

Regulation and Risk Assessment of Nanomaterials

- *Too Little, Too Late?*



Regulation and Risk Assessment of Nanomaterials
– Too Little, Too Late?

Steffen Foss Hansen

PhD Thesis
February 2009

Department of Environmental Engineering
Technical University of Denmark

Steffen Foss Hansen

Regulation and Risk Assessment of Nanomaterials
– *Too Little, Too Late?*

PhD Thesis, February 2009

The thesis will be available as a pdf-file for downloading from the homepage of the department: www.env.dtu.dk

Address: DTU Environment
Department of Environmental Engineering
Technical University of Denmark
Miljoevej, building 113
DK-2800 Kgs. Lyngby
Denmark

Phone reception: +45 4525 1600

Phone library: +45 4525 1610

Fax: +45 4593 2850

Homepage: <http://www.env.dtu.dk>

E-mail: reception@env.dtu.dk

Printed by: Vester Kopi
Virum, February 2009

Cover: Torben Dolin

ISBN: 978-87-91855-58-0

Table of Contents

Preface.....	I
Acknowledgements.....	IV
Abstract.....	V
Dansk sammenfatning.....	VII
1. Introduction.....	1
1.1 Objectives and outline.....	2
2. Development of nanotechnology and materials.....	5
3. Defining nanotechnology and nanomaterials.....	13
4. Regulation of nanomaterials.....	19
4.1 Registration, Evaluation and Authorization of Chemicals (REACH).....	19
4.2 Pharmaceutical regulation.....	22
4.3 Nanofood laws.....	24
4.4 Safety at Workplace Directives.....	25
4.5 Waste management of products containing nanoparticles.....	26
4.6 New nanospecific legislation vs. the “Incremental” approach and/or Voluntary Environmental Programs.....	27
5. Risk assessment of nanomaterials.....	29
5.1 Hazard identification.....	29
5.2 Dose-response assessment.....	44
5.3 Exposure assessment.....	46
5.4 Risk Characterization.....	56
6. Discussion.....	61
7. Conclusion.....	75
8. List of references.....	79
Appendix.....	111

Preface

This PhD thesis is based on research undertaken from 2005 to 2008 under the supervision of Associate Professor Anders Baun (Department of Environmental Engineering Technical University of Denmark). The work was done primarily at the Technical University of Denmark, but included a internship at the Project for Emerging Nanotechnologies, at the Woodrow Wilson International Center for Scholars in Washington, DC as well as a visiting research scholarship at the Work Department at the University of Massachusetts, Lowell and the Nanomanufacturing Center of Excellence.

This thesis comprises of six published papers as well as an analysis of the regulation and risk assessment of nanomaterials. Papers comprised in this thesis include:

1. **Hansen, S.F.**, Larsen, B.H., Olsen, S.I., Baun, A. 2007. Categorization framework to aid Hazard Identification of Nanomaterials. *Nanotoxicology* 1: 243-250.
2. **Hansen, S.F.**, Michelson, E., Kamper, A., Borling, P., Stuer-Lauridsen, F. & Baun, A. 2008, Categorization Framework to Aid Exposure Assessment of Nanomaterials in Consumer Products. *Ecotoxicology* 17 (5): 438-447.
3. Franco, A., **Hansen, S.F.**, Olsen, S.I., Butti, L. 2007. Limits and Prospects of the “Incremental Approach” and the European Legislation on the Management of Risks related to Nanomaterials. *Regulatory Toxicology and Pharmacology* 48: 171-183.
4. Baun, A., **Hansen, S.F.** 2008. Environmental Challenges for Nanomedicine. *Nanomedicine* 2(5): 605-608.
5. **Hansen, S.F.**, Tickner, J.A. 2007. The Challenges of Adopting Voluntary Health, Safety and Environment Measures for Manufactured Nanomaterials. Lessons from the past for more effective adoption in the future. *Nanotechnology Law & Business* 4 (3): 341-359.
6. **Hansen, S.F.**, Maynard, A., Baun, A., Tickner, J.A. 2008. Late Lessons from Early Warnings for Nanotechnology. *Nature Nanotechnology* 3 (8): 444-447.

Please note that the papers are not included in this web version but can be obtained from the library at Department of Environmental Engineering, Technical University of Denmark, Miljoevej, building 115, DK-2800 Kgs. Lyngby (library@env.dtu.dk).

Publications authored or co-authored in 2005-2008 and related to the topic of this thesis but not comprised in this thesis include:

Hansen, S.F., Rejeski, D. 2008. Applying the Chemical Policy Options to Emerging Technologies and Materials: Adaptations and Challenges. In J.A. Tickner and Y. Torrie (eds.). Options for State Chemicals Policy Reform A Resource Guide. Lowell Center for Sustainable Production, University of Massachusetts, Lowell. Available:<http://sustainableproduction.org/downloads/OptionsforStateChemicalsPolicyReform.pdf> (Accessed 18 Feb. 2008).

Grieger, K.D., **Hansen, S.F.**, Baun, A. 2008. The Known Unknowns of Nanomaterials: Describing and Characterizing Uncertainty within Environmental, Health and Safety risks. Environmental Science & Technology (Submitted).

Linkov, I., Steevens, J., Adlakha-Hutcheon, G., Bennett, E., Chappell, M., Colvin, V., Davis, M., Davis, T., Elder, A., **Hansen, S.F.**, Hakkinen, P., Hussain, S., Karkan, D., Korenstein, R., Lynch, I., Metcalfe, C., Ramadan, A., Satterstrom, F. K. 2008. Emerging Methods and Tools for Environmental Risk Assessment, Decision-Making, and Policy for Nanomaterials: Summary of NATO Advanced Research Workshop. Journal of Nanoparticle Research (In press).

Satterstrom, F. K., Arcuri, A.S.A., Davis, T.A., Gullledge, W., **Hansen, S.F.**, Shafy Haraza, M.A., Kapustka, L., Karkan, D., Linkov, I., Melkonyan, M., Monica, J., Owen, R., Palma-Oliveira, J.M., Srdjevic, B. 2008. Considerations for Implementation of Manufactured Nanomaterial Policy And Governance: Working Group Discussion Summary. In I. Linkov, J. Steevens (eds.), Nanotechnology Risks and Benefits. Springer: Dordrecht. 329-352.

Linkov, I., Satterstrom, F.K., Monica, J., **Hansen, S.F.**, Davis, T. 2008. Nano Risk Governance: Current Developments and Future Perspectives. Risk Analysis (Submitted).

Hansen, S.F., Baun, A., Michelson, E.S, Kamper, A., Borling, P., Stuer-Lauridsen, F. 2008. Nanomaterials in Consumer Products: Categorization and Exposure Assessment. In I. Linkov, J. Steevens (eds.), Nanotechnology Risks and Benefits. Springer: Dordrecht. 363-372.

Hansen, S.F., Krayner von Krauss, M. , Tickner, J.A. 2008. The Precautionary Principle and Risk-Risk Tradeoffs. Journal of Risk Research 11(4): 423-464.

Hansen, S.F., Tickner, J.A. 2008. Putting Risk–Risk Tradeoffs in Perspective: A Response to Graham and Wiener. Journal of Risk Research 11(4): 475-483.

Hansen, S.F., Kraye von Krauss, M.P., Tickner, J.A. 2007. Categorizing Mistaken False Positives in Regulation of Human and Environmental Health. *Risk Analysis* 27(1): 255-269.

Hansen, S.F., Kraye von Krauss, M.P., Tickner, J.A. 2007. Response to “Regulatory False Positives: True, False, or Uncertain?”. *Risk Analysis* 27(5): 1087–1089.

Hansen, S.F., Carlsen, L, Tickner, J.A. 2007. Chemicals Regulation and Precaution: Does the REACH proposal really incorporate the Precautionary Principle. *Environmental Science & Policy* 10:395-404.

Acknowledgements

There are a number of people that I would like to thank for their help and guidance in the past three years. First of all, I would like to thank my co-authors along with Dave Rejeski, Julia Moore, Alex Parlino and Yve Torrie. I would also like to thank Khara D. Grieger and Nanna B. Hartman and the staff at DTU Environment - especially Torben Dolin and Anne Harsting. Financial support from the Technical University of Denmark, the Otto Mønsted Foundation and the Frimodt-Heineke Foundation is greatly appreciated. Finally, I would like to thank Anders Baun, Joel A. Tickner, Gyde Hansen and Maria Eva Hansen.

Kgs. Lyngby,
December 2008

Steffen Foss Hansen

Abstract

Nanotechnology is the latest in a long series of technologies heralded as ushering in a new era and current and future applications of nanotechnology are expected to hold immense societal and environmental benefits. Concerns have been raised about the safety and regulation of nanomaterials following a number of studies which indicated that some nanomaterials can cause adverse effects on laboratory animals. Data on nanoparticles, such as increasing production volumes and commercialization, capabilities to cross biological barriers, and increased biological activities of nanoparticles when compared to bulk counterparts, have worried some scientists, policy-makers, members of the public and industry and investors about their potential impacts on the health and safety of both humans and the environment.

The aim of this PhD Thesis is to: 1) investigate whether existing regulation is adequate in the short and the long term, 2) explore the feasibility of risk assessment for the purpose of dealing with the complex emerging risks of nanomaterials, and finally, 3) provide recommendations on how to govern nanotechnologies.

The short and long term development of nanotechnologies and nanomaterials was investigated and an in-depth analysis was performed of key pieces of regulation in the EU such as REACH, pharmaceutical regulation, and the worker safety directives, and waste directives. The applicability of each of the four individual steps of risk assessment (i.e. hazard identification, dose-response assessment, exposure assessment, and risk characterization) was evaluated in the light of the current state of knowledge.

It is found that although nanomaterials might be covered by the general scope of many of the existing legislative frameworks it is often unclear, if current regulation is actually applicable when it comes to specific nanomaterials and their diverse applications. The main problems seem to be: that requirements to do safety evaluations are triggered by production volumes by tonnage not tailored to the nanoscale, the profound lack of (eco)toxicological data, and that no risk thresholds and occupational exposure limits cannot be established with existing methodologies.

So far, the only amendment that has been implemented is to annul the exemption status of carbon and graphite under REACH, which is deemed inadequate to address the potential risks of nanomaterials and the current regulatory uncertainty.

Several governments have opted to implement voluntary environmental programs (VEPs), arguing that this is the only viable proportional option for the time being. It is generally known that key elements of any successful VEP are: incentives to participate for various stakeholders, agency guidance and technical assistance, signed commitments and periodical reporting, quality of information, and transparency both in design, reporting and evaluation. However, many of these elements have not been fully addressed in the VEPs that are implemented currently on nanomaterials.

Each of the four steps, that together constitute the risk assessment framework hold a number of limitations as well. Toxicity has been reported on for multiple nanoparticles, but for most nanoparticles these need further confirmation before one can say that a hazard has been identified. It is currently impossible to systematically link reported nanoparticle properties to the observed effects for effective hazard identification. Although some studies have reported observing a dose-response relationship, it was unclear whether a no effect threshold can be established and what the best hazard descriptor(s) of nanoparticles is and what the most relevant endpoints are. The current lack of characterization of the nanoparticles tested in various studies makes it impossible to identify causality between observed hazards and specific physical and chemical properties. Several studies have tried to assess current and future consumer and environmental exposure for nanomaterials, but these should be seen as “proof of principle” rather than actual assessment of the exposure. Realistic exposure assessment is hampered by: paucity of knowledge, lack of access to information, by difficulties in monitoring nanomaterial exposure in the workplace and the environment, and by the fact that the biological and environmental pathways of nanomaterials are still largely unexplored. Risk characterization being at the end of the line, the sum or maybe even the power all of these limitations are conveyed to calculating risk quotients for nanomaterials.

It is concluded that we do not know enough to say that nanomaterials are safe, but that there is evidence that some nanomaterials are hazardous depending on their particle characteristics, how they are applied and how humans and the environment are exposed to them. Although recognizing that adaptations are needed, risk assessment has repeatedly been proposed by expert committees, policy-makers, members of industry and non-governmental organizations as means to inform decision-makers about the risks of nanomaterials. However, in this thesis, risk assessment is found to be inadequate to timely inform policy-makers about the health and environmental risks of nanomaterials, if not in the short term, then most definitely, in the long term. Risk assessment is not feasible for the purpose of dealing with the complex emerging risks of nanomaterials and will not be adequate to ensure a decision-making process that enables us to make informed decisions within a reasonable period of time. It is furthermore concluded that the existing regulation is not adequate to deal with nanomaterials in the short and the long term and that too little is being done currently to amend existing regulation through the incremental approach adopted by the EU and the voluntary program implemented in the UK. It is recommended that current regulation is adapted immediately to reflect the challenges posed by current nanomaterials and their applications. Risk assessment should be abandoned as the primary decision making tool. Alternative tools such as MultiCriteria Decision Analysis, Bayesian decision making and Adaptive management should be pursued to ensure and support transparent and informed decision-making processes.

Dansk sammenfatning

Nanoteknologi er den seneste i rækken af teknologier udråbt til at indvarsle en ny industriel æra. Nuværende og fremtidige anvendelser af nanomaterialer forventes at have store sociale og miljømæssige fordele, men der er blevet rejst bekymring omkring uønskede effekter af nanomaterialer efter et antal studier har vist, at nogle nanomaterialer kan forårsage skadelige effekter på laboratoriedyr. Generel viden om nanopartikler, så som stigende produktionsmængder, evne til at krydse biologiske membraner, og en øget biologisk aktivitet af nanopartikler sammenlignet med større partikler, har endvidere gjort videnskabsfolk, politikere, dele af befolkningen og industrien samt investorer bekymrede over nanopartiklers potentielle skadelige effekter på mennesker og miljø.

Formålet med denne Ph.d.-afhandling er: 1) at undersøge hvorvidt den nuværende regulering er tilstrækkelig på kort såvel som lang sigt, 2) at belyse anvendeligheden af risikovurdering til at håndtere komplekse og fremspirende risici relateret til nanomaterialer og 3) at give anbefalinger til hvorledes nanomaterialer kan reguleres.

I afhandlingen beskrives den kort- og langsigtede udvikling af nanoteknologi og der foretages en dybdeborende analyse af flere af de fundamentale dele af reguleringen i EU så som REACH, lægemiddellovgivningen og arbejdsmiljølovgivningen. Risikovurdering af kemikalier består af fire overordnede dele – farlighedsidentifikation, dosis-respons vurdering, eksponeringsvurdering samt risiko karakterisering. Anvendeligheden af hver af disse dele med hensyn til nanomaterialer bliver evalueret i lyset af den nuværende viden.

Resultatet af undersøgelse er, at selvom nanomaterialer falder ind under det overordnede formål af den nuværende regulering, så er det ofte uklart hvorvidt den nuværende regulering er anvendelig når det kommer til specifikke nanomaterialer og deres vidt forskellige anvendelser. Hovedproblemerne synes at være, at krav om sikkerhedsevalueringen udløses på baggrund af produktionsmængde i tons, den fundamentale mangel på (øko)toksikologiske data og at tærskelværdier for hvornår der ikke er nogen risiko og grænseværdier for arbejdsmiljø ikke kan fastsættes med nuværende metoder.

Det eneste tiltag, som er blevet implementeret indtil videre, er at slette karbon og grafit fra listen over stoffer, som er undtaget REACH. Dette vurderes at være utilstrækkeligt til at adressere de potentielle risici af nanomaterialer og den nuværende reguleringsmæssige usikkerhed.

I Storbritannien har man valgt at indføre frivillige aftaler med virksomheder, der producerer nanomaterialer, med det argument, at det er det eneste proportionale regulerings tiltag på nuværende tidspunkt. I et af studierne i denne afhandling er det beskrevet, hvorledes visse elementer skal være til stede for at frivillige aftaler bliver en succes. Disse elementer er: incitament til at deltage for forskellige interessenter,

myndighedsvejledning, underskrevne forpligtigelser og periodisk afrapportering, kvalitetssikring af information, og gennemskuelse i såvel design, rapportering som evaluering af disse frivillige aftaler. Mange af disse elementer er endnu ikke blevet fuldt ud implementeret i mange af de nuværende frivillige aftaler angående nanomaterialer.

Hvert enkelt element af risikovurdering for kemikalier har ligeledes en række begrænsninger. Selvom giftighed er blevet rapporteret for flere nanopartikler, så mangler disse observationer at blive bekræftet for de fleste partikler, før man kan sige, at egentlig farlighed er blevet identificeret. På nuværende tidspunkt er det umuligt systematisk at forbinde de rapporterede nanopartiklers karakteristika med de observerede skadelige effekter, hvilket igen begrænser mulighederne for effektiv farlighedsidentifikation. Selvom nogle studier har observeret et dosis-respons forhold er det uklart hvorvidt der findes en tærskelværdi for, hvornår der ikke er nogen effekt. Desuden er det ikke klarlagt hvad de(n) bedste farlighedsdeskriptor(er) for nanopartikler er og hvad der er de mest relevante biologiske effekt parametre. Den nuværende mangel på karakterisering af de testede nanopartikler i de fleste studier gør det umuligt at identificere sammenhænge mellem farlighed og specifikke fysiske og kemiske egenskaber ved partiklerne. Flere studier har forsøgt at vurdere den nuværende og fremtidige forbruger- og miljømæssige eksponering af nanomaterialer, men disse studier skal anses som værende af principiel karakter og ikke faktiske eksponeringsvurderinger. Realistiske eksponeringsvurderinger vanskeliggøres af: manglende viden og adgang til viden, problemer med at måle eksponering for nanomaterialer i arbejdsmiljøet og miljøet, og af det faktum, at de biologiske og miljømæssige eksponeringsveje for en stor del er uudforsket for nanomaterialer. Som det sidste led i risikovurderingsprocessen bliver summen - eller måske endda potensen - af alle disse begrænsninger opsamlet i risikokarakteriseringen og i udregningen af risikokvotienter for nanomaterialer.

Det konkluderes, at vi endnu ikke ved nok til at kunne sige om nanomaterialer er sikre. Desuden er der bevis for at visse nanomaterialer udgør en fare afhængig af deres partikelegenskaber, hvordan de anvendes, og hvorledes mennesker og miljø bliver eksponeret for dem. På trods af, at det generelt anerkendes, at ændringer til den nuværende kemikalie risikovurdering er påkrævet, så bliver risikovurdering gang på gang foreslået af diverse ekspertkomiteer, politiske beslutningstagere, repræsentanter for industrien og ikke-statslige organisationer som det primære middel til at informere beslutningstagere om nanomaterialers risiko. I denne afhandling findes det derimod, at risikovurdering er utilstrækkelig til rettidigt at informere beslutningstagere om de miljø- og sundhedsmæssige risici ved nanomaterialer. Det gælder dels på kort sigt og især på lang sigt. Risikovurdering er ikke anvendelig, når det drejer sig om at håndtere de komplekse risici ved nanomaterialer. Ej heller vil risikovurdering være tilstrækkelig til at sikre en beslutningsproces, som vil gøre det muligt at tage informerede beslutninger indenfor en rimelig tidsperiode. Det konkluderes endvidere, at den nuværende regulering er utilstrækkelig til at håndtere nanomaterialer på kort

såvel som på lang sigt, og at der på nuværende tidspunkt gøres for lidt for at modificere den nuværende regulering gennem den gradvise tilpasningspolitik, som man har valgt at følge i EU. Heller ikke den frivillige aftale, som man har valgt at indføre i Storbritannien, har vist sig at være effektiv.

Det anbefales, at den nuværende regulering ændres med det samme, så den reflekterer de udfordringer som nanomaterialer og deres nuværende og fremtidige anvendelser giver. Der bør ses bort fra risikovurdering som det primære beslutningsværktøj. Alternative beslutningsværktøjer som MultiCriteria Decision Analysis, Bayesian decision making and Adaptive management burde efterprøves for at sikre og understøtte gennemskuelige og oplyste beslutningsprocesser.

