-coated	Isoelectric point of 6.3 (negatively or positively charged when exposed to a pH either greater or less than 6.3, respectively)	-	-	Ex vivo - diffusion cell	H	SC, occasionally reaching the viable epidermis	19	✓	Baroli et al., 2007	15
Iron	4.9 ± 1.3	-	AOT-coated	-									
Polystyrene (FluoSpheres®)	200, 100 and 20	Spherical	Carboxylated, fluorescent	-	-	dH_2O	Ex vivo - diffusion cell	P	SC, only uppermost surface layers	16		Campbell et al., 2012	27
Copper oxide	35	-	-	Zeta potential peak at +26mV	-	ddH_2O or DMEM	Ex vivo - culture system	H	SC	20		Cohen et al., 2013	34
Zinc oxide	Range: 26-30	-	-	-	-	Suncreen formulation	Ex vivo - diffusion cell	H	SC, only uppermost surface layers	17		Cross et al., 2007	37
Zinc oxide	30	-	-	-	-	Suncreen formulation	Human	H	SC	20		Darvin et al., 2011	39
Multi-walled carbon nanotubes	-	Elongated tubular shape	PEGylated and non-PEGylated; ~ 4% carboxylated	-	-	Poroylene glycol, water	Ex vivo – diffusion cell	R/P	Permeated through rat skin	13		Degim et al., 2010	41

Chemical Composition	Primary Particle Size [nm]	Shape	Surface chemistry/ functionalisation	Surface Charge	Other Physico-chemical Properties	Vehicle	Model	Species [H/P/R]	Level of Penetration* [NP/SC/SG/SS /SGV/SP/SR]	Total Appraisal Score	High Interest	Ref.	Data-base ID
Double-walled carbon nanotubes	-	Elongated tubular shape	~1% carboxylated						Permeated through rat skin				
Titanium dioxide	20	Needle-shaped	Coated (no further description)	-	Crystal structure: rutile	Commercial sunscreens containing i) TiO2 only (TiA); ii) TiO2 and ZnO (TiB); and iii) rutile TiO2 (TiHB)	In vivo	H	SC, only uppermost surface layers	19		Filipe et al., 2009	47
Zinc oxide	20-60 nm	Spherical							SC, only uppermost surface layers				
Gold	12.6 ± 0.9	-	-	-	-	Synthetic Sweat	Ex vivo - diffusion cell	H	Dermis	23		Filon et al., 2011	48
Titanium dioxide	Length: 50-100 Diameter: 120-20	Spindle-shaped	Uncoated, and coated with aluminium hydroxide and stearic acid	-	-	Pentalan 408	In vivo - Direct application	R	ND	19		Furukawa et al., 2011	52
Titanium dioxide	21	Spherical platelets	-	-	Crystal structure: Mixture of rutile and anatase	Carbomergel, hydrophobic basisgel and poly-acrylategel	Human	H and P	SC (topmost 3-5 corneocyte layers)	23	✓	Gontier et al., 2008	53
Titanium dioxide	Length: 100 Diameter: 20	Rod-shaped	Aluminium oxide- and silica-coated		Crystal structure: rutile	Carbomergel, hydrophobic basisgel and poly-acrylategel							
Quantum dots (CdSe core/CdS shell)	37 ± 1	-	PEG-coated	-	-	Oil/water	In vivo - direct application	R	No consistent cadmium elevation detected in the sentinel organs of mice with intact, tape stripped, or acetone treated skin post application. In mice with dermabraded skin, however, cadmium elevations were detected in the lymph nodes and liver	24	✓	Gopee et al., 2009	54

Chemical Composition	Primary Particle Size [nm]	Shape	Surface chemistry/ functionalisation	Surface Charge	Other Physico-chemical Properties	Vehicle	Model	Species [H/P/R]	Level of Penetration* [NP/SC/SG/SS/SGV/SP/SR]	Total Appraisal Score	High Interest	Ref.	Data-base ID
Quantum dots (CdTe core)	Core: 3.5 Hydro-dynamic diameter: 9.5 ± 0.8	-	-	-	-	-	Ex vivo - Saarbrueck en	H	Distributed on skin surface, along edges on corneocytes. QD could only be found in deeper layers after massaging damaged skin for 10 min.	22		Gratieri et al., 2010	57
Zinc oxide	19 ± 8 and 110 ± 46	-	-	-	Enriched with ^{68}Zn (to either 51% or > 99%)	Oil/water	Human	H	Zn from the applied ZnO sunscreen was detectable in blood and urine	25	✓	Gulson et al., 2010	58
Zinc oxide	30	-	-	-	Enriched with ^{68}Zn	Oil/water	Human	H	Small levels of 68Zn detectable in the blood and urine	24		Gulson et al., 2012	59
Quantum dots (CdSe core/ZnS shell)	7 ± 2	Spherical	PEG-amine coated	Positively charged (unquantified)	-	-	Human	H	SC	19		Jeong et al., 2010	66
							In vitro - diffusion cell	H (EPiSkin)					
Polystyrene (Fluoresbrite®)	50	Spherical	-	-	-	PBS	Ex vivo - diffusion cell	R	No penetration in intact skin, SC-stripped skin or razor-treated skin. Permeated into the receiver compartment was only observed with needle-punctured skin.	21		Kimura et al., 2012	71
Fluorescein isothiocyanate-dextrans	2.8, 6.6, 9.0, 12.0, 21, 41.6	-							None				
Titanium dioxide	Not stated	-	-	-	-	Commercial sunscreen	Ex vivo - diffusion cell	P	No penetration, distributed into skin grooves and follicles but seldom migrated to viable epidermis or dermis	21		Kimura et al., 2012	71
Zinc oxide													
Titanium dioxide	-	-	-	-	-	Commercial sunscreen	In vivo – direct application	R (grafted with human skin)	NP	19		Kiss et al., 2008	73
	9						In vitro – single cell	H	-				