1. Introduction

It is hard to deny that most technologies have the potential to cause harm as well as do good. It is intuitive that the successful – and sustainable – use of any new technology will depend on discovering how to use it safely prior to its wide distribution. Yet history is replete with examples of technologies that were wielded without foresight, leaving a legacy of damage in their wake and often not achieving their full potential (EEA 2001).

Nanotechnology is the latest in a long series of technologies heralded as ushering in a new era – the “next industrial revolution”, according to some. Since 2000, nanotechnology has grown from little more than a gleam in the eyes of researchers to a technology projected to be worth \$2.6 trillion in manufactured goods in 2014 (Lux Research 2006). Current and future applications of nanotechnology are expected to hold immense societal and environmental benefits in regard to increased economic development and employment, improved materials using less resources and environmental remediation, along with new ways of diagnostics and medical treatments (RS & RAE 2004, Roco and Bainbridge 2005).

Nevertheless, as new materials based on nanoscale engineering move from the lab to the marketplace, have we learnt the lessons of past “wonder technologies”, or are we destined to repeat the mistakes of our predecessors?

Both the potential benefits of nanotechnology and potential hazards of some manufactured nanomaterials have been debated in recent years, especially following a number of studies, which indicated that some nanomaterials can cause adverse effects on laboratory animals (Oberdörster 2004, Lam *et al.* 2004, Pollard *et al.* 2008). Data on nanoparticles, such as increasing production volumes and commercialization, capabilities to cross biological barriers, and increased biological activities of nanoparticles when compared to bulk counterparts, have worried some scientists about their potential impacts on the health and safety of both humans and the environment.

Perhaps more than any preceding technology, the early development of nanotechnology has been characterized by discussions of potential risks. Early on in the development of the United States’ National Nanotechnology Initiative, addressing risks was an integral part of the government-led development process. In the UK, the Royal Society and Royal Academy of Engineering (RS & RAE) (2004) galvanized the development of cross-agency groups to address uncertainties regarding the risks of nanomaterials. Currently, most economies investing in nanotechnology pepper discussions with questions concerning potential risks – and how to manage them (Hansen *et al.* 2008a).

The first logical questions for many politicians, regulators, academics and members of the public have been:

- whether nanotechnology is safe;
- whether existing regulation is adequate in the short and in the long term;
- what is and should be done to learn more about protection of the public and workers from any potential environmental, health and safety risks related to nanotechnology and nanomaterials (RS & RAE 2004, Macoubrie 2005, Chaundry *et al.* 2006, Gavelin *et al.* 2007).

Currently, the short answer to these questions seems to be that “we do not know”, “probably not” and “not enough” which leads to another underlying and more urgent question of whether existing decision making tools such as risk assessment are adequate to ensure a decision making process that enables us to make informed decisions within a reasonable period of time, despite large uncertainties about the risks of nanotechnology and nanomaterials.

This thesis explores these and related questions.

1.1 Objectives and outline

The aim of this PhD thesis is to:

1. investigate whether existing regulation is adequate in the short and the long term;
2. explore the feasibility of risk assessment for the purpose of dealing with the complex emerging risks of nanomaterials, and finally;
3. provide recommendations on how to govern nanotechnologies protecting human health and the environment.

A fundamental aspect that has to be kept in mind is that nanotechnology is an emerging technology that it is developing with rapid speed in multiple directions and in many scientific fields and industrial sectors. Hence, one needs to take both current and reasonably foreseeable future developments and applications of nanotechnology and nanomaterials into consideration when discussing and trying to assess what the risks are and whether current regulation and decision making tools are adequate. The short and long term perspectives of nanotechnological development are introduced in section 2.

Before one can start discussing the risks of nanotechnology and/or nanomaterials and the suitability of current regulation and decision making tools, it is important to clarify the terminology used. The “nanorisk”-terminology has not always been consistent. Nanotechnology and nanomaterials have often been lumped together as one and the same notwithstanding that the term “nanotechnology” covers many fields of disciplines, research, applications, etc. and not just nanomaterials. A lot of advancement has been made recently to address the issue of diffuse terminology, which is introduced in section 3.

All governments bear the direct and indirect responsibility for protecting their citizens against risks, but because of lack of knowledge the proper regulatory response is not always obvious and views on how to regulate nanomaterials vary substantially, ranging from a “laissez-faire” attitude to a total moratorium on nanotechnology research, development and commercialization. In section 4 the applicability and feasibility of the existing regulation is investigated in the short and the long term, focussing especially on the new chemical legislation in Europe termed REACH.

In the past, science provided regulating authorities with essential information upon which they could base their regulatory decisions and justify these to the public. The complexity of risks in general and nanomaterials in particular makes it difficult for science to identify causality and provide clear answers immediately (if ever) (Weinberg 1985, Ruckelhaus 1985, Funtowicz and Ravetz 1992, Harremoës 2003). New ways to inform decision makers have been sought and decision support tools such as risk assessment have repeatedly been proposed as means to inform decision makers about the risks of nanomaterials. In section 5, the feasibility of applying risk assessment to nanomaterials is investigated and the state of knowledge is discussed within each of the four parts of chemical risk assessment i.e. hazard identification, dose-response assessment, exposure assessment, and risk characterization. Finally the pros and cons of existing regulation and risk assessment are discussed in section 6 and recommendations are provided.

2. Development of nanotechnology and materials

In order to assess the applicability of the current regulation and risk assessment in the short and the long term, insight is needed into current and future trends with regard to the development and commercialization of nanotechnology. If the current regulation and risk assessment is not able to deal with current nanomaterials, it is highly unlikely that it will be able to deal with future nanomaterials.

The development of nanotechnology has been rapid by almost any metric one can think of – governmental funding, number of research publications and industrial patents, among others. To begin with nanotechnological development was mostly driven by individual scientific breakthroughs such as the discovery of fullerenes, quantum dots and carbon nanotubes (Iijima, 1991) along with the inventions of the scanning tunneling microscopy and atomic force microscopy (Kroto *et al.* 1986, Iijima 1991, Binnig *et al.* 1982, 1986).

A turning point in science and technology policy in relation to nanotechnology was the increasing amount of government funding in the United States of America (USA) in the late 1990ties which finally lead to the launch of the National Nanotechnology Initiative (NNI) in 2000. Since then, almost every country on the globe has launched national initiatives or prioritizes research in nanotechnology, and the USA, Japan and the EU each spend more than a billion Euros a year on nano-related research.

Government funding of academic research has lead to an explosion in the number of scientific research publications in nanotechnology. Recently, Linkov *et al.* (2008a) performed an analysis of Science Citation Index database available through the Web of Science to map out nanotechnology application and data generation trends in regard to environment, health and safety. Using a wide range of search terms, they found that the total number of publications increased from just over 3,000 in 1995 to an estimated 52,000 in 2008. The number of papers that mention risk was less than 10 through 2003, however, since then it has been rapidly increasing and over 100 are expected to be published in 2008 (see figure 1).

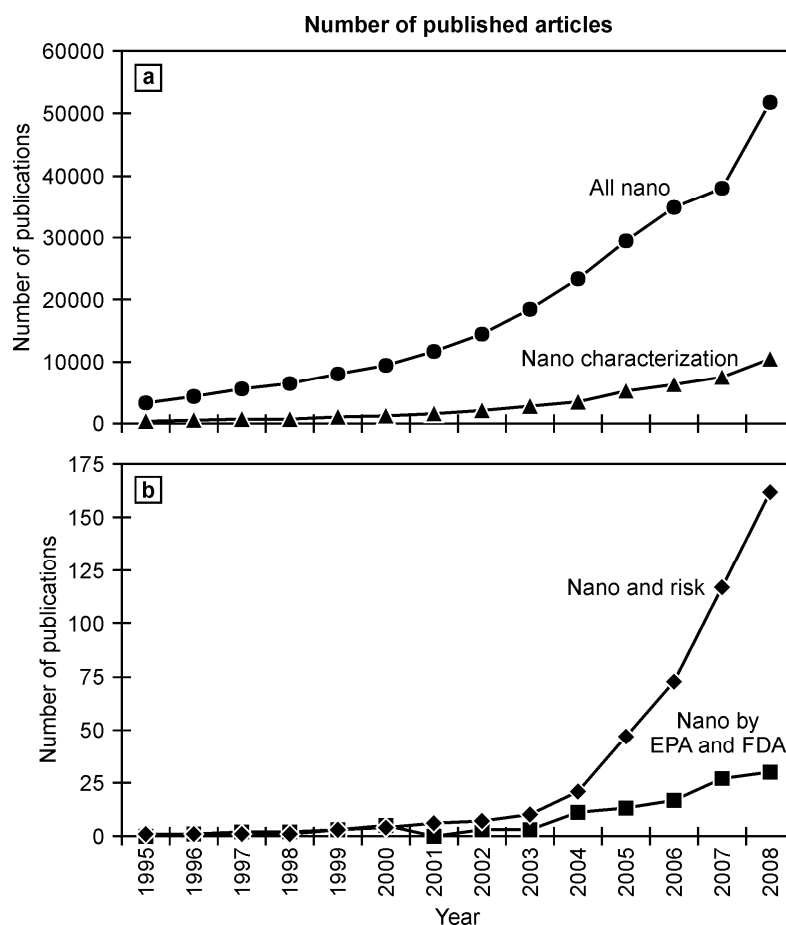


Figure 1: Number of journal articles on nanotechnology topics by year in Science Citation Index. “All Nano” corresponds to all published papers resulting from a search using the string “TS=(quantum dot OR nanostruc* OR nanopartic* OR nanotub* OR fullerene* OR nanomaterial* OR nanofib* OR nanotech* OR nanocryst* OR nanocomposit* OR nanohorn* OR nanowir* OR nanobel* OR nanopor* OR dendrimer* OR nanolith* OR nanoimp* OR nano-imp* OR dip-pen)” with document type = article (search string taken from Lux Research, 2007). “Nano Characterization” and “Nano and Risk” are a subset of papers that include nanomaterial characterization and risk. The final plot represents papers co-authored by scientists from U.S. Food and Drug Administration and U.S. Environmental Protection Agency. The number of articles published annually data for 2008 was estimated by multiplying the number of papers published in the first quarter by 4 (Linkov *et al.* 2008a).

Scientific activities sparked by Government funding have had a crucial role in knowledge creation and flows in nanotechnology although there is often some time-lag before scientific knowledge is diffused into useful inventions and applications (Igami and Okazaki 2007). Patent analysis is one way to examine how knowledge flows from science to technological development. In an analysis of patent applications to the European Patent Office (EPO), Igami and Okazaki (2007) found that there have been an increasing number of nanotechnology patents applications in the period of 1984-2002, especially after 1996 (see figure 2).

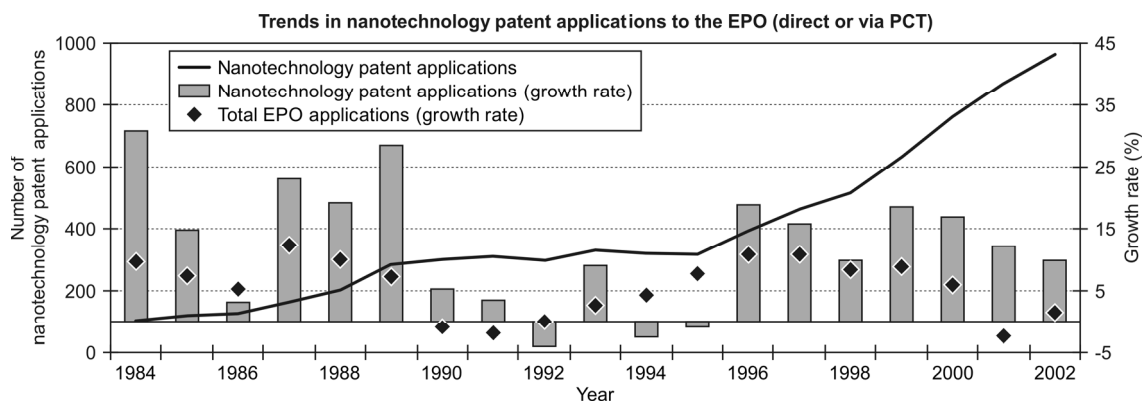


Figure 2: Trends in nanotechnology patent applications to the EPO (Modified from Igami and Okazaki 2007).

The USA, EU and Japan each account for about a third of the nanotechnology patent applications each whereas Germany, France and the United Kingdom are the leading countries in the European Union (Igami and Okazaki 2007).

The substantial increase in nanotechnological patent applications filed in the period from 1984-2002 manifests itself in most fields of applications e.g. electronics and nanomaterials except for environment and energy (Igami and Okazaki 2007). It is interesting to note that nanomaterials were the primary field of application for which patent applications were filed in 2002. It should however be noted that nanomaterials were defined fairly broadly under EPO and include among others: chemical or physical processes, nano- and/or microstructures, manufacture, shaping, or supplementary processes, coatings and crystal growth (Igami and Okazaki 2007).

The number of patents on nanomaterials as been increased globally as well – according to Royal Commission on Environmental Pollution (RCEP) (2008) the number of patents registered from 1990-2006 for nanoparticles, nanorod, nanowire, nanocrystal, nanotube or carbon nanotubes have more than doubled every 2 years.

Governmental funding and prioritization of nanotechnology since the mid-1990ties has lead to higher education and government sectors becoming important sources of knowledge in nanotechnology. This is reflected in the fact that comparatively larger shares of patents applications stem from government (5%) and higher education (8%) sectors. The business enterprise sector, however, still accounts for the majority of the applications (80%); it is approximately 10% lower than its share in overall EPO.

According to the Technology Transfer Center (2007) there are more than 300 nanotechnology companies in Europe, most of which are based in Germany and the UK. These companies range from multinationals to small and medium size companies and university spin-offs. They span over a wide range of sectors and applications (see figure 3) including textiles, anti-microbial wound dressings, paints and coatings, fuel catalysts and additives, lubricants, cosmetics, and food packaging (Chaundry *et al.* 2006).

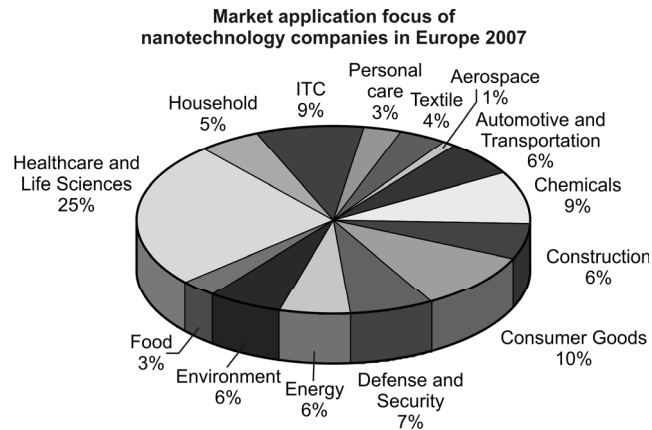


Figure 3: Market application focus of nanotechnology companies in Europe 2007 (Modified from Technology Transfer Center 2007)

Figure 3 shows the focus of market application of nanotechnology companies in Europe in 2007 for which nanotechnology products or platforms are a primary area of business, however, it is important to realize these numbers do not include tools and instrumentation companies or companies that use nanotechnology without having a nanotechnology business unit (Technology Transfer Center 2007).

Although much focus is and has been on research and development (R&D), nanotechnology is entering a new era in the sense that more and more emphasis is being put on commercialization of nanomaterials and products. In 2006, the Project for Emerging Nanotechnologies at the Woodrow Wilson International Center for Scholars launched an inventory of the products available online to consumers containing nanomaterials (the Woodrow Wilson inventory). Originally the inventory contained 212 different products in 2006, which has increased to 580 products in 2007 and 803 in 2008. Projections are that this number will continue to increase as the unique properties of nanomaterials are explored further and translated into commercial products. The products fall into a number of different product categories such as health and fitness, home and garden, electronics and computers, etc. (see figure 4).

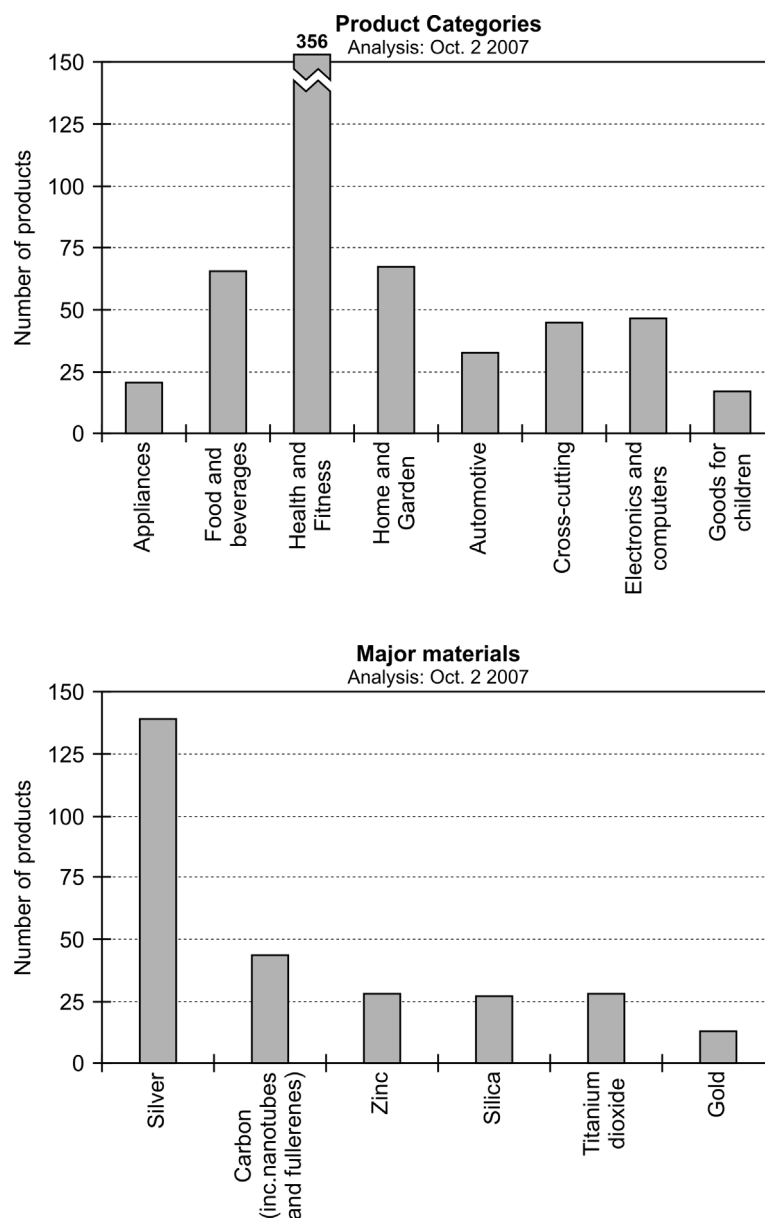


Figure 4: Top chart shows the distribution of the products in the inventory according to product category. The 580 products fall into a number of different product categories such as health and fitness, home and garden, electronics and computers, etc. Bottom chart shows the materials used in the various products listed in inventory. The main kinds of materials used are graphite (including carbon nanotubes and fullerenes), silver, silica, TiO₂, and ZnO. Source: <http://www.nanotechproject.org/consumer/analysis.html> (Accessed November 6 2007). All rights reserved by Woodrow Wilson International Center for Scholars (Hansen *et al.* 2008b).

From figure 4 one can see that nanomaterials are proclaimed to be used in products that fall mainly into the categories of health and fitness, food and beverages, and home and garden, whereas the silver and carbon are the nanomaterials mostly used (Hansen *et al.* 2008b).

The Woodrow Wilson inventory contains information such as product name, company, manufacturer or supplier, country of origin, and a short product description. However, it does not contain information about how many units are produced and sold of a given product or how much nanomaterial is used in each of the individual products. Such information is only available for a limited number of products, if producers make it available.

Very little is furthermore known about the production volumes at which nanomaterials are currently produced, although some scattered information is available. In 2001, the future global annual production of carbon-based nanomaterials was estimated to be several hundred tons, but already in 2003, the global production of nanotubes alone was estimated to be around 900 tons distributed between 16 manufacturers (Kleiner and Hogan 2003). The Japanese company, Frontier Carbon Corp, annually produces more than 40 tons of C₆₀ (Fujitani *et al.* 2008). It is estimated that the global annual production of nanotubes and fiber was 65 tons equal to €144 million worth and it is expected to surpass €3 billion by 2010 representing an annual growth rate of well over 60% (Cientifica 2006). Even though the information about the production of carbon-based nanomaterials is scarce, the annual production volumes of for instance quantum dots, nano-metals, and materials with nanostructured surfaces are completely unknown.

The development of nanotechnology is still in its infancy. Mihail Roco of the U.S. National Nanotechnology Initiative has projected four generations of nanotechnological development. The first generation ranged up to 2000 and consisted of simple “passive” nanostructures. The second generation, ranging from 2000-2005 included the development of “active (evolving function) nanostructures” such as for example targeted drugs and chemicals, light-driven molecular motors, nanoscale fluidics, laser-emitting devices, and adaptive structures.

In the third generation we will see “systems of nanosystems”. This generation is projected to range from 2005 to 2010 and will include the use various syntheses and assembling techniques such as bio-assembling, networking at the nanoscale and multiscale and hierarchical architectures, robotics on surfaces, modular nanosystems, chemo-mechanical processing of molecular assemblies, and quantum-based nanoscale systems. From 2010 to 2015/2020, a fourth generation is projected to involve the development of heterogeneous molecular nanosystems where each molecule in the nanosystem has a specific structure and plays a different role (Roco and Renn 2006).

In the light of these predictions the current production and use of nanomaterials is most likely not representative for the future use and production (RCEP 2008), but factual information is hard to obtain. The global market impact of nanotechnology is expected to reach 1 trillion US\$ by 2015, with around 2 million workers (Roco and Bainbridge 2001, Chaundry *et al.* 2006). The overall nanofood market (including packaging) is one of the areas of which a substantial growth is expected. According to

Helmut Kaiser Consultancy (cited in Boxall *et al.* 2008) it will reach US \$20.4 billion by 2010 from estimated \$7 billion in 2006. Cientifica (2006) estimate food applications of nanotechnologies in 2006 to be around \$410 million and that these will reach \$5.8 billion in 2012.

Besides application with regard to food packaging, processing, etc. projected applications include: remediation of contaminated soil and groundwater, fuel cells and batteries, medical applications, drinking water treatment, and weapons and explosives (Chaundry *et al.* 2006). Some estimates for the future manufacturing of nanomaterials have been made for instance by the Royal Society and the Royal Academy of Engineering.

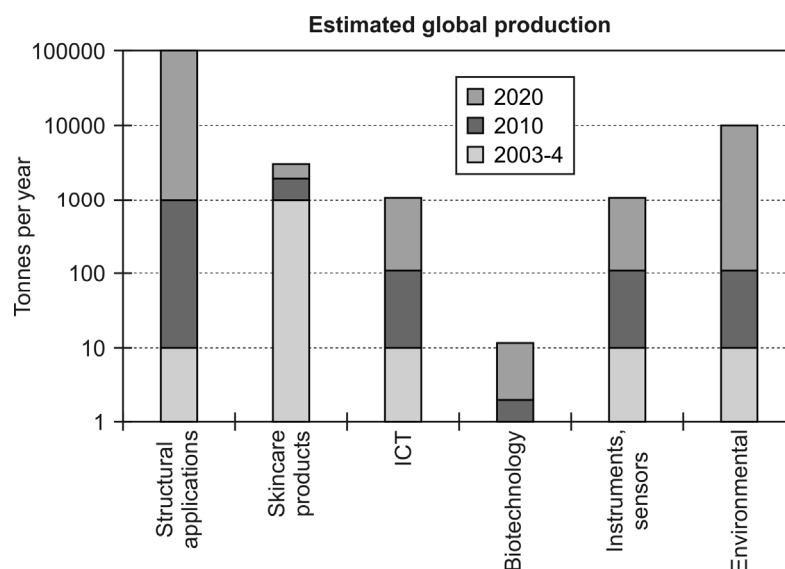


Figure 5: Global production in 2010 and 2020 estimated by RS & RAE (RS & RAE 2004)

According to the RS & RAE (2004), the largest growth in estimated global production is expected to be in structural applications such as ceramics, catalysts, coatings, film, etc. (see figure 5). Other areas where substantial growth is expected include single- and multi-walled nanotubes, TiO₂, zinc, and organic light-emitting diodes for ICT-applications, as well as nanoencapsulates, quantum dots, composites for nanobiotechnology.

Especially the market of carbon nanotubes is projected to grow substantially and production capacities have grown enormously within recent years. It is projected that sales will reach \$1-2 billion annually within the next four to seven years. End uses are primarily expected to be electronics and the automotive industry. The global enterprise Bayer AG have recently opened a 30 metric tons per year manufacturing plant in Germany bringing their total capacity up to around 60 metric tons, whereas the French based company Arkema are able to produce between 10 and 30 tons per year (Thayer 2007).

3. Defining nanotechnology and nanomaterials

Nanotechnology is often described as a cross disciplinary and enabling technology in the sense that it has roots and relevance in a wide range of scientific fields, including physics, chemistry, biology, material science, and electronics. This is reflected in the fact that the field of nanotechnology is very broad covering a wide range of different techniques, scientific and commercial applications and products as well as nanomaterials (RS & RAE 2004). A recent study of scientific publications via co-citation analysis identified approximately 30 research areas related to nanoscience and materials (Igami and Saka 2007).

A lot of effort has been put into the development of a standard terminology intended to support among others: patenting, commercialization, worker, public and environmental safety and testing, and legislation and regulation of nanotechnology (BSI 2007a).

The term “nanotechnology” was first used by Taniguchi in 1974 that was referring to the ability to engineer materials precisely at the nanometer level (RS & RAE 2004). However, putting forward a common “all embracing” definition of nanotechnology has been quite challenging.

One of the most cited definitions is the one applied by the U.S. National Nanotechnology Initiative (NNI) who defines nanotechnology as follows:

“Nanotechnology is the understanding and control of matter at dimensions of roughly 1 to 100 nanometers, where unique phenomena enable novel applications. (. . .) . At this level, the physical, chemical, and biological properties of materials differ in fundamental and valuable ways from the properties of individual atoms and molecules or bulk matter” (Nanoscale Science Engineering and Technology Subcommittee 2004).

Similar, the American Society for Testing and Materials International define nanotechnology as:

“A term referring to a wide range of technologies that measure, manipulate, or incorporate materials and/or features with at least one dimension between approximately 1 and 100 nanometers (nm). Such applications exploit the properties, distinct from bulk/macroscale systems, of nanoscale components” (ASTM Int’l 2006).

In 2004 the Royal Society and Royal Academy of Engineering found it useful to make a clear distinction between “nanoscience” and “nanotechnologies”. Nanoscience being defined as “...the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at a larger scale”. Whereas Nanotechnologies were defined as “...the design,

characterisation, production and application of structures, devices and systems by controlling shape and size at nanometer scale.” (RS & RAE 2004).

Although the term nanotechnology is often used in singular, it should probably be used in plural since the term covers several technologies as indicated by a number of the definitions above. Some the most well-known technologies and methods include chemical vapor deposition, atomic force microscopy and scanning probe- and tunneling microscopy but according to a recent standard on the terminology for nanofabrication and nanomaterials from the British Standard Institute (BSI) (2007b, c) the number of methods, processes and techniques easily exceeds 30. The techniques can, roughly speaking, be divided into so-called “top down” and “bottom up” approaches. Top down techniques involve starting from a larger unit of material, and etching or milling it down to smaller units of desired shape, whereas bottom up involves progressing from smaller sub-units (e.g. atoms or molecules) to make larger and functionally richer structures (RS & RAE 2004, BSI 2007b). Top down techniques include processes such as high-energy ball milling, etching, sonication, and laser ablation whereas bottom up techniques include sol-gel, chemical vapor deposition, plasma or flame spraying, supercritical fluid, spinning, and self-assembly (Biswas and Wu 2005). Both approaches hold specific challenges. Creating smaller and smaller structures with sufficient accuracy is a main challenge for top-down manufacturing, whereas the challenge for bottom up techniques is to make structures large enough and of sufficient quality (RS & RAE 2004).

Although the wording differs, most definitions of nanomaterials require that two criteria must be fulfilled in order to define a system or material as being related to nanotechnology: 1) It must have some structure in at least one dimension in the approximate range of 1-100 nm, and 2) this nanostructure must give the system properties differing from the bulk properties. Although this definition is broad, this does not make it diffuse, and for a given material or system it can be uniquely determined whether it involves nanotechnology or not (Hansen *et al.* 2007).

The number of nanomaterials that can be manufactured using top down and bottom up techniques is immense including, for instance, C₆₀, carbon nanotubes, micelles, self assemble monolayers, dendrimers, and aerogels in all kinds of size and shapes. Hence the nature of nanomaterials differs even more than the techniques.

A procedure for dividing nanomaterials into relevant subcategories has been developed by Hansen *et al.* (2007) in order to facilitate hazard identification and to focus the risk assessment procedures (see figure 6).

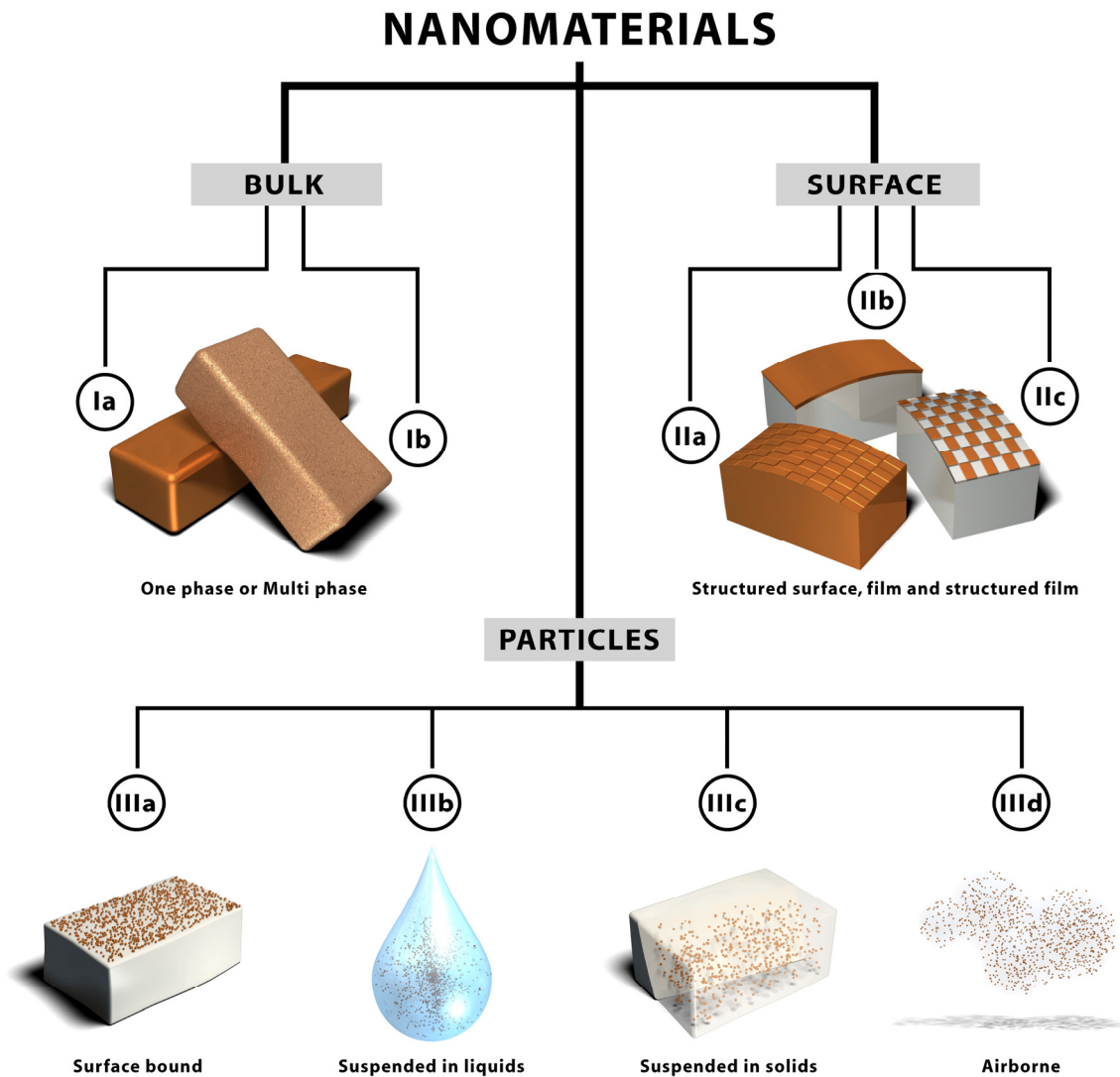


Figure 6. The categorization framework for nanomaterials. The nanomaterials are categorized according to the location of the nanostructure in the material (Hansen *et al.* 2007).

Hansen *et al.* (2007) suggest categorizing nanomaterials depending on the location of the nanoscale structure in the system. This leads to a division of nanomaterials into three main categories:

1. materials that are nanostructured in the bulk;
2. materials that have nanostructure on the surface and;
3. materials that contain nanostructured particles.

The main categories can be further divided into subcategories. The main category I contains materials that are nanostructured in three dimensions, i.e. in the bulk of the material. It is divided into two subcategories: Ia and Ib. The systems in Ia consist only of one type of material whereas systems in Ib are nanostructured throughout the bulk as well, but consist of two or more different constituents/materials. In category II the nanostructure is on the surface. The first subcategory, IIa, consists of materials where the surface is structured on the nanoscale, but the surface and bulk consist of the same

material. The second subcategory, IIb, covers un-patterned film of nanoscale thickness on a substrate of a different material. The third category, IIc, consists of patterned film on a substrate, where the film is either nanoscale in thickness, or the pattern has nanoscale dimensions along the surface. Category III contains nanoparticles, which Hansen *et al.* (2007) define as free structures that are nanosized in at least two dimensions much like the ASTM definition of nanoparticles (ASTM Int'l 2006). Nanostructured particles can have various forms and shapes and this category includes for example quantum dots, fullerenes, nanotubes and nanowires (Maynard and Aitken 2007). There are four subcategories of systems with nanoparticles, depending on the environment around the nanoparticles:

1. subcategory IIIa have nanoparticles bound to the surface of another solid structure;
2. subcategory IIIb consists of systems where nanoparticles are suspended in a liquid;
3. subcategory IIIc is nanoparticles suspended in solids
4. subcategory IIId, consists of airborne nanoparticles.

Using the framework for categorizing nanotechnology-based systems, it should be recognized that it is possible for a system to consist of nanostructured elements belonging to different categories. An example of this is car catalysts used to remove NO_x from car exhaust (Chorkendorff & Niemantsverdriet 2003). The chemical reaction that removes NO_x is catalyzed by Platinum and Ruthenium nanoparticles of 2-3 nm size. These nanoparticles are bound to the surface of a support material. This corresponds to a category IIIa system according to figure 6. At the same time, the support material is a nanoporous material consisting mostly of g-Al₂O₃ (70-85%) and other oxides such as cerium oxide or lanthanum oxide. Thus, the support structure is a category Ib system.

A major benefit of the proposed categorization framework is that it provides a tool for dividing nanosystems into identifiable parts and thereby facilitating evaluations of, for instance, relevant exposure routes or analysis of effect studies according to relevance of the material tested.

Using the framework, Hansen *et al.* (2008b) were able to categorize about 75% of the (at the time) 580 products in the consumer product inventory maintained by the Project for Emerging Nanotechnologies at the Woodrow Wilson International Center for Scholars. Figure 7 shows the distribution of all the products categorized according to the location of the nanostructure in the products. It was found that in 19% of the products the nanomaterial were nanoparticles bound to the surfaces. Nanoparticles suspended in liquids were used in 37% of the products, whereas 13% used nanoparticles suspended in solids. 1% were powders containing free potentially airborne nanoparticles, whereas we were not able to determine the location of the nanomaterial for 140 products given the available information from producers or through the data in the inventory.

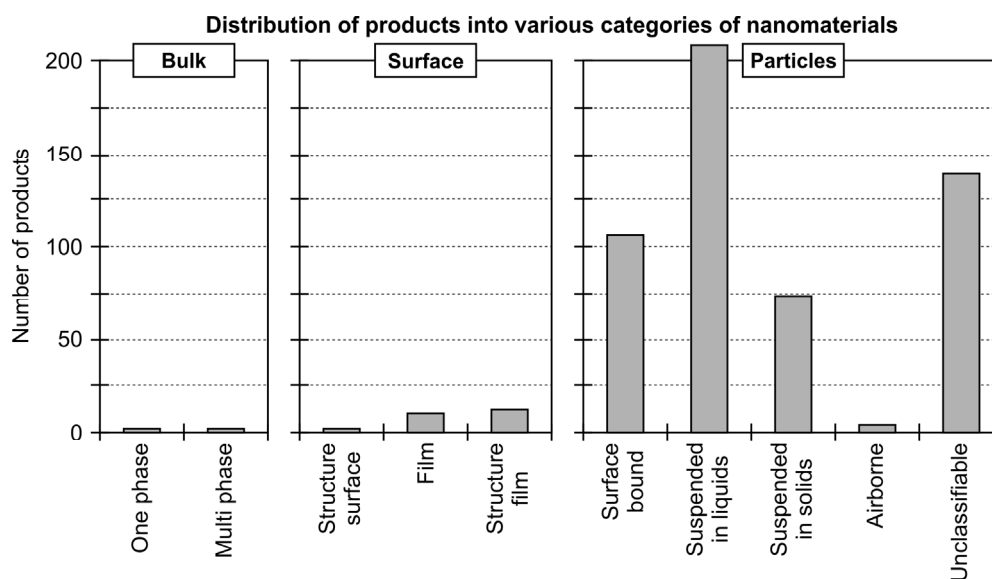


Figure 7: Products in the Woodrow Wilson inventory categorized depending on the location of the nanostructure. 13, 19, 37 and 1% of the products used nanoparticles suspended in solids, bound to the surfaces, suspended in liquids, and free potentially airborne nanoparticles, respectively (Hansen *et al.* 2008b).

Although they have the same size range and may have several physical characteristics in common, Oberdörster *et al.* (2007) proposed making a distinction between ultrafine particles that are generated as heterogeneous aggregates of primary particles and engineered nanoparticles that are generated as more monodispersed individual particles. This would divide ultrafine nanoparticles into two overall categories: natural and anthropogenically generated particles for which the later again can be divided into unintentionally and intentionally generated particles.

The framework proposed by Hansen *et al.* (2007) is limited to nanoparticles that have been humanly engineered intentionally - henceforth referred to as engineered nanoparticles - and does not include nanoparticles that are naturally occurring (e.g. forest fires and volcanoes) and unintentional human generated nanoparticles stemming from for instance internal combustion engines, power plants and incinerators (Oberdörster *et al.* 2007).