Chemical Composition	Primary Particle Size [nm]	Shape	Surface chemistry/ functionalisation	Surface Charge	Other Physico-chemical Properties	Vehicle	Model	Species [H/P/R]	Level of Penetration* [NP/SC/SG/SS /SGV/SP/SR]	Total Appraisal Score	High Interest	Ref.	Data-base ID
Latex copolymer particles	50, 100, 200 and 500	-	-	Positive, negative and neutrally charged (unquantified)	Fluorescent	H_2O	Ex vivo - diffusion cell	P	SC	19		Kohli et al., 2004	74
Gold	AuNP1: 6.00 ± 0.81 AuNP2: 6.00 ± 0.81 AuNP3: 14.90 ± 1.76 AuNP3: 14.90 ± 1.76	-	AuNP1: Dodecanethiol AuNP2: Lecithin AuNP3: Citrate ions AuNP3: Cetrimide	AuNP1: Uncharged AuNP2: -53.5 ± 1.44 mV AuNP3: -35.1 ± 1.87 mV AuNP3: Uncharged	-	AuNP1: Toluene AuNP2: Water AuNP3: Water AuNP3: Toluene	Ex vivo - diffusion cell	H	SC	23	✓	Labouta et al., 2011	75
Gold	AuNP-Aq:14.9 ± 1.8 AuNP-TOL: 6.0 ± 0.8	-	AuNP-Aq:citrate ions AuNP-TOL: dodecanethiol	AuNP-Aq: -35.1 ± 1.9 mV AuNP-TOL: Uncharged	-	AuNP-Aq: water AuNP-TOL: Toluene	Ex vivo - diffusion cell	H	10 cell layers deep and SG (Toluene treated group only)	21		Labouta et al., 2011	79
Gold	AuNP1 and AuNP2: 14.9 ± 1.8	-	AuNP1: citrate stabalised AuNP2: TGA and cetrimide coated	AuNP1: -35.1 ± 1.9 mV AuNP2: Uncharged	-	Urea, sodium lauryl sulphate, polysorbate 80 and DMSO	Ex vivo - diffusion cell	H	SC and DSL with increased penetration with DMSO in the case of hydrophilic particles only	21		Labouta et al., 2012	76
Gold	15	-	Citrate stabilised	Zeta potential: -35.1 ± 1.9 mV	Hydrophilic	Cell culture Medium	In vitro – six well plate	H (skin equiva-lents)	SC	20		Labouta et al., 2013	81
Polymer (prepared from Resomer)	320	-	-	-	Labelling: 5-fluoresc-einamine	Hydrogel formulation	Human	H	SC and hair follicles	13		Lademann et al., 2006	83

Chemical Composition	Primary Particle Size [nm]	Shape	Surface chemistry/ functionalisation	Surface Charge	Other Physico-chemical Properties	Vehicle	Model	Species [H/P/R]	Level of Penetration* [NP/SC/SG/SS /SGV/SP/SR]	Total Appraisal Score	High Interest	Ref.	Data-base ID
Polymer	320	-	-	-	Labelling: 5-fluoresc-einamine	3% hydroxyl-ethylcellulose hydrogel (Natrosol type 250 M pharma)	Human	H	The nanoparticles were stored in the hair follicles up to 10 days, while the non-particle form could be detected only up to 4 days.	22		Lademann et al., 2007	85
							In vitro	P	Penetration into the hair shaft and increased with massage				
Cobalt	80	-	-	-	-	Synthetic Sweat	Ex vivo - diffusion cell	H	Penetration - Results showed a progressive penetration of cobalt into the receiving fluid which increased with time and that this was greater for the abraded skin than for the undamaged skin.	23		Filon et al., 2013	88
Silver	25 ± 7.1	-	Polyvinylpirrolidine-coated	-	-	Synthetic Sweat	Ex vivo - diffusion cell	H	SC, and upper layers of the epidermis. Penetration through damaged skin was five times greater than through intact skin.	20		Larese et al., 2009	89
Gold	Length: 40 Diameter: 18	Rod-shaped	CTAB-, CTAB-PSS-, and CTAB-PSS-PDADMAC-coated	CTAB Au: +31.3 CTAB-PSS Au: -66.3 CTAB-PSS-PDADMAC Au: +61.1	-	Cell Culture Media	Ex vivo - diffusion cell	R	SC, uniform distribution in upper epidermis with percutaneous absorption	21		Lee et al., 2013	93
Gold	10, 30 and 60	-	-	-	-	Saline/ Sodium Citrate	Ex vivo - diffusion cell	H	SC, no significant penetration into the lower layers	27		Liu et al., 2012	96