Nowack and Bucheli (2007) also propose dividing nanoparticles into “natural” and “anthropogenic” particles depending on their source of origin. Both of these categories can be separated into carbon-containing and inorganic nanoparticles. The first category includes “*biogenic, geogenic, atmospheric and pyrogenic particles such as fullerenes and carbon nanotubes of geogenic or pyrogenic origin, biogenic magnetite or atmospheric aerosols (both organic, such as organic acids, and inorganic, such as sea salt).*” The second category of anthropogenic nanoparticles include particles inadvertently formed as a by-product, or produced intentionally, i.e. engineered or manufactured nanoparticles.

Various international standardization institutes have shifted attention from trying to define nanotechnology to defining the nature of the many different kinds of nanoparticles. Recently the BSI (2007d) published a standard on the terminology on “carbon nanostructures” defining, for instance, carbon nanotubes as hollow nanorods of carbon with nanorods being defined as nano-object with two similar external dimensions in the nanoscale and the third dimension significantly larger. The International Organization for Standardization (ISO) (2008) has also published definitions on nanotubes, nanorods, and quantum dots, for instance defining the later as crystalline nanoparticles that exhibits size-dependent properties due to quantum confinement effects on the electronic states.

The many and sometimes conflicting definitions raise the question/problem of how to resolve the fact that we now have many potential competing standards in an area that needs greater certainty and not additional confusion (Monica 2008). Recently, concerns have been raised that this could have further complicated the efforts to develop sensible, effective policy (Center for Responsible Nanotechnologies 2008).

4. Regulation of nanomaterials

The first logical questions for many politicians, regulators, academics and members of the public, have been whether existing regulation is adequate in the short and the long term and what should be and is being done to address any potential regulatory gaps related to nanotechnology and nanomaterials (RS & RAE 2004, Macoubrie 2005, Chaundry *et al.* 2006, Gavelin *et al.* 2007).

Opinions on the applicability of the existing regulation differ substantially and so does views on which regulatory options best address the current lack of information about environment, health and safety risks of nanomaterials, as well as the regulatory uncertainty and concerns expressed by the politicians, members of the public and industry, and investors. Some argue that a complete new regulatory framework is needed, whereas others argue in favor of implementing a total moratorium on nanotechnology research, development and commercialization. Again others adopt a “laissez-faire” attitude.

Understanding the limitations of the current regulation in regard to nanomaterials is a starting point in the process towards adapting existing laws and facilitating discussion about which kind of regulatory options is best to address these.

The identification of gaps or limitations of the current regulation of nanomaterials has been subject to intense international scrutiny. Some have used a very horizontal approach to evaluate the applicability of the current regulation to nanomaterials as a whole without any specific technology, nanomaterials, application, product or sector in mind (CEC 2008a). Others have analyzed the current regulation using a sector-by-sector approach in regard to current and future applications of nanomaterials (Chaundry *et al.* 2006), whereas others have looked at the regulation of specific commercialized products along their life-cycle (Franco *et al.* 2007). In the following, the results of these will be presented with regard to some of the major regulatory frameworks relevant to nanomaterials. These include REACH, pharmaceutical regulation, food laws, worker safety directives, and environmental legislation i.e. waste directives.

4.1 Registration, Evaluation and Authorization of Chemicals (REACH)

June 1, 2007, a new chemical legislation on the manufacturing and commercialization of chemical substances in the European market went into force. The new regulation, termed Registration, Evaluation and Authorization of CHEmicals (REACH), establishes an authorizing system that requires the registration and evaluation of existing and new chemical substances (EP & CEU 2006).

In a horizontal scoping study, Chaundry *et al.* (2005) assessed the gaps in environmental regulation in the UK and the EU, media by media and sector by sector focusing on current and future products and applications of nanomaterials. In total 16 different sectors were included among others coatings, construction, and cosmetics.

One of the limitations identified by Chaundry *et al.* (2005) was related to whether a nano-equivalent of a substance with different physicochemical and (eco) toxicological properties from the bulk substance would be considered as the same or as new substances under REACH. REACH defines a substance as *“a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition.”* (EP & CEU 2006).

Whether nanomaterials is considered to be a equivalent or different to the bulk material will have a major impact on the requirements put on manufacturers prior to placing nanomaterials on the market. If a nanomaterials is considered to be a different substance, hazard information would have to be generated for the registration dossier if produced in more than 1 tons/year. On the other hand, if the nanomaterial are considered to be the same as a registered bulk material, the appropriateness of the hazard information data would be open to discussion (Chaundry *et al.* 2006). Recently the European Commission published a review of the applicability of REACH arguing that although there is no specific provisions in REACH referring to nanomaterials the definition of “a chemical substance” covers nanomaterials (CEC 2008a). The Commission further states that: *“When an existing chemical substance, already placed on the market as bulk substance, is introduced on the market in a nanomaterial form (nanoform), the registration dossier will have to be updated to include specific properties of the nanoform of that substance. The additional information, including different classification and labelling of the nanoform and additional risk management measures, will need to be included in the registration dossier. The risk management measures and operational conditions will have to be communicated to the supply chain”*. It is, however, highly unclear how companies should do this. Companies are urged to use already existing guidelines, however, both the Commission of the European Communities (CEC 2008a) as well as the its Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR 2007) and others have pointed out that current test guidelines that support REACH are based on conventional methodologies for assessing chemical risks and may not be appropriate for assessing risks associated with nanomaterials. This means that although manufacturers and importers might be required to provide a Chemical Safety Assessment (if they produce or import nanomaterials in volumes more than 10 tons) they cannot rely on the toxicological profile of the equivalent bulk material and they cannot use existing test

and risk assessment guidelines since these might not provide any meaningful results or be practically applicable due to the limitations of conventional methods.

Until recently carbon and graphite were exempted from registration under REACH, however this exemption was redrawn to address concern raised about carbon based nanomaterials (C & EN 2008). Companies will now have to register these materials if produced in quantities above one ton per producer or manufacturer per year. If it is produced in quantities greater than 10 tons per year a Chemical Safety Assessment has to be undertaken and if it meets the criteria for classification as dangerous or a PBT (Persistent, Bioaccumulable and Toxic) or vPvB (very Persistent and very Bioaccumulable), the manufacturer is required to develop exposure scenarios and undertake risk characterisation(s). So far no nanomaterial has been classified as such. It should be noted that a Chemical Safety Assessment can also be required if a nanomaterial is selected for further evaluation by a Member State or by the European Chemicals Agency due to specific concerns; or if a substance is a CMR (Carcinogenic, Mutagenic, or toxic for Reproduction), PBT, vPvB, ED (Endocrine Disrupting), or substance of equivalent concern.

Even despite the recent amendments to REACH withdrawing carbon and graphite from the list of exemptions, it is highly unclear whether this would involve any additional obligations from producers of C₆₀ and carbon nanotubes as well as from producers or importers of the final products containing C₆₀ and carbon nanotubes, given what is known and accessible information about production, product contents, and expected consumer exposure (Franco *et al.* 2007). Another issue raised by Franco *et al.* (2007) is whether the annual production should be measured including impurities. All carbon based nanomaterials contain some degree of impurities due to the manufacturing process, however, a substance with a different degree of purity and composition can be classified as the same substance, provided hazardous properties do not differ significantly. This means that for instance, raw soot, NanomBlack® and purified fullerenes from the production of C₆₀ could all be classified as the same, provided that the hazardous properties do not differ significantly.

Although there is no tonnage related exemption under REACH for authorization, restriction or classification and labeling requirements, a second limitation of REACH is that “*Substances manufactured or imported in volumes of less than 1 tons/year do not need to be registered*” and hence producers or importers are not required to provide toxicological data and assess environmental exposure. As noted by Chaundry *et al.* (2006) and Franco *et al.* (2007) this threshold would hardly be reached for many nanoparticles. Chaundry *et al.* (2006) estimates that the majority of applications is likely to fall outside the scope of REACH on the basis of the low tonnage currently used in gram to kilogram quantities. Furthermore, the usually low concentration of nanoparticles in the final article is likely to exclude many nanoengineered articles from the REACH legislation, since no registration is required when the concentrations of a

substance is lower than 0.1% w/w. However, a general lack of access to information about product formulations and nanoparticles concentration hampers determination of concentrations of substances by weight (Franco *et al.* 2007).

4.2 Pharmaceutical regulation

The application of engineered nanoparticles for medical use offers immense benefits within areas like diagnosis, targeted drug delivery, and drug development and have been marketed for more than 17 years (NanoRoad SME 2006, EGE 2007, Gaspar 2007). Well-described and understood medicinal products containing nanoparticles in the form of liposomes, polymer protein conjugates, polymeric substances or suspensions have been given Marketing Authorizations within the EU under the existing regulatory framework e.g. Regulation 726/2004 on authorization and supervision of medicinal products for human and veterinary use, Directive 2001/83/EC on medicinal products for human use, Directive 93/42/EEC concerning medical devices, Directive 90/385/EEC relating to active implantable medical devices, and Directive 98/79/EC on in vitro diagnostic medical devices (EP & CEU 2004, Council of the European Communities 1990, 1993, 1998, 2001).

There is no specific mentioning of nanomedicine in the EU legislation on medicinal products and devices, tissue engineering and other advanced therapies. None of these regulations or directives was written with nanomedicinal applications in mind and although their scope covers nanomedicine they have been accused for being general and non-specific and fraught with concerns and difficulties when it comes to dealing with drugs more complex than traditional ones (Editorial 2007, D'Silva and Van Calster 2008).

The use of nanoparticles in nanomedicine has not been subject to much regulatory scrutiny since existing laws and regulatory instruments are believed also to cover medical products based on nanotechnology. The extensive testing requirements prior to marketing of medicine may also contribute to the notion that the potentially negative effects will be discovered prior to marketing, that patients are adequately informed about negative side-effects, and that benefits outweigh the risks or the adverse effects, should such be found to occur (EGE 2007, N&ET Working Group 2007) .

However, concerns have been raised that risk assessment, safety and quality requirements for medicine have to be fulfilled by conformity to established quality systems and published product standards that may not be suitable designed to address various aspects relating to nanomedicine. According to the European Medicines Agency (EMA) (2006), this might be especially relevant when it comes to novel applications of nanotechnology such as nanostructure scaffolds for tissue replacement, nanostructures that allow transport across biological barriers, remote control of

nanoprobes, integrated implantable sensory nanoelectronic systems and multifunctional chemical structures for drug delivery and targeting of disease.

It is furthermore unclear whether novel nanomedicine is to be regulated as a medicinal product or as a medical device (EGE 2007). Currently the mechanism of action is key to decide whether a product should be regulated as one or the other, however, nanomedicinal products may exhibit a complex mechanism of action combining mechanical, chemical, pharmacological and immunological properties, and combining diagnostic and therapeutic functions. Hence many of these novel applications are likely to span regulatory boundaries between medicinal products and medical devices (EGE 2007, EMEA 2006).

Another aspect of nanomedicine is, however, often neglected (Linkov *et al.* 2008b). It is the question what happens after the prescribed use when residues of nanomedicine enter the environment? It is not difficult to imagine that residues of nanomedicine or nano-sized drug carriers could have unexpected effects on the environment, just as it has been the case for conventional medicine for which a rapidly increasing number of laboratory studies show ecotoxicological effects (Fent *et al.* 2006). However, to the best of our knowledge no study exploring the environmental effects of nanomedical products has been published to date (Hansen *et al.* 2007). In the EU, all new marketing authorization applications are required to undergo an environmental risk assessment following a tiered assessment procedure. One significant difference in the EU risk assessment approach for medical products compared to that of industrial chemicals is the inclusion of a pre-screening stage involving a rough calculation of the predicted environmental concentration for surface water with an action limit of 0.01 ppb (EMEA 2006). Thus, if the estimated environmental concentration is below this value and “no other environmental concerns are apparent” (European Medicines Agency 2006), no further actions are to be taken for the medical product in terms of environmental risk assessment.

These approaches, in which the estimated environmental concentrations are used as trigger values for further action, are problematic if the current regulation of medical products is transferred directly to regulation of nanomedical products. The concentration limits are not set science-based and can by no means be interpreted as “environmentally safe concentrations” for medical products in general (also mentioned in the European guidelines (European Medicines Agency 2006) nor for nanomedical products in specific. Here a pre-defined action limit will be especially problematic since the new properties of nano-based products are expected to also affect their environmental profiles, as argued by several authors, e.g. Zhang *et al.* (2007) and Baun *et al.* (2008).

Although an initial weighing of benefits versus harm may be needed to avoid over-regulation, it should be stressed that the evaluation of environmental fate and effects of engineered nanoparticles used in medical products is significantly different

from that of conventional pharmaceuticals. The establishment of a fixed value for a level-of-no-concern, where the benefits outweigh the risks, is not scientifically justifiable given the present level of knowledge. Not only is the amount of laboratory data limited and field studies and exposure models nearly non-existing – we may not even know the ecotoxicological endpoints to investigate and how to measure exposure to engineered nanoparticles in the environment.

Many of the shortcomings to existing approaches for determining the ecotoxicity apply for nanomedical products as well as for nanoparticles. For two parameters used today to identify environmentally hazardous compounds, i.e. persistence and bioaccumulation, the SCENIHR concluded that: “*The criteria used for persistence, bioaccumulation and toxicity (PBT) assessment applied for substances in soluble form should be assessed for applicability to nanoparticles*” (SCENIHR 2007). Thus, the present use of the octanol-water coefficient (as a surrogate value for bioaccumulation data to signify environmental concern) in the EU guidelines for risk assessment of medical products should not be transferred to a regulation of nanomedicine unless strong scientific evidence supports this (Handy *et al.* 2008, Baun *et al.* 2008).

4.3 Nanofood laws

In the EU, food and food packaging are regulated by a number of laws, directives and regulations such as EU Food Law Regulation and the EU Novel Foods Regulation (EP & CEU 2002). The EU Food Law Regulation requires all food to be safe, something which – as an overarching principle – applies to all foods and food packaging containing nanomaterials as well, but has been criticized for being too loose (FOE 2008). None of the existing EU regulations applicable to agriculture, food or food packaging currently consider or mention nanoscale products or materials. If a substance has already been approved for use as food ingredients, additives or packaging in its bulk form, it can also be used in this nano form since there is no regulatory trigger to require new safety assessment or labeling due to particle size (FOE 2008).

The existing regulation regarding food additives is in the process of being updated in the EU. During this process the European Parliament’s Committee on Environment, Public Health and Food Safety last year stated that it wanted separate limit values for nanotechnologies and that the permitted limits for an additive in nanoparticle form should not be the same as when it is in traditional form (Halliday 2007).

The EU Novel Foods Regulation requires mandatory pre-market approval of all new ingredients and products. Recently, the European Commission adopted a proposal to revise the Novel Foods Regulation with a view to improving the access of new and innovative foods to the EU market (CEC 2008b). In the revised regulation the definition of novel food includes foods modified by new production processes, such as

nanotechnology and nanoscience, which might have an impact on the food itself. Once the European Commission receives an application for authorization of a novel food and its use as an ingredient, the European Food Safety Authority (EFSA) evaluates whether or not it presents a danger to consumers or misleads them. The regulation requires assessments by EFSA on the composition, nutritional value, metabolism, intended use and the level of microbiological and chemical contaminants. Studies on the toxicology, allergenicity and details of the manufacturing process may also be considered. However, the regulation makes no distinction in relation to particle size, and hence nanoparticles will not require new safety assessments if the substance has already been approved in bulk form. EFSA is currently preparing a scientific opinion on the potential risks arising from the use of nanotechnology in food. A Draft Opinion was published for public consultation in which EFSA concludes that that nanotechnology aspects shall be considered when risk assessment guidance documents in the food and feed area are reviewed, and among others recommend that risk assessment of nanomaterials in the food and feed area should consider the specific properties of nanomaterials in addition to those common to the equivalent non-nanofoms (EFSA 2008).

4.4 Safety at Workplace Directives

There is no direct reference to the potential exposure of engineered nanoparticles in the Safety at Workplace Directives or in the communitarian and national legislation on the protection of workers' health at workplaces (Franco *et al.* 2007). However, according to the Commission of the European Communities (2008) they fully apply to nanomaterials and “...employers, therefore, must carry out a risk assessment and, where a risk is identified, take measures to eliminate this risk.”

The Framework Directive 89/391 as well as Directive 98/24 on the risks associated with chemical substances set guidelines to establish Occupational Exposure Limits (OELs) for workers (Council of the European Communities 1989, 1998). However, there are three main problems associated with the establishment of OELs for workers at this point:

- 1) The establishment of OELs is typically based on a complete risk assessment procedure which is presently not possible for engineered nanoparticles;
- 2) OELs are based on mass concentration being a proper metric for toxicity, but the most optimal parameter(s) to determine nanoparticle toxicity is still undefined;
- 3) Nanoparticles are not easily detected and monitored in the workplace and it is unclear whether existing personal protective equipment is adequate (Franco *et al.* 2007).

At the moment, manufactures refer to OELs set for metal dusts or dusts of other compounds (e.g. graphite or carbon black instead of C₆₀ and carbon nanotubes). However, given the recent concern about the toxicity of airborne carbon nanotubes,

OELs defined for related bulk substances (e.g. graphite), are not representative and should not be used since properties displayed by nanoparticles differ substantially from those of their bulk material (Lam *et al.* 2004).

Problems associated with establishing OELs are reflected in many of the Material Safety Data Sheets (MSDSs) made available by the producers, many of which classify carbon nanotubes as graphite. In addition, a list of exposure control measures are recommended in the MSDS if “engineering controls do not ensure that the OEL is not exceeded”, while the absence of any OEL is mentioned a few lines below. These problems and inconsistencies reveal serious gaps in the Safety at Workplace regulation, considering that the MSDSs are essential in passing information about risk and safety down the supply chain and that they also provide workers and emergency personal with information about the risks, protective equipment and proper handling of a substance (Franco *et al.* 2007).

4.5 Waste management of products containing nanoparticles

Waste containing nanoparticles are produced at different phases of the life cycle, from by-products generated during manufacturing and purification processes to nanoengineered goods, becoming waste at the end of their lifetime. There are no specific references to engineered nanoparticles in existing laws and hence nano-waste are tackled by waste management regulations in a non-specific way (Franco *et al.* 2007). In general, nanoparticles will follow the material or the substance in which they are contained and their fate depends on the way this waste is treated. In some cases the nano-waste can fall within a particular waste category, such as if C₆₀ is used in oil lubricants and the exhausted oil lubricant is specifically regulated (Franco *et al.* 2007). In other cases nanoparticles which are contained in products are typically disposed of in landfills or incinerated. Very little is known about the long-term behavior of nanoparticles in a landfill. Release depends on the nanoparticles’ mobility as well as on the degradability of the host material for fixed particles. When products are incinerated, the thermal properties of nanoparticles determine their fate. For example, a study by Cataldo (2002) demonstrated that C₆₀ molecules are much easier to degrade than carbon nanotubes. Further, in a combustion chamber C₆₀ behaves like graphite, while carbon nanotubes, similar to diamonds, are stable until very high temperatures.

If a certain nano-waste falls within the scope of Council Directive 1991/689 on the management of hazardous waste (Council of the European Communities 1991), more severe obligations would apply. Again, the lack of (eco)toxicological data makes it difficult to state if nanoparticles meet the criteria of hazardousness (Franco *et al.* 2007).