Chemical Composition	Primary Particle Size [nm]	Shape	Surface chemistry/ functionalisation	Surface Charge	Other Physico-chemical Properties	Vehicle	Model	Species [H/P/R]	Level of Penetration* [NP/SC/SG/SS /SGV/SP/SR]	Total Appraisal Score	High Interest	Ref.	Data-base ID
Titanium dioxide	Length: 50-150 Diameter: 20-30	Needle-shaped	Alumina-coated	-	-	Suncreen formulation	Ex vivo – diffusion cell	P	SC	22		Miquel-Jeajean et al., 2012	101
Titanium dioxide	Length: 50 Diameter: 10	Rod-shaped	Hydrated silica, dimethicone/methicone copolymer and aluminium hydroxide coated	-	Crystal structure: Rutile Specfic surface area: 100 m^2/g.	Oil/water	In vivo - direct application	P	SC, 13 layers in UVB -damaged skin; 7 layers in normal skin; minimal transdermal absorption	26	✓	Monteiro-Riviere et al., 2011	107
Zinc oxide	Mean: 140 Range: 60-200	-	Uncoated, and coated with triethoxycaprylylsilane		Specific surface area: 12-24 m^2/g				SC, localised in upper 1-2 layers in both UVB-damaged and normal skin; minimal transdermal absorption				
Titanium dioxide	Length: 50 Diameter: 10	Rod-shaped	Hydrated silica, dimethicone/methicone copolymer and aluminium hydroxide coated		Crystal structure: Rutile		In vitro - diffusion cell		SC, localised in upper 9 layers in normal skin; 13 layers in UVB-damaged skin; minimal transdermal absorption				
Zinc oxide	Mean: 140 Range: 60-200	-	Uncoated, and coated with triethoxycaprylylsilane		-				SC, up to 10 layers deep in UVB-damaged skin; on or immediately above the surface in normal skin uppermost layers, minimal transdermal absorption.				
Quantum dots (CdSe/ZnS core/shell)	Range: 14-15	-	DLHA-coated	Zeta potential: -40 mV	-	Glycerol	In vivo - direct application	R	No UVR = SC, UVR = scarce but higher levels of penetration through the SC	23	✓	Mortensen et al., 2012	113
Quantum dots (CdSe/ZnS core/shell)	Range: 20-33	-	DLHA-coated	Zeta potential: - 20 mV	-	Glycerol	In vivo - direct application	R	Dermis	22		Mortensen et al., 2008	114

Chemical Composition	Primary Particle Size [nm]	Shape	Surface chemistry/ functionalisation	Surface Charge	Other Physico-chemical Properties	Vehicle	Model	Species [H/P/R]	Level of Penetration* [NP/SC/SG/SS/SGV/SP/SR]	Total Appraisal Score	High Interest	Ref.	Data-base ID
Quantum dots (CdSe/ZnS core/shell)	12-20	-	DHLA-functionalised	- 45 mV in water, - 40 mV in glycerol, -35 mV in OPTI-MEM	-	50:50 Glycerol/ DHLA mixture	In vivo	R	Folds and hair follicles	20		Mortensen et al., 2009	117
							In vitro – single cell	H	-				
Quantum dots (CdSe/ZnS core/shell)	19	-	DHLA-coated	Zeta potential: -45 ± 5 mV	-	Cell Cultue Media	In vitro - single cell	H	Differentiated keratinocytes were less effected by UVB exposure and more resistant to QD penetration and uptake	22		Mortensen et al., 2012	115
Silica	70, 300 and 1000	Spherical	Plain, non-porous	Zeta potential: 70 nm: -21.6 ± 4.5 mV 300 nm: -31.3 ± 6.5 mV 1000 nm: -37.7 ± 4.6 mV	Amorphous Fluorescent, (ref-F)-labelled Specific surface area: 70 nm: 43 m_2/g 30 nm: 10 m_2/g 1000 nm: 3 m_2/g	PBS with 10% isopropyl myrisate	In vivo - direct application	R	Penetrated the SC and entered the skin, lymph node, liver, cerebral cortex and hippocampus.	23	✓	Nabeshi et al., 2011	120
Quantum dots (composition not stated)	35		PEG-coated	-	-								
Quantum dots (CdSe/ZnS core/shell)	PEG: 35 PEG-amine: 15 PEG-carboxyl: 14	Spherical	PEG-, PEG-amine-, PEG carboxyl- coated	Zeta potential PEG: -9.6 ± 4.3 mV at pH 7.0; -2.7 ± 6.0 mV at pH 8.3. PEG-amine: -16.8 ± 5.4 mV at pH 7.0; -7.8 ± 8.1 mV at pH 8.3. PEG-carboxyl: -44.0 ± 10.9 mV at pH 7.0; -30.9 ± 2.8	-	Borate buffer	Ex vivo - diffusion cell	H	SC, only for PEG-QD at pH 8.3	25		Prow et al., 2012	137