4.6 New nanospecific legislation vs. the “Incremental” approach and/or Voluntary Environmental Programs

A number of limitations were identified in section 4.1-4.5 above. First of all, although nanomaterials might be covered by the general scope of many of the existing legislative frameworks, it is often unclear if nanoparticles actually are covered specifically by current legislation when it comes to specific nanomaterials and applications. The main problems seem to be that metrology tools are unavailable, that thresholds are not tailored to the nanoscale, but based on bulk material, profound lack of (eco)toxicological data, and that no risk thresholds and occupational exposure limits cannot be established with existing methodologies.

Given the gaps and limitations of the current regulation, the questions on whether or not and how to regulate the manufacture and commercialization of nanomaterials have become subject to heated debate internationally, and the answer has been hard to come by, due to the pervasive uncertainty about the environmental and health risks of nanomaterials.

During the last few years, several legislative policies have been proposed and discussed by public institutions like, i.e. the European Commission, Health and Consumer Protection Directorate General (2004), RS & RAE (2004), U.S. Environmental Protection Agency (U.S. EPA) (2007) and non-governmental organizations like i.e. ETC Group (2003) and Environmental Defense (Balbus *et al.* 2007). The different views on how to regulate nanomaterials vary substantially, ranging from a “laissez-faire” attitude to a total moratorium on nanotechnology research, development and commercialization. A number of different kinds of regulatory options are available for decision makers with regard to nanomaterials. These include implementing a new regulatory framework, various command-and-control measures, voluntary environmental programs, etc.

Currently, the possibility of a specific regulation on nanomaterials is considered unfeasible in the European context, due to the difficulty of establishing links between strikingly different pieces of legislation and the need to negotiate internationally in order to establish a sensible specific, in-depth regulatory process (European Commission, Health and Consumer Protection Directorate General 2004). Instead, the European Commission has adopted a so-called “incremental approach”, which focuses on adapting existing laws to regulate nanotechnologies. This approach is defined as the launch of a process which uses existing legislative structures to the maximum, revisits them, and, when appropriate only, amends them in order to deal with nanomaterials.

However, so far, the only amendments that have been implemented in the EU are to cut carbon and graphite from the list of substances exempted from registration under REACH – arguably dissatisfactory to address the current regulatory uncertainty and the concerns expressed by the politicians, members of the public, industry and investors and insurers.

Several governments have opted to implement voluntary environmental programs (VEPs), arguing that this is the only viable proportional option for the time being (Hansen and Tickner 2007, DEFRA 2006a, 2006b, US EPA 2007, Weiss 2005). One such VEPs is the Voluntary Reporting Scheme for Nanomaterials established by the Department for Environment Food and Rural Affairs (DEFRA) in the UK. The reporting scheme for engineered nanoscale materials was established in 2006 in response to a highly cited Royal Society and Royal Academy of Engineering report (RS & RAE 2004) and the UK Government response to that report (HM Government 2005). Both of these reports identified large areas of uncertainty about: 1) the risks posed by engineered nanomaterials, and 2) the types of nanomaterials that will become most widespread within industry in the UK. The purpose of the DEFRA scheme is to, “*develop a better understanding of what types of engineered nanoscale materials are likely to be produced in the UK, and to build up an understanding of their properties and characteristics so that the potential hazard, exposure and risk associated with these materials may be determined.*” (DEFRA 2006) The program is intended to run for two years. It begins by engaging industry to submit existing data on the characteristics of engineered nanoscale free materials, including information on material characterization, hazard, use and exposure potential, and risk management practices. Submission of all available information is encouraged and lack of a complete package of data should not keep companies from reporting under the scheme (DEFRA 2007). However, DEFRA does not request that industry develop new data and even discourages industry from generating any additional data that would require animal testing (DEFRA 2006). After more than 2 years of implementation only eleven submissions have been received by DEFRA, two from academia and nine from industry (DEFRA 2008). The results to date have been disappointing given that a recent report have identified more than 60 companies in the UK for which nanotechnology is the primary area of business whereas another report found that 372 companies there involved in micro- and nanomanufacturing in the UK (NMT Network 2005, Technology Transfer Center 2007).

It remains to be seen whether and to what extend voluntary measures will be enough to generate the up-to-date and relevant health and safety information needed to perform risk assessments to support and implement regulations. It is generally known that key elements of any successful VEP are: incentives to participate for various stakeholders, agency guidance and technical assistance, signed commitments and periodical reporting, quality of information, and transparency both in design, reporting and evaluation. However, Hansen and Tickner (2007) recently found that many of these elements have not been fully addressed in the VEPs that are implemented currently on nanomaterials – neither in the UK or anywhere else.

5. Risk assessment of nanomaterials

The analysis of the applicability of the current regulation on nanomaterials showed that among other (eco)toxicological data and risk assessments are often necessary to support and implement regulations.

At the moment, risk assessment methodologies are being discussed, evaluated and refined with great vengeance internationally with the hope that in the future, we will be able to perform complete scientifically valid quantitative risk assessments of nanomaterials. It is generally assumed that once such risk assessments have been completed, this will lead to informed risk management decisions protecting human health and the environment while reaping the benefits of nanotechnology for society. When risk assessment of nanomaterials is discussed, it is often in the context of previous experience with chemical risk assessment, consisting of four parts – hazard identification, dose-response assessment, exposure assessment, and risk characterization. In Europe, legislation for controlling the production, use and release of chemical substances is based on risk assessment, as described in detail in the “Technical Guidance Document” (TGD) (European Commission JRC 2003a). The TGD totals a staggering 1000 pages and is issued by the European Commission to help competent authorities to carry out risk assessments. It includes extensive technical details for conducting hazard identification, dose (concentration) – response (effect) assessment, exposure assessment and risk characterization in relation to human health and the environment (European Commission JRC 2003a, 2003b, 2003c, 2003d).

The purpose of this chapter is not to do a complete risk assessment in accordance to the TGD, but to assess the applicability of each of the four elements of risk assessment with regard to nanomaterials. In this process, the current state of knowledge will be addressed and the most significant findings will be highlighted in relation to each of the four parts of risk assessment.

5.1 Hazard identification

Hazard identification is defined as the “*identification of the adverse effects which a substance has an inherent capacity to cause*” (European Commission JRC 2003a). However, until recently the potential negative effects of nanomaterials on human health and the environment were rather speculative and unsubstantiated. This has changed within the past few years and a number of laboratory studies have indicated that exposure to some nanoparticles can lead to adverse effects in the lungs and the brain of test animals (Lam *et al.* 2004, Oberdörster 2004, Pollard *et al.* 2008).

In a recent review, Hansen *et al.* (2007) identified 428 studies reporting on the toxicity and ecotoxicity of nanoparticles. The studied materials have mainly been

nanoparticles suspended in water belonging to category IIIb in figure 6 (Oberdörster 2004, Sayes *et al.* 2005), but also some studies with airborne nanoparticles (category IIIc in figure 6) have been carried out (Baggs *et al.* 1997, Cheng 2004, Baker *et al.* 2005).

Figure 8 shows the distribution of these studies divided into cytotoxicity, mammalian toxicity, DNA damage, microbial test and ecotoxicity. In total, the studies reported the observed adverse effects of 965 tested nanoparticles of various chemical compositions.

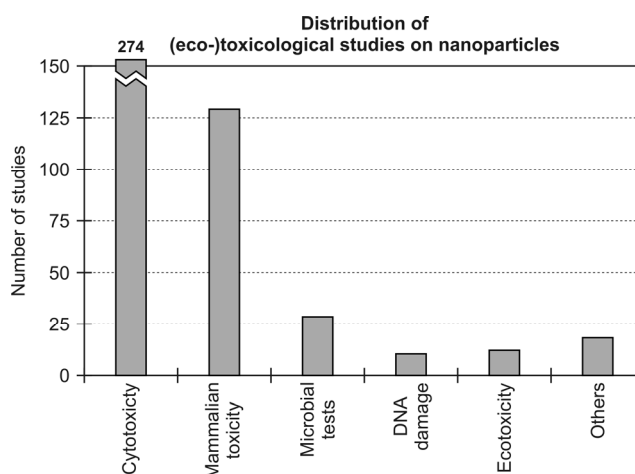


Figure 8: Distribution of studies on nanoparticles describing cytotoxicity, mammalian toxicity, microbial tests, DNA damage, and ecotoxicity of engineered nanoparticles. Total number of studies: 472 (Hansen *et al.* 2007a)

The scientific literature on the environmental, health and safety of nanomaterials has been reviewed multiple times by both national and international scientists, agencies, and governments. In the following, a number of significant scientific studies and findings in regard to hazard identification of C₆₀, carbon nanotubes, quantum dots, and nanometals will be introduced.

5.1.1 C₆₀

Studies on C₆₀ can be divided into three categories: 1) studies on C₆₀ suspended by sonication or suspended in various solvents, 2) studies on hydroxylated C₆₀ and 3) studies on functionalized C₆₀. The number of *in vivo* toxicity studies is limited for all three categories. Gharbi *et al.* (2005) and Mori *et al.* (2006) observed no acute or subacute effects in rats exposed to C₆₀ in a dose of 2 g/kg body weight (bw) 14 and 21 days post exposure, respectively. Sayes *et al.* (2007a), however, did observe an increase in the percentages/numbers of Bronchoalveolar lavage (BAL)-recovered neutrophils (i.e. white blood cells) after intratracheally instillation of C₆₀ and hydroxylated C₆₀ i.e. C₆₀(OH)₂₄ just 1 day post-exposure. Sayes *et al.* (2007a) also observed a significant

increase in lipid peroxidation values and an increase in level of glutathione (GSH), after 1 week. Lai *et al.* (2000) also observed a significant increase in lipid peroxidation products after intravenous administration of 1 mg C₆₀(OH)₁₈ per kg into male mongrel dogs previously induced with infusion/reperfusion injury. In contrast to Sayes *et al.* (2007a), Lai *et al.* (2000) observed a decrease in the GSH level in intestinal tissue. In one of the only studies so far investigating the embryo-/fetotoxicity on nanoparticles, Tsuchiya *et al.* (1995) observed shrunken membrane and narrow blood vessels on the yolk sac on 11 day pregnant mice and embryo death 18 hours (h) after intraperitoneal (i.p.) injection of between 25-137 mg C₆₀ per kg.

Adverse effects of functionalized C₆₀ have been observed as well for instance by Chen *et al.* (1998) and Yamago *et al.* (1995). Chen *et al.* (1998) observed a LD₅₀ of 600 mg C₆₀((CH₂)₄SO₂Na)₄₋₆/kg bw in female rats after intraperitoneal (i.p.) administration of between 0 and 2,500 mg/kg bw for 2 weeks. Whereas Yamago *et al.* (1995) observed symptoms of discomfort and weight loss in female mice after a single intraperitoneal injection of between 200-500 mg/ per kg.

The cytotoxic effects of C₆₀ have been studied extensively *in vitro* using a number of different cell strains, different test procedures including a range of different solvents to get C₆₀ into suspension. Adelman *et al.* (1994) observed a reduction of the viability of bovine alveolar macrophages compared to control after exposure to sonicated C₆₀ along with increased levels of cytokine mediators of inflammation (i.e. TNF, IL-6 and IL-8) whereas Baierl *et al.* (1996) and Porter *et al.* (2006) found that C₆₀ and raw soot was not toxic towards bovine- and human alveolar macrophages. The alveolar macrophage serves as the first line of cellular defense against respiratory pathogens (Rubins 2003) and hence studies reporting on the effects on alveolar macrophages are of special interests.

Studies on the cytotoxicity of C₆₀ towards cancer cell are ambiguities and while some have reported observing no signs of cytotoxicity of any kind after exposure to fairly high concentrations of C₆₀, suspended in toluene, methanol and by sonication (Baierl *et al.* 1996, Levi *et al.* 2006), others do observe cytotoxicity of C₆₀ suspended in tetrahydrofuran (THF) (Burlaka *et al.* 2004, Isakovic *et al.* 2006a, b). However, these studies are hard to interpret since they often use different transformed and/or damaged cell strains and different ways of suspending C₆₀ – some even consider the cytotoxicity of C₆₀ under the influence of light.

Surface chemistry has been found to have an important influence on the toxicity of C₆₀. Dose-dependent cytotoxicity of hydroxylated C₆₀ and functionalized C₆₀ has been observed for instance by Yamawaki and Iwai (2006) who observed a dose-dependent decrease in cell density and lactate dehydrogenase (LDH) release in human umbilical vein endothelial cells cavity after exposure to C₆₀(OH)₂₄. Sayes *et al.* (2004) found that the toxicity of C₆₀ towards human skin fibroblasts and liver carcinoma cells varied by seven orders of magnitude depending on the number of functional groups.

Aggregates of C₆₀ were found to be substantially more toxic than highly soluble derivatives such as C₃-, Na+2-3[C₆₀O7-9(OH)12-15](2-3)-, and C₆₀(OH)₂₄. Rouse *et al.* (2006) observed a dose-dependent decrease in the viability of human epidermal keratinocytes after exposure to C₆₀-phenylalanine while exposing HeLa cells to di-, tri-, quadrimalonic acid C₆₀ was observed by Yang *et al.* (2002) to cause irradiation- and dose-dependent cytotoxicity.

A number of studies also report finding no cytotoxic effects on macrophages, human keratinocytes, human skin and lung MC and PBB cells, and human fibroblasts cells after exposure to C₆₀((CH₂)₄SO₃Na)₄₋₆, C₆₀[C(COOH)₂], Polyhydroxy C₆₀, and N-ethyl-polyamino C₆₀ (Chen *et al.* 1997, Fumelli *et al.* 2000, Ryan *et al.* 2007).

A number of studies have reported hydroxylated C₆₀ being able to reduced cell and neuronal death induced by: Doxorubicin (Bogdanovic *et al.* 2004), sodium nitroprusside (SNP) and H₂O₂ (Chen *et al.* 2004), NMDA, amino-3-hydroxy-5-methyl-4-isoxazole propionic acid or kainite (Dugan *et al.* 1996), iron (Lin *et al.* 1999), serum (Lu *et al.* 1998), UVB-irradiation (Xiao *et al.* 2005). Others have reported that glutathione, ascorbic acid and α -tocopherol were capable of inhibiting membrane damage induced by hydroxylated C₆₀ (Kamat *et al.* 2000).

A number studies on the environmental effects of C₆₀ have been done as well. Oberdörster (2004) observed significant increase in lipid peroxidation of the brain of juvenile largemouth bass after exposure to uncoated fullerenes (99.5%) in concentrations of 0.5 and 1 ppm after exposure for 48 h. The C₆₀ were dissolved in THF which have since then led to some discussion about whether C₆₀ or the THF was responsible for the effects observed (Zhu *et al.* 2006, Henry *et al.* 2007).

Henry *et al.* (2007) compared the effect on larval zebrafish *Danio rerio* of 1) 99.5% C₆₀ that had been prepared in water through stirring and sonication and 2) 99.5% C₆₀ that had been dissolved in THF. Henry *et al.* (2007) observed no affect on the survival of larval zebrafish *Danio rerio* after exposure to stirred and sonicated C₆₀ in concentrations up to 25% vol/vol for 72 hours. No death was observed in zebrafish *Danio rerio* < 5% vol/vol with LC₅₀ = 3.1% (95% CI, 2.3–4.2%) for C₆₀ dissolved in THF whereas lethal effects were observed within 60 min. above > 5% vol/vol, in addition to arched backs, severe yolk-sac and pericardial edema. Henry *et al.* (2007) also observed significant changes in expression of 271 genes compared to only 10 for stirred and sonicated C₆₀.

Zhu *et al.* (2006) compared the ecotoxicity on *Daphnia magna* of hydroxylated C₆₀ with C₆₀ dissolved in THF and found an LC₅₀ > 35 ppm for hydroxylated C₆₀ after 48 hours compared to LC₅₀ = 0.8 ppm for the THF-dissolved C₆₀. 100% mortality was furthermore observed in fathead minnow after exposure to 0.5 ppm THF-dissolved C₆₀ for 6-18 hours, whereas no obvious physical effects were observed in fathead minnow after exposure to 0.5 ppm for 48 hours. Elevated lipid peroxidation was, however,

observed in the brain and gill along with increased expression of CYP2 family isozymes in the liver (Zhu *et al.* 2006, Oberdörster *et al.* 2006).

Lovern and Klaper (2006) also compared sonicated C₆₀ with C₆₀ dissolved in THF. Despite a great variation in mortality in *Daphnia magna*, they reported observing a LC₅₀ of 7.9 ppm for sonicated C₆₀. The lowest- and no observed effect concentration (LOEC and NOEC) was reported to be 0.45 ppm and 0.18 ppm, respectively. For C₆₀ dissolved in THF, a dose-dependent increase in mortality was observed after 48 hours, and LC₅₀, LOEC, and NOEC was found to be sustainably lower i.e. 460, 260 and 180 ppb, respectively. A number of behavioral changes have been reported as well. For C₆₀ dissolved in THF, Lovern *et al.* (2007) observed a significant increase in hopping rate, heart rate as well and the number of cycles per minute in appendage movement compared to control and 30 nm TiO₂ after exposure to 260 ppb.

In a long-term study Oberdörster *et al.* (2006) investigated the effect of hydroxylated C₆₀ on the reproduction and survival rate of *Daphnia magna* and observed an increased cumulative mortality and significant delay in molting and reduced offspring after exposure to 1-5 ppm for 21 days.

The hypothesis that C₆₀ could act as a vector for the transport of other toxic chemicals has also been investigated. Baun *et al.* (2008) investigated whether C₆₀ affected the ecotoxic effects of known environmental pollutants such as phenanthrene and pentachlorophenol. It was found that the toxicity of phenanthrene was increased towards algae and *Daphnia magna* following sorption to C₆₀ aggregates. In contrast, Baun *et al.* (2008) found that the toxic effect of pentachlorophenol decreased when C₆₀ was added.

The effect on various kinds of bacterial strains has also been tested. Chiron *et al.* (2000) tested micronized C₆₀ and observed no effect on microbial growth of 22 collection strains including 6 strains of *S. typhimurium*, 5 strains of *E. coli*, and 2 strains of *P. aeruginosa*, *S. aureus* and *L. monocytogenes* (43.2 µg/mL). Babynin *et al.* (2002) found that the occurrence of mutations in *S. typhimurium* strain BA13 depends on the type of molecular group with which fullerene interacts whereas Fortner *et al.* (2005) observed that bacteria growth was media dependent. No growth was observed for either *E. coli* DH5α or *B. subtilis* CB315 exposed to > 0.4 mg/L C₆₀ at pH= 7 under anaerobic or aerobic conditions using a Minimal Davis media. When using a Luria broth media, growth was observed when exposed to ≥ 2.5 mg/L. For C₆₀ suspended in THF, Lyon *et al.* (2005, 2006) observed minimal inhibition concentrations of 0.5-1 mg/L and 1.5-3 mg/L for *E. coli* and *B. subtilis*, respectively. Similar observations have been reported for various functionalized C₆₀ (Tsao 2002, Mashino *et al.* 2003a, b, Tegos *et al.* 2005, Fang *et al.* 2007, Tang *et al.* 2007).

5.1.2 Carbon nanotubes

A number of studies have been done on the cytotoxicity of single-walled carbon nanotubes (SWCNTs). Cui *et al.* (2005) observed a dose-and time dependent inhibition of cell proliferation, and a decrease in cell adhesive ability in human embryo kidney 293 cells after exposure to SWCNT in concentrations between 0.78125-200 µg/mL for up to 5 days. Shvedova *et al.* (2003) observed oxidative stress and cellular toxicity towards human epidermal keratinocytes (HaCAT), after 2 to 18 hours exposure to unrefined SWCNT (30% iron) in concentrations ranging from 0.6-0.24 mg/mL. This lead to speculation about whether adverse effects observed were caused by SCWNTs or iron residues.