Chemical Composition	Primary Particle Size [nm]	Shape	Surface chemistry/ functionalisation	Surface Charge	Other Physico-chemical Properties	Vehicle	Model	Species [H/P/R]	Level of Penetration* [NP/SC/SG/SS /SGV/SP/SR]	Total Appraisal Score	High Interest	Ref.	Data-base ID
				mv at pH 9.0.									
Silica	42 ± 3, 75 ± 6, 190 ± 9 and 291 ± 9	-	Uncoated and (3-amino-propyl)-trimethoxysilane (APS) coated	Uncoated: *42 nm:* -22 ± 3 mV at pH = 7; -2 ± 1 mV at pH = 7.4. *75 nm:* -45 ± 4 mV at pH = 7; -2 ± 1 mV at pH = 7.4. *190 nm:* -56 ± 5 mV at pH = 7; -1 ± 2 mV at pH = 7.4. *291 nm:* -48 ± 2 mV at pH = 7; aggregated at pH = 7.4. APS-coated: *42 nm:* +12 ± 2 mV at pH = 7; +4 ± 1 mV at pH = 7.4. *75 nm:* +11 ± 3 mV at pH = 7; +2 ± 2 mV at pH = 7.4. *190 nm:* +10 ± 1 mV at pH = 7; +1 ± 2 mV at pH = 7.4. *291 nm:* +10 ± 2 mV at pH = 7; aggregated at pH = 7.4.	Amorphous Labelled: FITC	PBS	Ex vivo - SC removed	H	SC with only 42 nm particles penetrating to the lower epidermis	24		Rancan et al., 2012	138
						Cell Culture Media	In vitro - single cell		Positive charge resulted in higher cell uptake in cultured cells, lower in primary cells.				

Chemical Composition	Primary Particle Size [nm]	Shape	Surface chemistry/ functionalisation	Surface Charge	Other Physico-chemical Properties	Vehicle	Model	Species [H/P/R]	Level of Penetration* [NP/SC/SG/SS/SGV/SP/SR]	Total Appraisal Score	High Interest	Ref.	Data-base ID
Quantum dot (CdSe/ZnS core/shell)	13.6 ± 0.4	-	DHLA-coated	-26.2 mV	-	Thioglycolate-containing depilatory agent	Human Ex vivo	H	SC	25		Ravichandran et al., 2011	140
Fullerene-substituted phenylalanine (Baa) derivative of a nuclear localization peptide sequence (Baa-Lys(FITC)-NLS)	3.5	-	-	-	-	PBS	Ex vivo - diffusion cell	P	SG	22	✓	Rouse et al., 2007	143
Quantum dots	Spherical: 4.6 Ellipsoid: Major axis 12, minor axis 6	Spherical and elliptical core/shell shapes	PEG-, PEG-amine-, PEG carboxyl- coated	PEG: neutral PEG-amine: positive PEG-COOH: negative (unquantified)	-	Borate buffer	Ex vivo - diffusion cell	P	SC, some penetration to epidermis/dermis	24	✓	Ryman-Rasmussen et al., 2006	144
Titanium dioxide	Particle size of TiO2 in raw material and formulation: Sub-micron TiO2: 300-500 Uncoated nano-TiO2: 30-50 Coated nano-TiO2: Diameter: 20-30 Length: 50-150	-	Micron-TiO2: uncoated Nano-TiO2: uncoated and dimethicone/methicone copolymer-coated	-	Crystal structure: Micron-TiO2: rutile Uncoated nano-TiO2: Mixture of rutile and anatase Coated nano-TiO2: rutile	Sunscreen formulation	In vivo - direct application	P	SC, some penetration to dermis	20	✓	Sadrieh et al., 2010	148
Silver	Unwashed/uncoated: 20, 50, and 80 Washed/uncoated: 20, 50, and 80 Carbon-coated: 25 and 35	-	Uncoated, and coated with polyaromatic graphitic carbon	-	-	-	In vivo - direct application	P	SC	20		Samberg et al., 2010	150

Chemical Composition	Primary Particle Size [nm]	Shape	Surface chemistry/ functionalisation	Surface Charge	Other Physico-chemical Properties	Vehicle	Model	Species [H/P/R]	Level of Penetration* [NP/SC/SG/SS /SGV/SP/SR]	Total Appraisal Score	High Interest	Ref.	Data-base ID
Titanium dioxide	Non-coated: 35, 250; With alumina/silica /silicon coating: 35; Mixture of almina coated and silicon coated particles, dispersed in cyclo-pentasiloxan: 10 x 100	-	Uncoated, alumina -, silica-, and silicon coated	-	Crystal structure: rutile	Oil/water	Ex vivo - diffusion cell	P	No penetration	22		Senzui et al., 2010	156
Gold	15 ± 2.30, 102 ± 5.56 and 198 ± 7.56	Spherical	-	Zeta potential: 15 nm: -42.9 102 nm: -40.8 198 nm: -35.9	-	dH2O	Ex vivo - diffusion cell	R	Size dependent permeation and penetration of epidermis/ dermis	20		Sonovane et al., 2008	161
Zinc oxide	21	Ellipsoid	-	-	-	Zinclear_60C CT formulation	Ex vivo - diffusion cell	H	SC, no detectable absorption in the viable epidermis	21		Song et al., 2011	164
Upconversion (UC) nanoparticles (fluoride nanocrystal $NaYf_4$)	UC1: 32 UC2: 8	-	Oleic-acid capped	-	UC1: Doped with Yb^{3+} ions and Er UC2: Doped with Yb^{3+} ions and Tm	Oil formulation	Ex vivo – diffusion cell	H	SC and where needle punctures were made, deeper penetration of 8nm particles occured	24		Song et al., 2013	163
Zinc oxide	19	-	-	-	-	Hydrophobic basis gel	Human	H	SC, no detectable absorption into the SS on longer application time	19	✓	Szikszai et al., 2011	168
Zinc oxide	8	-	-	-	-	Hydrophobic basis gel	Human	H	SC, removal of SC partially or entirely did not cause penetration into the deeper dermal layers	18		Szikszai et al., 2010	169
Titanium dioxide	-	-	-	-	-	Suncreen formulation	Human	H	Non-significant increase in dermal levels of Ti compared to control	19		Tan et al., 1996	236
Dextran beads	500, 1000, 2000 and 4000	-	-	-	-	-	Ex vivo	H	SC, only small proportion of applied beads penetrated to SC and penetration appeared random in flexing skin. No	20	✓	Tinkle et al., 2003	172

Chemical Composition	Primary Particle Size [nm]	Shape	Surface chemistry/ functionalisation	Surface Charge	Other Physico-chemical Properties	Vehicle	Model	Species [H/P/R]	Level of Penetration* [NP/SC/SG/SS /SGV/SP/SR]	Total Appraisal Score	High Interest	Ref.	Data-base ID
									penetration observed when applied to non-flexing skin.				
Titanium dioxide	< 1 nm	-	-	-	Surface area: 300 m^2/g;	Sodium lauryl sulphate suspension	In vitro – diffusion cell	H	NP	18		Van der Merwe et al., 2009	179
Magnesium oxide	Thickness: 7nm Length and width: 100-2oo nm	Hexagonal platelets			Surface area: > 200 m^2/g				NP				
Silver	Not stated	-	-	-	-	Acticoat dressings	Human	H	Silver (ions) released from wound dressings were detectable in patient serum suggesting systemic availability	16		Vlachou et al., 2007	182
Polymer	40, 750 and 1500	-	-	-	Fluorescent	PBS	Ex vivo	H	Size dependent penetration (40 nm) of the perifollicular dermis	20		Vogt et al., 2006	183
Titanium dioxide	5 ± 1, 10 ± 1 25 ± 5 60 ± 10 90 ± 10 21	-	Hydrophobic Hydrophobic Hydrophilia Hydrophobic Hydrophobic Hydrophilia	-	Crystal structure/ Surface area: Anatase: 200 m_2/g Anatase: 160 m_2/g Rutile: 80 m_2/g Rutile: 40 m_2/g Rutile: 40 m_2/g Anatase/rutile mixture: 50 m_2/g	Carbopol 940 and triethanol-amine in dH_2O	In vivo - direct application	R	Skin penetration (depth not detailed) and systemic absorption	22		Wu et al., 2009	190
									Basal cell layer, but not in the dermis				
						Caprylic/ capric triglyceride with 1% Tween 80	Ex vivo - diffusion cell	P	SC, trace amounts of Ti in uppermost layers only				
Polystyrene	28.8 28.0 30.9	Spherical	Covalently bound fluorescein methacrylate (FMA) and dispersed Nile Red (NR): PS PS-FMA PS-FMA-NR	-	-	-	Ex vivo - diffusion cell	P	SC, did not penetrate beyond the superficial SC, showed some affinity for hair follicles, and the associated	22		Wu et al., 2009	192