Sayes *et al.* (2006a) found that cytotoxicity towards human dermal fibroblasts was dependent on the density of surface functionalization. Underivatized SWCNT was the most cytotoxic whereas a concentration dependent decrease in the viability of fibroblasts was observed for SWCNT functionalized with phenyl-SO₃H, phenyl-(COOH)₂ and phenyl-SO₃Na. This decrease did however not exceeding 50%.

Lam *et al.* (2004) tested SWCNT of different purity, i.e. raw and purified nanotubes containing (29.6% and 2.14% Fe) and nickel-containing nanotubes (25.99% Ni). Lam *et al.* (2004) exposed mice to concentrations between 0 and 0.5 mg in durations of 7 and 90 days and found that all nanotubes induced dose-dependent granulomas and interstitial inflammation. The fact that purified nanotubes produced prominent granulomas indicates that nanotubes – and not the residue metals – are the cause of these lesions. The results by Lam were supported by observations made by Warheit *et al.* (2004) who also observed pulmonary grandulomas in rats after exposure to SWCNT soot (5% Ni and Co) in concentrations of 1 and 5 mg/kg for time durations of 24 hours up to 3 months. However, effects observed by Warheit *et al.* (2004) were non-dose dependent in contrast to Lam *et al.* (2004). Absence of pulmonary biomarkers suggests a potentially new mechanism of pulmonary toxicity and injury induced (Warheit *et al.* 2004).

The number of studies reporting on the toxicity of multi-walled carbon nanotubes (MWCNTs) is far less, compared to SWCNTs. Carrero-Sanchez *et al.* (2006) observed lethal effects of 30, 60, and 90% in male inbred CD1 mice after intratracheal administration of a single dose of 1, 2.5, and 5 mg/kg along with signs of pulmonary distress, mainly dyspnea. Multiple granulomas were observed in the lung interstitium and globet cell hyperplasia after 15 d (50-150 µL/ 24 h-30 days post treatment). Muller *et al.* (2005) observed dose-dependent increase in LDH release in female Sprague-Dawley rats after intratracheal instillation which was more marked in the ground MWCNTs (0.5-5mg/60d) than in MWCNTs.

Recently, Pollard *et al.* (2008) compared the toxicity of four kinds of MWCNT of various diameters, lengths, shape and chemical composition by exposing the mesothelial lining of the body cavity of three mice with 50 mg MWCNT for 24 hours or

7 days. This method was used as a surrogate for the mesothelial lining of the chest cavity. It was found that long MWCNTs “*produced length dependent inflammation, FBGCs and granulomas that were qualitatively and quantitatively similar to the foreign body inflammatory response caused by long asbestos*”. Only the long MWCNTs caused significant increase in polymorphonuclear leukocytes or protein exudation. The short MWCNTs failed to cause any significant inflammation at 1 day or giant cell formation at 7 days. Pollard *et al.* (2008) also found that water-soluble components of MWCNT did not produce significant inflammatory effects 24 hours after injection, which rules out that residue metals were the cause of the observed effects such as others previously had speculated on the basis on *in vitro* studies (Shvedova *et al.* 2005, Kagan *et al.* 2006).

Plenty of cytotoxicity tests have been carried out on MWCNTs. Bottini *et al.* (2006) for instance, found that MWCNT was more cytotoxic when oxidized towards viability of Jurkat T leukemia cells whereas Clopek *et al.* (2006) and Monteriro-Riviere *et al.* (2005) observed a decrease of the viability of human osteoblastic lines and human epidermal keratinocytes.

Similar to observations made on the ecotoxicity of C₆₀, Roberts *et al.* (2007) observed peak survival in *Daphnia magna* at 0.5 mg/L whereas mortality of 20% and 100% were observed at 10 and 20 mg/L, respectively, after 96 hours of exposure to 0-20 mg of 85% SWCNT coated with lysophosphatidylcholine. Smith *et al.* (2007) tested the ecotoxicity of SWCNT dissolved through a combination of sodium dodecyl (SDS) and sonication and they observed a dose-dependent rise in ventilation rate, gill pathologies (oedema, altered mucocytes, hyperplasia), and mucus secretion with SWCNT precipitation on the gill mucus of juvenile rainbow trout. They also observed dose-dependent changes in brain and gill Zn or Cu, partly attributed SDS, a significant increase in Na⁺K⁺-ATPase activity in the gills and intestine, a significant dose-dependent decrease in thiobarbituric acid reactive substances (TBARS), especially in the gill, brain and liver, and a significant increase in the total glutathione levels in the gills (28%) and livers (18%), compared to the solvent control (15 mg/L SDS). TBARS are indicators of lipid peroxidation and oxidative stress. Finally, Smith *et al.* (2007) observed increasing aggressive behavior, possible aneurisms or swellings on the ventral surface of the cerebellum in the brain and apoptotic bodies and cells in abnormal nuclear division in liver cells.

Cheng *et al.* (2007) observed significantly delayed hatching of zebrafish (*Danio rerio*) embryos from the blastula period following exposure to 120-360 mg SWCNT/L for 4-96 hours whereas carbon black and double walled carbon nanotubes had no influence on hatching at either of the tested concentrations i.e. 20-360 mg/L. The hatching delay observed for SWCNT did not influence the hatching success rate and survival of the embryos exposed to between 20-360 mg/L up to 96 hours postfertilization.

Templeton *et al.* (2006) compared “As prepared” SWCNT with electrophoretically purified SWCNT and the fluorescent fraction of nanocarbon byproducts. Templeton *et al.* (2006) observed an average cumulative life-cycle mortality of $13 \pm 4\%$, while mean life-cycle mortalities of 12 ± 3 , 19 ± 2 , 21 ± 3 , and $36 \pm 11\%$ were observed for 0.58, 0.97, 1.6, and 10 mg/L. Exposure to 10 mg/L showed: 1) significantly increased mortalities for the naupliar stage and cumulative life-cycle, 2) a dramatically reduced development success to 51% for the nauplius to copepodite window, 89% for the copepodite to adult window, and 34% overall for the nauplius to adult period, and 3) a significantly depressed fertilization rate averaging only $64 \pm 13\%$. They also observed that exposure to 1.6 mg/L caused a significant increase in development rate of 1 day faster, whereas a 6 day significant delay was seen for 10 mg/L. For the electrophoretically purified SWCNT, Templeton *et al.* (2006) observed a naupliar, copepodite, and adult-stage mortality of 13 ± 2 , 3 ± 0 , and $0 \pm 0\%$, respectively, after 28 d and a significant 1 day delay in development rate at 1.6 mg/L after exposure to between 0.58-10 mg/L. For the fluorescent fraction of nanocarbon byproducts, the cumulative life-stage mortality averaged $10 \pm 3\%$. For the two highest concentrations tested (i.e. 1.6 and 10 mg /L), nauplius-to-adult stage mortalities were increased with average full life-cycle mortalities of $81 \pm 7\%$ whereas development success in treatments ranged between 81 and 95%. For the copepodite-to-adult stage transition, the three highest exposures of 0.97, 1.6 and 10 mg/L resulted in significantly reduced development success, whereas this was the case for all concentrations when considering the full life-cycle development success from the nauplius-to-adult stage.

5.1.3 Quantum dots

The toxicity of quantum dots (QDs) has been found to differ among others depending on the constituting metals, size, metal ratio, surface charge and coating of the QDs. Larson *et al.* (2003), for instance, observed no ill effects on mice after intravenous injection of CdSe/ZnS ($\sim 1 \mu\text{M}$ and 20 nM), and a number of studies have reported observing no effect on quantum dots such as CdSe and CdSe/ZnS on various cell lines (Chen and Gerion 2004, Chan *et al.* 2006, Delehanty *et al.* 2006). Delehanty *et al.* (2006) did, however, observe dose-dependent cell proliferation for CdSe/ZnS after incubation of COS-1 or HEK 293T/17 cell lines, just as Zhang *et al.* (2007) have reported observing in human hepatoma HepG2 cells for CdTe.

The cytotoxicity of QDs was found to be influenced by the exposure to light and temperature dependent. Green and Howman (2005), for instance, observed 56% damaged DNA after incubation and exposure to UV light for 0-60 min. vs. 29% when incubated in the dark for CdSe/Zn. Whereas Chang *et al.* (2006) found that CdSe/CdS was cytotoxicity to cancer cells at 37 °C, but not at 4 °C. Quantum dots are often coated in order to improve their biocompatibility and to reduce their toxicity (Hardman, 2006). Adverse cytotoxic effects have been reported on in mouse lymphocytes after exposure

to CdSe/ZnS coated with albumin fraction V (Hoshino *et al.* 2004). Kirchner *et al.* (2005) and Lovrić *et al.* (2005) observed significant decrease in cell metallic activity and marked cytotoxicity in NRK fibroblasts, MDA-MB-435S breast cancer cells, Chinese ovary cells (CHO), rat basophil leukemia (RBL) cells and rat pheochromocytoma cells (PC12) and N9 murine microglial cells for not only QD of different sizes coated with MPA but also of dots coated with Cysteamine hydrochloride. Uncoated CdTe QDs were cytotoxic at 1 µg/mL. Derfus *et al.* (2004) found that the cytotoxicity of CdSe coated with TOPO could be reduced by capping them with ZnS, MAA (mercaptoacetic acid) and bovine serum albumin (BSA) whereas Lovrić *et al.* (2005) also observed that cytotoxicity was more pronounced with smaller positively charged QDs (2.2 ± 0.1 nm) than with larger equally charged QDs (5.2 ± 0.1 nm) at equal concentrations. In contrast Jaiswal *et al.* (2003) found that CdSe/ZnS coated with DHLA had no effect on mammalian cells and Ballou *et al.* (2004) observed no signs of necrosis or decreased viability of mice after injection of QD coated with amphiphilic polyacrylic acid polymer and PEG-amine groups at QD concentrations of 20 pmol QD/g animal weight.

Only a limited number of studies have been done of the ecotoxicity of QDs. Dubertret *et al.* (2002) observed some abnormalities in cell size, cell death, and cell movement after injected in frog embryos at higher concentrations ($> 5 \times 10$ QDs per cell) 10-15 nm CdSe/ZnS quantum dots coated with n-poly(ethylene glycol) phosphatidylethanolamine and phosphatidylcholine.

5.1.4 Nanometals

A number of different nanometals have been tested in (eco)toxicity tests and in the following the findings regarding nano- Titanium dioxide, Zink, Silicon dioxide and Iron oxide will be presented.

Titanium dioxide

Various effects have been observed in various strains of rodents after exposure to TiO₂ and several studies report differences in the responses for TiO₂ particles smaller than 100 nm compared to those found for larger particles.

After long-term and daily exposure Oberdörster *et al.* (2004) observed progressive increase in total cells lavaged in rats and a significantly prolonged pulmonary retention and sustained impairment of alveolar macrophages for 20 nm particles (23.5 mg/m^3) compared to the 250 nm particles (23.5 mg/m^3). Ferin *et al.* (1992) and Baggs *et al.* (1997) also observed greater alveolar epithelial thickness and fibrosis in rats for 20 nm particles than for 250 nm particles, whereas Wang *et al.* (2007a) found a significantly higher level in the liver coefficients in female mice after a single dose oral administration of 80 and 25 nm particles compared to control and 155 nm particles.

When comparing anatase TiO₂ particles of 20 and 250 nm Oberdörster (2000) found that the former generated a much greater pulmonary-inflammatory response in rats after exposure to equal mass doses. When the dose is expressed as a function of mass and surface particle number it showed huge differences in the dose-response curve of the two materials, but when expressed in terms of particle surface area, both forms of TiO₂ followed a similar dose-response curve. Donaldson *et al.* (2002) reported observing a similar correlation for carbon black, when studying the ability of nano- and micron particles to cause inflammatory effect in rats (Donaldson *et al.* 2002). Warheit *et al.* (2006) and Sayes *et al.* (2007b) reported, however, not to have observed any association with surface area when evaluating biological response in rats after exposure to nano-sized TiO₂, SiO₂ and other particles.

A number of factors have been hypothesized to influence the toxicity of TiO₂ besides surface area. These include crystalline structure and shape and exposure route (e.g. intratracheal vs. oral vs. inhalation). Oberdörster *et al.* (1992) observed that anatase and rutile TiO₂ caused different responses in rats after intratracheal instillation. For anatase TiO₂, a significant increase in total lavagable cells, polymorphonuclear leukocytes (PMN) and total protein content was observed whereas a low level of PMN response was observed for rutile particles.

Warheit *et al.* (2007a) observed no difference in the effects caused by spherical dots versus nanorods of TiO₂ – both caused a short-lived 24 hours post-exposure, increase in the percentages/numbers of BAL-recovered neutrophils, BAL fluid LDH and BAL fluid micro-protein in rats after intratracheally instillation of 5 mg/kg after 24 hours and 3 months post-exposure. Warheit *et al.* (2007b) furthermore observed that a temporary increase in percentages of BAL-recovered neutrophils for \approx 98 and 88 wt% TiO₂ 24 hours after intratracheal instillation. For 100% wt, inflammation was still evident after 3 months for rats exposed to 5 mg/kg.

The influence of coatings has been investigated as well by Höhr *et al.* (2002) who observed a lower non-significant total cell number and influx of neutrophils (PMN) in female rats after intratracheal instillation of 1 mg coated particles compared to untreated particles. No difference was observed with 6 mg or surface area > 600 cm².

Questions have been raised about the biological relevance of intratracheal instillation since it bypasses upper respiratory tract defenses and does not deposit particles evenly in the lung similar to what would be observed after inhalation (Oiser *et al.* 1997). Observations made by Ferin *et al.* (1991) might support this argument. They observed that intratracheal instillation of 20 nm particles caused a more pronounced effect in rats (i.e. increased number of leukocytes) compared to inhalation. Oiser and Oberdörster (1997) contradict these findings in the sense that they observed significantly elevated levels of total protein following inhalation compared to intratracheal instillation.

The cytotoxicity of TiO₂ has been found to be dependent on crystalline structure, size and purity as well. Sayes *et al.* (2006b) observed a time-dependent decrease in human dermal fibroblasts (HDF) for anatase TiO₂ (30 µg/mL/1-48 h) and found a LC₅₀ = 3.6 µg/mL, but for rutile TiO₂, the LC₅₀ was substantially higher and equal to 550 µg/mL. with LC₅₀ of anatase/rutile TiO₂ falling between the two.

Zhu *et al.* (2006) observed a more severe toxic effect (LC₅₀= 100 µg/mL) and cell apoptosis on Chinese hamster ovary tumor cells after 24 hours, whereas growth inhibition was the only effect observed in human kidney epithelium 293T cells (LC₅₀= 300 µg/mL). Wang *et al.* (2007b) observed a time- and dose-dependent decrease in the viability of WIL2-NS human lymphoblastoid cells ranging from 2-62% (0-130 µg/mL/6-48 h) whereas Rahman *et al.* (2006) observed significant increase in micronuclei in Syrian hamster embryo cells after treatment with 1.0 µg/mL ≤ 20 nm particles whereas > 200 nm particles had no effect (0.5-10 µg/mL/12-72h).

Limbach *et al.* (2007) found promoted increase in reactive oxygen species (ROS) in human lung epithelial A459 cells that increased with the purity of the particles (30 µg/mL/4 h). This observation was supported by Long *et al.* (2006) who observed a rapid (< 5 min) and sustained (120 min) release of ROS at non-cytotoxic concentrations (2.5-120 ppm/1-18 h) in BV2 brain microglia.

Vileno *et al.* (2007) observed the generation of ROS in human skin fibroblasts CCL-110 as well as cellular stiffness under illumination with UVA (8 and 20 mW/cm² for up to 180 s) whereas no cellular stiffness was observed without illumination.

The UV-reactive of TiO₂ has been a subject of several studies and although Uchino *et al.* (2002) and Gurr *et al.* (2005) found no additional effect of photoactivation against human bronchial epithelial BEAS-2B-cells and Chinese hamster ovary cells, evidence indicates that the cytotoxicity of TiO₂ is influenced by UV-irradiation. Cai *et al.* (1991, 1992) and Huang *et al.* (59) observed a survival rate higher than 90% in HeLa cells and U937 cells exposed to more than 360 µg/cm³ for 24 hours but observed 75-90% killing of cells after 10 min. exposure of UV light. Sayes *et al.* (2006b) found that UV illumination (10 W/cm²) of TiO₂ made cell death increase by 20%. Anatase TiO₂ produced a high LDH release in cells which increased when exposed to UV light and decreased mitochondrial activity and enhanced IL-8 in both HDF and A549 cells. Anatase/rutile TiO₂ produced a biological response less than that of anatase, but greater than rutile TiO₂.

Ivankovic *et al.* (2003) observed that the survival fraction for squamous Carcinoma SCVII cells not only differed between highly-dispersed and lowly-dispersed, but also differed when exposed to UV-irradiation. The cytotoxicity was higher for anatase and brookite TiO₂ particles of different sizes after being irradiated with 7-8 mV cm⁻² for 10 min. which again did not differ much for iron-doped anatase TiO₂ nanoparticles. Iron-doped Anatase + rutile nanoparticles of different sizes were, however, found to be sustainably less toxic. Cytotoxicity differed most between highly

dispersed and low dispersed nanoparticles. For 20 nm anatase/rutile TiO₂, survival fraction ranged between 0.768 (highly-dispersed) and 0.366 (lowly-dispersed) whereas it was 0.970 (highly-dispersed) and 0.418 (lowly-dispersed) for 17 nm iron-doped anatase/rutile TiO₂.

The effects of TiO₂ and irradiation on bacteria have also been studied. Maness *et al.* (1999) observed that *E. coli* K-12 cell death was dependent on cell concentration (9.1×10^2 - 5×10^8) after 30 min. irradiation (8 W/m) (0.1-1 mg/mL), whereas Rincón and Pulgarin (2003) found that cell survival was time-dependent in the presence of sunlight. Nakagawa *et al.* (1997), however, observed no significant increase in the frequency of revertant colonies in *S. typhimurium* strain TA100, TA98 and TA102 exposed to light (0-5 J/cm²).

Finally, in a (eco)toxicity study on *Daphnia magna*, Lovern and Klaper (2006) compared 30 nm size TiO₂ nanoparticles with TiO₂ of the same size dissolved in THF and sonicated 100-500 TiO₂ nanoparticles in a 48 hour immobilization test. For TiO₂, a dose-dependent increase in mortality in *D. magna* (0-10 ppm) was observed and LC₅₀ was estimated to be 5.5 ppm with LOEC and NOEC being equal to 2.0 and 1.0 ppm, respectively. No significant difference was observed in *D. magna* exposed to TiO₂ prepared with THF and TiO₂ alone whereas the mortality varied across concentrations ranging from (50 ppm-500 ppm and never exceeded 10% for the sonicated TiO₂. Zhang *et al.* (2007) investigated the effect of exposing carp to a combination of TiO₂ and cadmium salts and observed a 2.5-fold increase in cadmium accumulation (Zhang *et al.* 2007).

Zink

In one of the only studies in which humans have been exposed directly to nanoparticles, Beckett *et al.* (2005) reported observing no difference in 6 women and 6 men 24 hours after exposure to a total of 500 µg Zn/m³ over the course of 3 times 2 hours over 3 d. On the other hand Gordon *et al.* (1992) reported observing symptoms such as fever, chills, sore throat, chest tightness and headache in 4 human volunteers after exposure of 5 mg/m³ for 2 h. They also observed a significant increase in total cells, lactate dehydrogenase (LDH), β-glucuronidase, and protein content in male Hartley guinea pigs and Fisher 344 rats after 4 and 24 hours post-nose-only exposure of 5 mg/m³. Additionally, they found that β-glucuronidase was decreased in New Zealand rabbits exposed to 5.0 mg/m³ after 2 hours, whereas no increase was observed in total cells, LDH, and protein content. Wang *et al.* (2006a) observed some death and severe symptoms of lethargy, anorexia, vomiting, diarrhea, and loss of body weight in mice after gastrointestinal administration, whereas limited effect was observed for micro-Zn.

Sayes *et al.* (2007b) observed an increase in the production of LDH levels in immortalized rat lung epithelial cells after 1 hour exposure to Zn at 520 µg/cm² and

LDH levels were observed to be increased at concentrations ≥ 0.052 and $0.52 \mu\text{g}/\text{cm}^2$ after 4 and 24 h, respectively, but not after 48 hours and no increase in LDH levels were observed in primary rat alveolar macrophages.

Gojova *et al.* (2007) observed a 20 and 50% cell death at 10 and 50 $\mu\text{g ZnO}/\text{mL}$ respectively and viability was observed to be $89 \pm 1\%$ and $82 \pm 3\%$ after 4 hours. Long *et al.* (2006) observed 15-50% cell death after exposure to concentrations in the same range, i.e. 50-100 $\mu\text{g}/\text{mL}$. As with TiO_2 , the UV reactivity of ZnO has been investigated. Dufour *et al.* (2006) observed that the dose needed to reach 40–60% concentration-dependent cytotoxicity in CHO cells decreased if cells were exposed to particles and irradiated simultaneously or had been pre-irradiated. Finally, Brayner *et al.* (2006) reported observing 15% growth inhibition *E. coli* strain MG1655 in a bacterial test on ZnO_2 .

Silicon dioxide

In the literature, the toxicity of TiO_2 has often been compared to Si and SiO_2 . When comparing Si particles of different average sizes (i.e. 12, 50, 300, and 534 nm), Warheit *et al.* (2006) found a higher number of cells recovered by Bronchoalveolar lavage (BAL) from the lungs of rats after intratracheally instillation of 5 mg/kg 534 nm particles than with any of the other sizes of particles. For the 50 nm particles, a substantial lung inflammatory response was observed in rats at 24 hours post-exposure followed by a 15–20% increase in polymorphonuclear leukocytes 3 months post-exposure, whereas 534 nm particles produced persistent pulmonary inflammatory responses, with the 5 mg/kg producing $> 40\%$ neutrophils after 3 months post-exposure. 300 nm particles produced a reduced inflammatory response. A significant greater pulmonary inflammatory response in the lungs of rats was also reported for 1.6 μm particles compared to 50 nm particles (Warheit *et al.* 2007b).

Cytotoxic effects have been reported as well. Chen and Mikecz (2005) observed a significant inhibition in replication and transcription in human epithelial HEp-2 cells for 70 nm particles after exposure to 25 $\mu\text{g SiO}_2/\text{mL}$ for 24 h. Lin *et al.* (2006) tested amorphous SiO_2 and observed a dose- and time dependent decrease in the viability of human bronchoalveolar carcinoma A549 cells. For 15 nm particles cell numbers decreased to 82.8%, 67.1%, and 54.6% for 10, 50, and 100 $\mu\text{g}/\text{mL}$ respectively after 72 hours and there was no significant difference between 15 and 46 nm particles.

Various kinds of coatings – including succinic acid (Jovanovic *et al.* 2006), dimethylamino groups (Bertazza *et al.* 2006), chitosan (Chang *et al.* 2007) and TiO_2 , Mn, Cu, Fe (Limbach *et al.* 2007) – have been reported to affect the hemolytic activity of red blood cells, ROS generation in human lung epithelial A459 cells and the viability of human oesophageal squamous cell carcinoma KYSE-510 and various kinds of human skin fibroblast human epithelial cells.

Iron oxide

Only a limited number of studies have been done on nano-Fe *in vivo*. For instance Brusentov *et al.* (2004) reported LD₅₀ of crystal γ -Fe₂O₃ and FeO coated with Dextran to be 5 g/kg after intravenous and intraperitoneal injection into mice. Bourrinet *et al.* (2006) studied the effects of Dextran coated Fe₃O₄ as well and reported observing a slightly significant increase in aortic blood flow at 13 mg/kg in rats and swollen snout and/or paws, dark body areas, labored respiration, and red crust around the nose and loss in body weight after intravenous bolus administration. Bourrinet *et al.* (2006) also reported observing ataxia, hypoactivity, exophthalmos, emesis, salivation, lacrimation, discolored and mucoid feces, injected sclera, and yellow eyes in dogs after a single-dose exposure of 20 and 200 mg Fe/kg through intravenous bolus administration and a significant increase in fetal skeletal malformations in rats and rabbits. Zhou *et al.* (2003) observed a significant elevation in total protein within the lavage fluid in male Sprague Dawley rats after inhalation of γ -Fe₂O₃ for 6 hours per day for 3 days. Gojova *et al.* (2007) observed no increase in ICAM-1, IL-8, or MCP-1 mRNA levels in human aortic endothelial cells and viability was observed to be $88 \pm 4\%$ and $92 \pm 3\%$ at 10 and 50 μ g Fe₂O₃/mL, respectively after exposure of 4 hours. Limbach *et al.* (2007) tested Fe₂O₃ of different purity and observed no increase in ROS level in human lung epithelial A459 cells (30 μ g/mL/4 h). Pisanic *et al.* (2007) and Auffan *et al.* (2006) observed that Fe₂O₃ and γ Fe₂O₃ coated with DMSA caused a significant dose-dependent decrease in viability and cell detachment in rats' PC12 pheochromocytoma clonal cells, and in human dermal fibroblasts. Gupta and Curtis (2004) and Gupta and Gupta (2005) observed no cytotoxicity or increase in the number of attached InfinityTM telomerase-immortalized primary human fibroblasts cells for Fe₃O₄ coated with Poly(ethyleneglycol) (PEG) and Pullulan in concentrations up to 20 mg/mL. They did, however, observe an up to 64% significant decrease in the number of attached cells for uncoated particles exposed to just 0.1 mg/mL. Hilger *et al.* (2003) observed a time-dependent decrease in the survival of human adenocarcinoma cells (BT-20) for both positively and negatively charged Fe₃O₄ particles with sizes varying from 8 to 220 nm in pure form and with various forms of surface chemistry. On the other hand Cheng *et al.* (2005) and Yu *et al.* (2006) observed limited and no difference in the viability of Cos-7 monkey kidney cells, human breast cancer SK-BR-3 cells and human dermal fibroblasts. Muller *et al.* (2007) only observed slight 10% fluctuations in the viability of human monocyte macrophages for Fe₃O₄ coated with Dextran.

Other metals

Most studies performed explored the hazards of nano-TiO₂, Zn, Si/SiO₂ and Fe although adverse effects have been observed on rodents, guinea pigs, and cells for other nanometals including Cu, Ag, Au, Ni, Se, Mg, CdO, and CdCl₂ (see Serita *et al.* 1999, Zhang *et al.* 2001, Casse *et al.* 2002, Alessandrini *et al.* 2003, Zhang *et al.* 2004, Berry

et al. 2003, 2004, Goodman *et al.* 2004, Jia and Chen 2005, Zhang *et al.* 2005, Bhattacharya *et al.* 2004, 2007, Connor *et al.* 2005, Pernodet *et al.* 2006, Niidome *et al.* 2006, Huff *et al.* 2007). Zhang *et al.* (2001), for instance, found the acute toxicity in mice of Se decrease 7-fold when coated with BSA. LD₅₀ was found to be 113 and 15.7 mg Se/kg bw respectively, whereas Cui *et al.* (27) observed severe toxicological effects and heavy injuries on kidney, liver and spleen after exposure via oral gavage to Cu, including that male mice seemed to suffer more than female mice. LD₅₀ was reported by Cui *et al.* to be 413 (95% CI 305–560) mg/kg bw.

Yang and Watts (2005) tested both pure 99.6% Al₂O₃ as well and various surface modifications of Al₂O₃ on the relative root growth (RRG) in *Zea mays* (corn), *Glycine max* (soybean), *Brassica oleracea* (cabbage), and *Daucus carota* (carrot). For pure 99.6% Al₂O₃ RRG was significantly (avg. $p=0.015$) inhibited compared to control after exposure to 2 mg/mL for 24 h. The RRG of cucumber was found to be 1.24- and 1.21-fold of that of Al₂O₃ particles alone for Al₂O₃ loaded with 10.0% and 100.0% monomolecular layer of Phen. No toxicity towards cucumber was observed in regard to root growth (2 mg/mL of Al₂O₃ nanoparticles loaded with 432.4% monomolecular layer of Phen). No detectable effect on root growth (avg. $p=0.84$) in corn, soybean, cabbage, and carrot compared to control exposed to 2 mg/mL of Al₂O₃ loaded with 10.0% monomolecular layer of Phen for 24 hours. Recently, Seeger *et al.* (2008) tested the effects on willow trees after exposure to 25 and 100 nm TiO₂-particles and reported observing no significant effect on growth, transpiration and water use efficiency in concentrations up to 100 mg/L after 2 weeks. Agglomeration and sedimentation of nanoparticles was observed to be more rapid in the presence of tree root.

Microbial toxicity has been tested for a number of nanoparticles on a number of bacterial strains. The most studied is probably nano-Ag. Alt *et al.* (2004) observed a dose dependent antibacterial effect on *S. epidermidis* EDCC 5245, and methicillin-resistant *S. epidermidis* ECCC 5130 and reported that bone cement with 1% nano-Ag completely inhibited proliferation of *S. epidermidis* EDCC 5245, methicillin-resistant *S. epidermidis* EDCC 5130 and methicillin-resistant *S. aureus* EDCC 5246. Baker *et al.* (2005) observed dose-dependent increase in antibacterial behavior against *E. coli* (0–0.114 mg/mL) whereas Morones *et al.* (2005) observed no significant growth above 75 µg/mL in *E. coli*, *V. cholera*, *P. aeruginosa* and *S. typhus* (0–100 µg/mL/30 min.). Pal *et al.* (2007) found that the growth of *E. coli* ATCC 10536 was dependent on the shape of the nano-Ag and most outspoken for triangular and spherical particles for which significant and complete growth inhibition was observed. Some colonies were able to grow even in presence of 100 µg rod shape Ag particles. Sondi *et al.* (2004) observed a 70 and a 100% inhibition of growth in *E. coli* strain B at 10 and 50–60 µg Ag cm⁻³ respectively in Luria broth medium. Thill *et al.* (2006) – for CeO₂ nanoparticles – observed a concentration-dependent decrease in the percentage of *E. coli* bacteria colony forming units. 50% decrease was observed at 5 CeO₂ ppm, however, the

presence of particles in the Luria broth growth medium did not influence the growth of the cells after 1-5 hours when exposed to 0.46-500 mg/L. Finally, Tang *et al.* (2007) observed no effect on *E. coli* K12 and *S. oneidensis* MR-1 after exposure to Au in concentration of up to 80 mg/L.

5.2 Dose-response assessment

According to the TGD a dose-response assessment involves “...an estimation of the relationship between dose, or level of exposure to a substance, and the incidence and severity of an effect” (European Commission JRC 2003).

Several of the studies mentioned above have reported observing dose-response relationship. This goes for, especially, *in vitro* studies on among other C₆₀, single- and multiwalled carbon nanotubes, and various forms of nanometals. Normally, dose refers to ‘dose by mass’, however, based on the experiences gained in biological tests of nanoparticles, it has been suggested that biological activity of nanoparticles might not be mass-dependent, but dependent on physical and chemical properties not routinely considered in toxicity studies (Oberdörster *et al.* 2005b). For instance, Oberdörster *et al.* (1996, 2007) and Stoeger *et al.* (2006, 2007) found that the surface area of the nanoparticles is a better descriptor of the toxicity of low-soluble, low toxicity particles, whereas Wittmaack (2007a, b) found that the particle number worked best as dose metrics. Warheit *et al.* (2007a, b) found that toxicity was related to the number of functional groups in the surface of nanoparticles.

However, it is still an open question which properties determine or influence the inherent hazards of nanoparticles partly due to the general lack of characterization of the nanoparticles tested (Hansen *et al.* 2007).

Physical and chemical properties such as particle size, size distribution, number concentration, agglomeration state, shape, crystal structure, chemical composition, surface area, surface chemistry, surface charge, porosity, and method of synthesis are in the literature proposed as properties that need to be considered (Oberdörster *et al.* 2005a, b, Lam *et al.* 2006, Warheit 2008). However, many of the proposed physical and chemical properties are overlapping, or are only applicable to nanoparticles and not to nanomaterials in general and/or are rarely reported on in eco(toxicological) studies. Table 1 summarizes the number of studies that reported different characteristics in percentage. The table shows that there is a large difference in which characteristics have been reported in the literature.

Table 1: The percentage of studies on nanoparticles that provide information on specific risk characteristics. See supporting information of Hansen *et al.* (2007) for references and data compilation.

	No. of compounds	Chemical comp.	Size	Shape	Crystal struc.	Surface area	Surface charge	Surface chem..	Solu-bility	Adhesion
C _{xx}	210	100	17	8	2	6	7	4	7	-
SWCNT	64	100	45	39	2	14	2	20	-	2
MWCNT	39	100	56	33	8	23	-	5	-	-
QDs	73	100	71	10	-	-	27	85	-	-
N-metals	275	100	96	39	24	33	17	25	4	-
Others*	304	100	76	12	.3	12	30	26	2	-

*) Others include polymers, in-organic nanoparticles, carbon black, and soot

The majority of all the studies report the chemical composition of the nanoparticles tested. C₆₀ behaves neither as a simple molecule or as a bulk solid (Fortner *et al.* 2005), however only 17% of the studies testing the toxicity of C₆₀ report the size distribution, and only 8% report on the shape of the aggregates of C₆₀. The greater portion of the studies on SWCNT report on the surface chemistry, whereas almost none of the studies on C₆₀ and MWCNT have done so. Surprisingly, only about half of the studies on SWCNT reported on the size even though the unique features of SWCNT vary with the diameter and length of the SWCNT. Furthermore, only one study reported the crystal structure for the SWCNT tested, although it is well known that several properties of the SWCNT depend on the crystal structure (i.e. armchair (n,n), zigzag (n,0), or choral (2n,n) SWCNT). And only one study reported the adhesion by which the nanomaterial is held together, although adhesion is important to the stability of the individual particles as well as aggregates of particles. In the case of quantum dots, for example, it has been reported that the shell protecting the toxic core of quantum dots can be degraded or broken down (Jordan *et al.* 1996, Berry *et al.* 2003, Derfus *et al.* 2004).

When estimating the hazard of nanomaterials – instead of just generating a laundry list of properties that one could imagine influences the (eco)toxicity of nanoparticles – Hansen *et al.* (2007) propose considering the information needed in order to describe a nanomaterial from a physical and chemical perspective. This would lead to the following properties as being important: 1) Chemical composition, 2) Size, 3) Shape, 4) Crystal structure, 5) Surface area, 6) Surface chemistry, 7) Surface charge, 8) Solubility, and 9) Adhesion – defined as the force by which the nanoparticles and its components are held together (Beck-Speier *et al.* 2001, Berry *et al.* 2004, Cheng 2004, Lockman *et al.* 2004, Nigavekar *et al.* 2004, Sayes *et al.* 2004, Baker *et al.* 2005, Martin *et al.* 2005, Fortner *et al.* 2005, Brunner *et al.* 2006). For reviews of methods for estimating these properties see for instance Tsuji *et al.* (2006), Maynard and Aitken (2007), and Powers *et al.* (2006). Recently, the Organisation for Economic Co-operation and Development (OECD) published a list of nanomaterials and endpoints selected for phase one of the OECD testing program adding additional properties such as dustiness, representative transmission electron microscopy (TEM) picture(s), and photocatalytic activity without making clear how these should be measured or reported (OECD 2008).

Although so far, only a few studies have reported to have observed adverse effects of engineered nanoparticles on humans or the environment, a fair number of studies have been published on nanoparticles and cytotoxicity. The number of studies on mammalian toxicity is still limited and only a very limited number of studies address the ecotoxicity of nanoparticles. It is, however, important to notice that the vast majority of the 428 studies reviewed demonstrate some degree of adverse effects on tested animals or cell lines (Hansen *et al.* 2007). Endpoints reported on vary substantially and reading across and interpreting the results of the various studies is hard at the moment since nanomaterials tested differ substantially in regard to: 1) physical-chemical properties such as chemical composition and shape and 2) endpoints tested for and duration of exposure and methods (e.g. assays) and standards used (Hansen *et al.* 2007). Preliminary results, however, indicate that *in vitro* testing may not always predict hazards accurately whereas the number of large *in vivo* studies has been limited and difficult to reproduce (RCEP 2008, CCA 2008). For many nanoparticles it is not clear whether a threshold exists (by any metric) below which exposure ceases to cause adverse effects (Wittmaack 2007, Hansen *et al.* 2007, RCEP 2008). It is furthermore not clear whether the endpoints tested and reported on actually are the most sensitive or the most relevant, or whether new biological endpoints might show to be more relevant (RCEP 2008).

Recently, an expert committee gathered by the Council of Canadian Academies (CCA 2008) argues that “...based on the current understanding of toxicological sciences, there are no new biological endpoints caused by the exposure to nanomaterials”. However, it cannot be ruled out that nanoparticles are harmful by some other paradigm not yet explored (Pollard *et al.* 2008, RCEP 2008). Most of the studies done so far follow a more or less traditional paradigm and one can hardly expect that new endpoints will spring from our current understanding and/or can be detected by using traditional toxicological methods (RCEP 2008).

5.3 Exposure assessment

Exposure is a key element in risk assessment of nanomaterials since it is a precondition for the potential toxicological and ecotoxicological effects to take place. If there is no exposure – there is no risk. According to the Technical Guidance Document exposure assessment involves “...an estimation of the concentrations/doses to which human populations (*i.e.* workers, consumers and man exposed indirectly via the environment) or environmental compartments (aquatic environment, terrestrial environment and air) are or may be exposed.” (European Commission JRC 2003a).

Completing a full exposure assessment requires extensive knowledge about among others manufacturing conditions, level of production, industrial applications and uses, consumer products and behavior, and environmental fate and distribution.

Such detailed information is not available and so far no full exposure assessment has been published for any one or more nanomaterials. This may partly be due to difficulties in monitoring nanomaterial exposure in the workplace and the environment, and partly due to the fact that the biological and environmental pathways of nanomaterials are still largely unexplored (CCA 2008). Some efforts have been made to assess occupational-, consumer- and environmental exposure, however, both in regard to assess the level of exposure and to assess the applicability of current exposure assessment methods and guidelines.

5.3.1 Occupational exposure

For workers the primary route of exposure is assumed to be through inhalation and/or dermal contact after the manufacturing process of a nanomaterial for instance when a reaction chamber is opened, a product is dried, during the handling of products after their manufacture. Exposure is less likely during the manufacturing process itself since most nanomanufacturing processes are performed in a closed reaction chamber. However, unexpected system failure such as rupture of a seal could happen (Biswas and Wu 2005, Franco *et al.* 2007, Fujitani *et al.* 2008).

Occupational exposure to ultrafine particles has a long history but for the moment, it is unclear to what extent analogies can be drawn to nanomaterials. According to Biswas and Wu (2005), active operations in production will lead to high spikes of ultrafine particle number concentration. Once these operations stop, a gradual decay will be observed due to primarily coagulation, evaporation, dilution, and/or deposition. The effects of spatial and temporal changes are important as well in order to evaluate exposure accurately. Whereas the fraction of the total ultrafine particle number concentrations generally decreases, fine particle number concentrations increases with time and distance from the point of emission (Biswas and Wu, 2005).