Chemical Composition	Primary Particle Size [nm]	Shape	Surface chemistry/ functionalisation	Surface Charge	Other Physico-chemical Properties	Vehicle	Model	Species [H/P/R]	Level of Penetration* [NP/SC/SG/SS/SGV/SP/SR]	Total Appraisal Score	High Interest	Ref.	Data-base ID
Poly(methyl methacrylate)	79.0 99.9 68.5		Covalently bound fluorescein methacrylate (FMA) and dispersed Nile Red (NR): PMMA PMMA-FMA PMMA-FMA-NR						hydrophobic active (NR) is released from the nanoparticle and is able to diffuse into the deeper layers of the skin				
Polysytrene	PS-NH_3^+: 115 PS-CO_2-: 87	-	NH2- and COOH-functionalised	PS-NH_3^+: 48 ± 9 mV PS-CO_2-:-21 ± 3 mV	Labelled: N-(2,6-diisopropylphenyl)perylene-3,4-dicarboximine		In vitro	P	SC of cationic form	22		Wu et al., 2009	191
Poly-(L-lactide)	138		-	-34 ± 5 mV					NP				
Fullerenes (pristine C_{60})	Not stated	-	-	-	-	Different types of solvents used: toluene, cyclohexane, chloroform and mineral oil	In vivo - direct application	P	SC	21	✓	Xia et al., 2010	193
						Toulene, cyclohexane, chloroform or mineral oil	Ex vivo - diffusion cell	P	SC, but very little with mineral oil				
Titanium dioxide	20	-	-	-	-	Pentalan 408	In vivo - direct application	R	SC, not detected in deeper tissue	16		Xu et al., 2011	194
Quantum dot (CdSe/CdS core/shell)	Length: 8.40 ± 1.9 Diameter: 5.78 ± 0.97	Nail-shaped	PEG-coated	-	-	H_2O	Ex vivo - diffusion cell	P	SC, uppermost layers and near hair follicles	23		Zhang et al., 2008	203
Quantum dot (CdSe/CdS core/shell)	Spherical: diameter 4.6 nm; Ellipsoid: 6 nm (minor axis) by 12 nm (major axis)	Spherical and Ellipsoid	-	-	-		In vivo	R	SC	22		Zhang et al., 2008	201

Chemical Composition	Primary Particle Size [nm]	Shape	Surface chemistry/ functionalisation	Surface Charge	Other Physico-chemical Properties	Vehicle	Model	Species [H/P/R]	Level of Penetration* [NP/SC/SG/SS /SGV/SP/SR]	Total Appraisal Score	High Interest	Ref.	Data-base ID
Zinc oxide	Mean: 26-30	-	-	-	-	Commercial sunblock	In vivo	H	SC	19		Zvyagin et al., 2008	205
							In vitro	H	-				
Silica	25	-	-	-	Fluorescent	0.9% NaCl supplemented with gentamycin	Ex vivo - diffusion cell	H	Decreasing silica content and penetration with increasing depth and they concluded that deposition mainly occurred in the epidermis with potentially minor deposition in the upper dermis although the authors point to the need for more exact measurements.	23		Staronova et al., 2012	231
Zinc oxide	19 ± 8, 110 ± 46	-	-	-	Enriched with ^{68}Zn to 51% or >99%;	Oil/water	Human	H	Small amounts of Zn from ZnO particles detected in blood and urine.	23		Gulson et al., 2010	234

Key:

- Not Described.

Level of Penetration: No Penetration, SC=Stratum Corneum, SG=Stratum Granulosum, SS=Stratum Spinosum, SGV=Stratum Germinativum (also called Stratum Basale); Dermis Stratum SP=Papillare, SR=Stratum Reticulare.

Species: H=Human, P=Porcine, R=Rodent.

Appendix 3: Summary Table of Dermal Penetration Test Methods

Method Type	Assay	Species	Particle Type	Detection Method	Weight of Evidence Paper	High Interest	Reference	Database ID
Human	Direct application	Human	ZnO	ICP-MS			Gulson et al., 2008	235
Human		Human	TiO_2	TEM, SEM, RBS, PIXE			Gontier et al., 2008	53
Human	Direct application	Human	ZnO	Stable isotope tracing using MC-ICP-MS	✓	✓	Gulson et al., 2010	58
Human	Direct application	Human	ZnO	Stable isotope tracing, using MC-ICP-MS	✓		Gulson et al., 2012	59
Human	Direct application	Human	Quantum dots	Confocal microscopy, TEM	✓		Jeong et al., 2010	66
Human	Direct application	Human and in vitro pig	Polymer Nanoparticles	Laser scanning microscopy; Fluorescence spectrometry	✓		Lademann et al., 2007	85
Human	Direct application	Human	TiO_2	UV-Vis Spectroscopy, X-ray fluorescence			Popov et al., 2006	134
Human	Direct application	Human	Cd QD	Fluorescence spectrometry	✓	✓	Ravichandran et al., 2011	140
Human	Direct application	Human-diseased skin	ZnO	STIM, PIXE	✓	✓	Szikszai et al., 2011	168
Human	Direct application	Human	ZnO	Photon induced X-ray spectroscopy, scanning transmission ion microscopy	✓		Szikszai et al., 2010	169
Human		Human	Ag	ICP-MS	✓		Vlachou et al., 2007	182
In vivo- direct application	Direct application	Rodent	TiO_2	Light microscopy, TEM-EDX, and confocal laser scanning microscopy	✓		Adachi et al., 2010	3
In vivo- direct application	Subcutaneous injection	Rodent	Polyethylene-glycol coated Nanoparticles	TEM	✓		Baik et al., 2008	12
In vivo- direct application	Direct application	Rodent	TiO_2	Histology	✓		Furukawa et al., 2011	52
In vivo- direct application	Direct application	Rodent	Polyethylene-glycol coated CdSe QD	Confocal fluorescence microscopy; ICP-MS.	✓	✓	Gopee et al., 2009	54
In vivo- direct application	Diffusion cell	Pig	TiO2 and ZnO	TEM, SEM, TOF-SIMS, ICP-MS	✓	✓	Monteiro- Riviere et al., 2011	107
In vivo- direct application	Direct application	Rodent	Cd/Se/ZnS core/shell QD	Phase contrast and fluorescent microscopy, TEM-EDX	✓	✓	Mortensen et al., 2012	113
In vivo- direct application	Direct application	Rodent	QD	Fluorescence confocal microscopy, TEM with EDAX analysis	✓		Mortensen et al., 2008	114
In vivo- direct application	Diffusion cell, Rodent epidermal cells and EpiDERM model	Rodent	SWCNT	-	✓		Murray et al., 2009	118
In vivo- direct application	Direct application	Rodent	Silica and PEG QD	TEM	✓	✓	Nabeshi et al., 2011	120
In vivo- direct application	Direct application	Pig	TiO_2	ICP-MS, TEM and SEM-EDX	✓	✓	Sadrieh et al., 2010	148
In vivo- direct application	Single cell	Pig	Ag	Light microscopy, TEM, DLS	✓		Samberg et al., 2010	150
In vivo- direct application	Direct application	Rodent	TiO_2	TEM	✓		Wu et al., 2009	190
In vivo- direct application	Diffusion cell	Rodent	Fullerenes	HPLC-UV/VIS analysis of tape strips	✓	✓	Xia et al., 2010	193
In vivo- direct application	Direct application	Rodent	TiO_2	TEM, ICP-MS	✓		Xu et al., 2011	194

Method Type	Assay	Species	Particle Type	Detection Method	Weight of Evidence Paper	High Interest	Reference	Database ID
In vivo- direct application	**NANODERM REPORT**				✓	✓	Butz et al., 2007	213
Ex vivo- Skin absorption model	Diffusion cell	Rodent	Polystyrene	Confocal laser scanning microscopy	✓		Alvarez- Roman et al., 2004	8
Ex vivo- Skin absorption model	Diffusion cell	Human	Meghemite and Fe	TEM, ICP-OES			Baroli et al., 2007	15
Ex vivo- Skin absorption model	Diffusion cell	Rodent	Polystyrene	Laser scanning confocal microscopy	✓		Campbell et al., 2012	27
Ex vivo- Skin absorption model	Diffusion cell	Human	CuO	Electron and light microscopy			Cohen et al., 2013	34
Ex vivo- Skin absorption model	Diffusion cell	Human	Au	TEM, ICP-MS	✓		Filon et al., 2011	48
Ex vivo- Skin absorption model	Diffusion cell	Human	QD	Multi-photon- and confocal laser scanning microscopy	✓		Gratieri et al., 2010	57
Ex vivo- Skin absorption model	Diffusion cell	Rodent	TiO_2 and ZnO	Confocal laser scanning microscopy, Fluorescence microscopy	✓		Kimura et al., 2012	71
Ex vivo- Skin absorption model	Diffusion cell	Pig	Latex	Fluorescence microscopy	✓		Kohli and Alpar, 2004	74
Ex vivo- Skin absorption model	Diffusion cell	Human	Au	Multiphoton laser scanning microscopy with pixel analysis	✓	✓	Labouta et al., 2011	75
Ex vivo- Skin absorption model	Diffusion cell	Human	Thiol coated Au NP	Fluorescence microscopy			Labouta et al., 2011	78
Ex vivo- Skin absorption model	Diffusion cell	Human	Co NP	ICP-MS, TEM, Electro thermal atomic absorption spectroscopy (ETAAS)	✓		Filon et al., 2013	88
Ex vivo- Skin absorption model	Diffusion cell	Human	Polyvinylpirrolidine coated Ag	TEM, Electro thermal atomic absorption spectroscopy (ETAAS)	✓		Larese et al., 2009	89
Ex vivo- Skin absorption model	Diffusion cell	Rodent	Ag	TEM with Fourier Transform image analysis	✓		Lee et al., 2013	93
Ex vivo- Skin absorption model	Diffusion cell	Human	Ag	Multiphoton tomograph-fluorescence lifetime imaging microscopy	✓		Liu et al., 2012	96
Ex vivo- Skin absorption model	Diffusion cell	Human	Lead sulfide QD	Reflectance and fluorescence confocal microscopy (near-IR)			Mortensen et al., 2011	112
Ex vivo- Skin absorption model	Diffusion cell	Human	QD	Laser-scanning confocal microscopy	✓		Prow et al., 2012	137
Ex vivo- Skin absorption model	SC removed	Human	Silica	Confocal laser scanning microscopy, TEM	✓		Rancan et al., 2012	138
Ex vivo- Skin absorption model	Diffusion cell	Pig	Fullerenes	Confocal scanning microscopy, TEM	✓	✓	Rouse et al., 2007	143
Ex vivo- Skin absorption model	Diffusion cell	Pig	QD	Confocal-differential interference contrast microscopy and fluorescence measurements	✓	✓	Ryman-Rasmussen et al., 2006	144
Ex vivo- Skin absorption model	Diffusion cell	Pig	TiO_2	ICP-MS, SEM-EDS	✓		Senzui et al., 2010	156
Ex vivo- Skin absorption model	Diffusion cell	Rodent	Ag	UV-Vis Spectroscopy, ICP-MS, TEM, energy-dispersive x-ray spectroscopy	✓		Sonavane et al., 2008	162
Ex vivo- Skin absorption model	Diffusion cell	Human	ZnO	Non-linear optical microscopy	✓		Song et al., 2011	164

Method Type	Assay	Species	Particle Type	Detection Method	Weight of Evidence Paper	High Interest	Reference	Database ID
Ex vivo- Skin absorption model	Diffusion cell	Human	Dextran beads	Laser scanning confocal microscopy, SEM	✓	✓	Tinkle et al., 2003	172
Ex vivo- Skin absorption model	SC removed	Human	Polymer NP	Fluorescence and laser scanning microscopy	✓		Vogt et al., 2006	183
Ex vivo- Skin absorption model	Diffusion cell	Pig	Polystyrene	Laser scanning confocal microscopy, DLS	✓		Wu et al., 2009	192
Ex vivo- Skin absorption model	Diffusion cell	Pig	PEG coated QD	Laser scanning confocal microscopy, ICP-MS	✓		Zhang et al., 2008	203
Ex vivo- Skin absorption model	Diffusion cell	Human	Silica	Laser scanning confocal microscopy	✓		Staronova et al., 2012	231
In vitro- Skin absorption model	Single cell	Keratinocyte	CdSe/ZnS core/shell QD	Fluoroescence microscopy	✓		Mortensen et al., 2012	115
In vitro- Skin absorption model	Single cell	HEK	QD	Laser scanning confocal microscopy, TEM	✓	✓	Ryman-Rasmussen et al., 2007	146
In vitro- Skin absorption model	Single cell	HEK	Fullerenes	TEM	✓		Saathoff et al., 2011	147
In vitro- Skin absorption model	Single cell	Human epidermal cells	TiO_2	TEM	✓		Shukla et al., 2011	159

Appendix 4: Link to the MS Access database

The MS Access database accompanying this report can be accessed through the hyperlink below:

HYPERLINK TO MS ACCESS DATABASE

Dermal absorption of nanomaterials

This report and accompanying MS Access database systematically evaluates the reliability and relevance of the existing scientific literature regarding dermal absorption of nanomaterials. The results seem to suggest that absorption of nanoparticles through the skin is possible although occurs to a very low degree. The report makes specific recommendations for future testing approaches and highlights the need for more robust and harmonised testing.

Rapporten og den tilhørende MS Access-database gennemgår systematisk pålideligheden og relevansen af den eksisterende videnskabelige litteratur vedrørende hudgennemtrængelighed af nanomaterialer. Resultaterne tyder på, at absorption af nanopartikler gennem huden er mulig, om end i meget lav grad. Rapporten indeholder konkrete anbefalinger til fremtidige testmetoder og fremhæver behovet for mere robust og harmoniseret testning.

Danish Ministry of the Environment
Environmental Protection Agency

Strandgade 29
1401 Copenhagen K, Denmark
Tel.: (+45) 72 54 40 00

www.mst.dk