The information and data publicly available about current levels of worker exposure to nanomaterials is very limited. This includes valuable information such as what kinds of nanomaterials workers are exposed to, where and how, the concentrations by dose or by particles number they are exposed to and what kinds of protective measures are used or are available (ICON 2006).

Maynard *et al.* (2004) performed one of the first exposure measurements of unprocessed airborne SWCNT at four facilities that were using either the HiPCO or laser ablation production methods. Measurements were taken to assess the propensity for aerosol particles to be released during agitation and to measure the size of particles released into the air while SWCNT material was removed from production vessels and handled prior to processing. Airborne concentrations of SWCNT were estimated to be then lower than $53 \mu\text{g}/\text{m}^3$ whereas glove deposits of SWCNT during handling were estimated at between 0.2 mg and 6 mg per hand (Maynard *et al.* 2004).

Han *et al.* (2008) reported observing MWCNTs in the range from 172.9 to 193.6 MWCNTs/cm³ in a MWCNT research facility where the researchers handled unrefined materials. Gravimetric concentrations of total dust ranged from 0.21 to 0.43 mg/m³. After implementation of protective control measures such as enclosing and ventilating the furnace these decreased to 0.018-0.05 MWCNTs/ cm³.

Using various measuring instruments simultaneously Bello *et al.* (2008) evaluated the potential exposure to MWCNTs during CVD growth in a university research lab, and during subsequent handling as the CNTs are removed from the furnace and detached from the growth substrate. In contrast to Maynard *et al.* (2004) and Han *et al.* (2008), Bello *et al.* found no increase in the total particle number concentration and any particle size range during furnace operations compared to background. Total particle number concentration was found to be between 4000-7000 particles/cm³ which is considered to be normal since ranges measured in the indoor environment commonly varies between 2000 and 10,000 particles/cm³. Bello *et al.* did not find evidence either of individual or bundles of CNTs in various air samples or in the personal filter.

Fujitani *et al.* (2008) compared the particle size distributions and morphology of aggregated/agglomerated fullerenes at Frontier Carbon Corporation in Japan, during work and non-work periods as well as an agitation process, and compared it to nearby outdoor air. They found that the particle number concentration of particles with a diameter <50 nm was not larger during the removal of fullerenes from a storage tank for bagging and/or weighing than in the non-work period. However, the concentration of particles with a diameter >1000 nm was observed to be larger during the non-work period. Similar to Maynard *et al.* (2004), Fujitani *et al.* (2008) found that the use of a vacuum cleaner reversed these observations.

A significant concern is related to the processing including drilling and cutting of nanomaterial hybrid composites. Bello *et al.* (2008) investigated the airborne exposures generated in a research lab during the dry and wet cutting of composites consisting of advanced fibers and polymer matrix with and without CNTs. No significant difference was observed compared to background during wet cutting, which is the usual procedure for such composites. Compared to background particle levels, dry cutting did, however, generate statistically significant quantities of nanoscale and fine particles regardless of the composite type i.e. CNT-carbon, CNT-alumina, and their respective base composites (Bello *et al.* 2008).

Current worker exposure monitoring and assessments are hampered by the lack of one sampling method that can be used to characterize exposure (NIOSH 2006, Maynard and Aitken 2007). Some methods monitor and measure nanoparticles using mass concentration monitoring. This is, however, insufficient because of the low mass of nanoparticles. There exist relatively few methods for measuring the surface area of nanoparticles in real-time in the workplace, and each of them has limitations when it comes to measuring the surface area of particles above 100 nm (NIOSH, 2006). Other

methods are based on real-time measurement of nanometer aerosol concentrations, however, many of these have limited sensitivity to detect small particles and cannot distinguish between engineered nanomaterials and other solid particles. Concurrent particle sampling by filter and particle characterization is needed, but this again would rely on statics and area sampling methods and hence only lead to estimates of the level of worker exposure (NIOSH 2006, Fujitani *et al.* 2008).

5.3.2 Consumer exposure

The number of products available to the consumer entailing nanomaterials or based on nanotechnology has increased rapidly within the last couple of years. The nature of the products is diverse and so is the nature of consumer exposure.

In a first attempt, Hansen *et al.* (2008b) used the Technical Guidance Document for exposure assessment of nanoparticles in a number of consumer products. These products represent a facial lotion, a sunscreen lotion, a fluid product for outdoor surface treatment, and a spray product for indoor surface treatment. The calculations done by Hansen *et al.* (2008b) were based on default values and equations taken from Part 1, Appendix II – “Consumer Exposure”, in the TGD (European Commission JRC 2003a). In cases where the TGD did not contain any data, the default values were based partly on information from knowledgeable stakeholders and partly on estimated values. Very few producers/distributors provide information about the content of the nanomaterials in the products. However, using the best estimates available and/or worst-case assumptions, Hansen *et al.* (2008b) estimate consumer exposure to be 26, 15, and 44 $\mu\text{g}/\text{kg}$ bw/year for a facial lotion, a fluid product, and a spray product containing nanoparticles, respectively. From a survey on the industrial production and application of nanotechnology in the Danish industry, it is known that producers of sun lotions use 10-20 nm TiO_2 particles with a specific surface area of 50-200 m^2/g as UV absorber and that the nanoparticles are present in concentrations up to 10% (Tønning and Poulsen 2007). The route of exposure for a sun lotion will mainly be dermal contact. Intake of smaller quantities by contact with the area around the mouth is not taken into consideration. As the product is a “leave on” product, which should neither be diluted when used nor washed off, the quantity of active substance on the skin (A_{der}) for an adult can be estimated to be $A_{\text{der}} = 800$ mg for a sun lotion containing 10% of nanomaterial. Assuming that all nanoparticles penetrate the skin the potential uptake per kilogram body weight per day ($U_{\text{der, pot}}$) can be estimated. $U_{\text{der, pot}}$ is equal to 40 mg/kg bw/day nano- TiO_2 for women if the sun lotion contains 10% nanoparticles. For men $U_{\text{der, pot}}$ is equal to 34 mg/kg bw/day. The conversion of the value of applied sun lotion in an adult compared to a child can be calculated using Equation 3 in Table 1 of Hansen *et al.* (2008b). The quantity of the active substance on the skin per application for a 2 year old child is found to be $A_{\text{der}} = 260$ mg for a particle concentration of 10%. This value is about 3 times less than for an adult. $U_{\text{der, pot}}$ would, on the other hand, be 2 times

higher i.e. 63 mg/kg bw/day. It should be noted that these uptake values are worst-case scenarios assuming full skin penetration of nanoparticles. In its latest opinion on the safety of nanomaterials in cosmetic products, the European Scientific Committee on Consumer Products stated that there is inadequate information on, among other issues, uptake via physiologically normal and compromised human skin (Scientific Committee on Consumer Products 2007). The extent to which nanoparticles actually penetrate the skin is currently a matter of considerable debate internationally and will probably depend on specific particle properties and the local environment in which they are used.

The assessment of the overall consumer exposure has been hampered by both lack of information and lack of access to information about which and how many products are commercially available that contain nanomaterials or are based on nanotechnology, the content of nanosized materials for most products and consumption rates of nanoproducts and consumer behavior. Hansen *et al.* (2008b) propose a framework to aid exposure assessment in consumer products. The framework is based on categorizing consumer products according to location of the nanomaterial and grouping products into three different exposure categories:

1. expected to cause exposure
2. may cause exposure
3. no expected exposure to the consumer

Products that would typically fall under the first category are products with “nanoparticles suspended in liquids” or “airborne nanoparticles” whereas products with “surface-bound nanoparticles” and “nanoparticles suspended in solids” would fall into the second and third category, respectively.

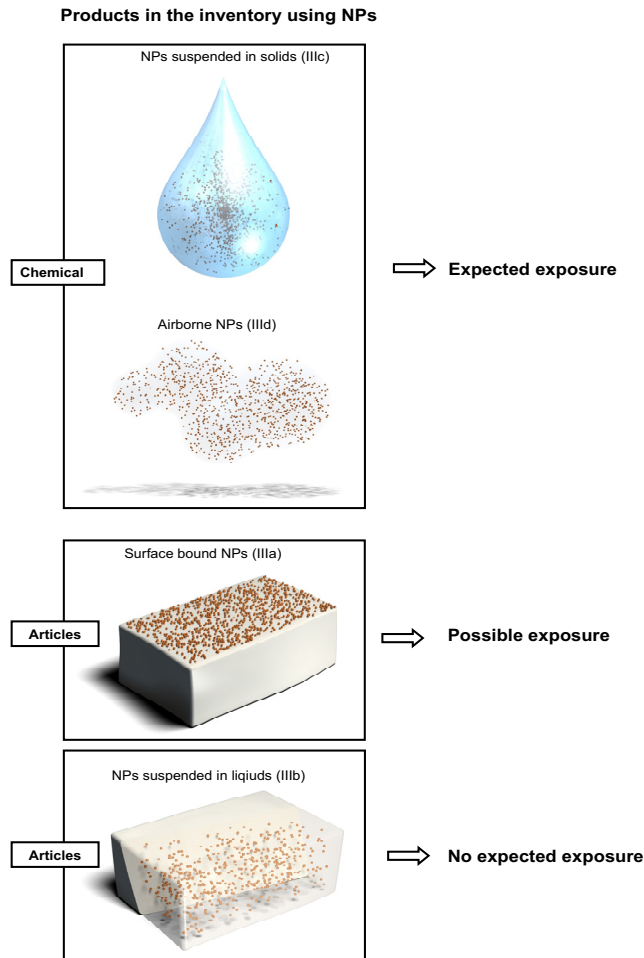


Figure 9: Distribution of the products with no, possible and expected exposure within each of the various product categories depending on the location of the nanomaterial in the product (Hansen *et al.* 2008c)

Using the data behind figure 7 on the distribution of products into various categories of nanomaterials in combination with the exposure grouping illustrated in figure 9, it is found that expected consumer exposure is highest for products in the products categories “Appliances” and “Health and Fitness” (see figure 10). Expected exposure is 36% for products that fall into the category of home and garden whereas it is 58% for cross-cutting products. For the other categories of products, the expected exposure ranges between these two percentages except for appliances for which exposure is only expected for 17% of the products. Possible exposure percentages are equally high ranging between 20-30% except for food and beverages, and electronics and computers for which about 10% fall into the category of possible exposure.

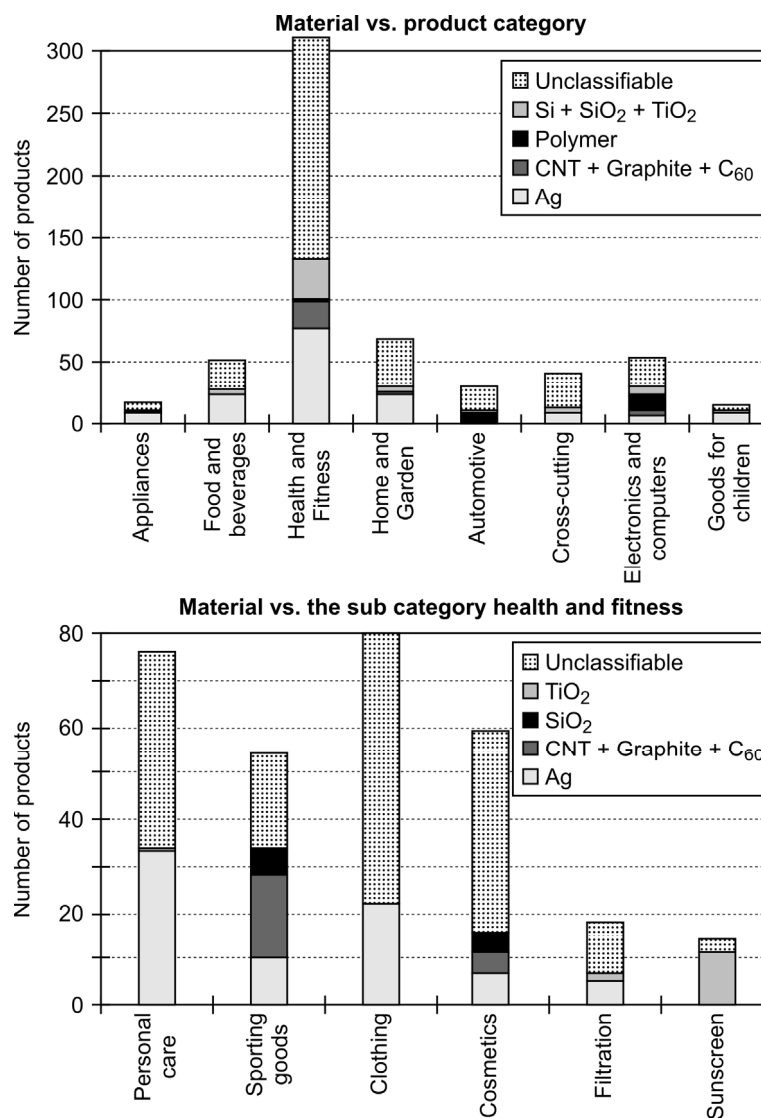


Figure 10: Distribution of the products with no-, possible- and likely exposure within each of the various products categories (Hansen *et al.* 2008b).

The exposure grouping is based on the physical state in the application phase when the consumer exposure is expected to be highest. It should be noted that some consumer products will change their exposure potential during the product life-cycle, e.g. for paints where nanoparticles will be in liquid form when the paint is applied but in solid form once the paint has dried. In this case the major consumer exposure is expected to be from the liquid paint, but weathering and physical abrasion of the dried surface could potentially lead to an exposure.

A comparison of the exposure grouping with the materials used (see figure 4 and 10, and Table 1 in Hansen *et al.* 2008b) shows that the expected and possible exposure is highest for Ag, TiO₂, and ZnO (see figure 11). It is also interesting to note that the category of unclassified products, for which we do not have information on the material

used, is the one containing the highest number of products for which consumer exposure is expected. This lack of information about which kinds of nanomaterials are used must raise concerns not only because of the potential exposures but also about the potential hazards of these products to the consumer.

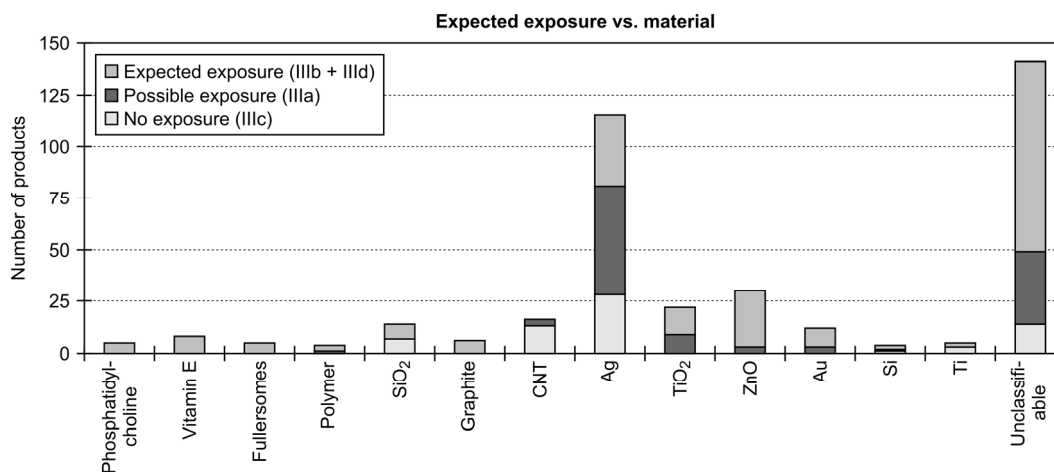


Figure 11: A comparison between the exposure categorization and the nanomaterials used (Hansen *et al.* 2008b)

5.3.3 Environmental exposure

Exposure of nanomaterials to the environment seems inevitable with the increasing production volumes and the increasing number of commercially available products containing nanomaterials or based on nanotechnology.

Environmental routes of exposure are multiple and can stem from:

- operations related to the production of nanomaterials such as cleaning of production chambers;
- spills from production, transport, and disposal of nanomaterials or products;
- the use and disposal of products containing nanoparticles including incomplete waste incineration and landfills;
- wastewater overflow and ineffective sewage treatment plants (STP) unable to hold nanoparticles back or degrade them;
- degradation of products containing nanomaterials (Biswas and Wu 2005, RS & RAE 2004, Boxall *et al.* 2008).

The total load to the environment from current uses of nanomaterials is unclear and analytical methods to detect and quantify environmental concentrations of nanoparticles have yet to become available (Muller and Nowack 2008, Luoma 2008). However various estimates have been made both for individual products, nanomaterials and applications as well as product types.

Envirox

Recently, Park *et al.* (2008) completed a hazard and risk assessment of a nanoparticulate cerium oxide-based diesel fuel additive known as Envirox. Envirox is diluted with diesel to extend and improve fuel burn yielding a final concentration of CeO₂ in the diesel fuel of 5 mg/L. Park *et al.* (2008) estimated the ambient level of cerium by the use of modeling studies and drawing on historical data on atmospheric monitoring before and after Envirox was introduced into the diesel fuel used in buses in London and Newcastle in 2003 and 2005, respectively. A significant fourfold increase from 0.145 ± 0.064 to 0.612 ± 0.287 ng/m³ was observed in the cerium concentrations in the ambient air in Newcastle following the introduction of Envirox whereas no increase was observed in the samples taken from London. The lack of increase observed in London might be reflected in the low percentage of buses using Envirox passing the sites in London where the samples were taken

Using various models, Park *et al.* (2008) also calculated the cerium emissions to the atmosphere for all EU Member States up to year 2020 for a baseline- and a diesel particulate trap application scenario under various best- and worst-case conditions – assuming a 5 ppm concentration of cerium oxide in the diesel fuel and that newly registered passenger cars, light- and heavy duty vehicles will be equipped with a filter operated on Envirox.

Park *et al.* (2008) found that using traps to retain cerium emissions in conjunction with Envirox would reduce car emissions by more than 70%, buses by 85%, and heavy trucks by almost 90% by 2020 taking 1990 as a baseline. The introduction of traps will reduce particular matter by more than 70,000 tons per year whereas 75 tons of cerium would be emitted to the atmosphere. In the worst-case, assuming that all diesel fuel is doped with cerium and fully emitted to the atmosphere < 1255 tons would be emitted.

The urban population of the EU will be exposed to about 0.2 g cerium oxide per year, whereas the rural areas and the average emissions along the highways would be about 6.6 g and 0.3 g cerium oxide per square kilometer per year.

Ignoring that traps might capture much of the particulate emission, Boxall *et al.* (2008) also estimated the emission of cerium oxide in vehicle exhausts. Assuming that using cerium oxide as an additive in all diesel fuel at 10 ppm would lead to an emission rate of 10 ppm from diesel fuel vehicles, Boxall *et al.* found an emission of 0.0010 g/km for the passage of 1000 vehicles. The concentration at 50 m from the road would be $0.0002 \mu\text{g}/\text{m}^3$, whereas it would be three fold higher at 5 m. The annual emission of cerium oxide from all vehicles in the UK was estimated to be 161 kg based on the estimated vehicle emission rates and the total annual mileage for vehicles.

Park *et al.* (2008) also modeled the possible soil contamination along a typical highway assuming that all cerium oxide emissions would accumulate over a 40-year period and they found that estimated concentrations vary between 0.28 and 1.12 $\mu\text{g}/\text{g}$,

depending on the soil depth at a distance from the edge of the highway. In comparison Boxall *et al.* (2008) estimated concentration in soil to be $< 0.01 \mu\text{g}/\text{kg}$

Nanosilver

Luoma (2008) estimated mass release of silver from silver socks, silver wash machines and swimming pools assuming that 10 and 30% of the population in the U.S. use silver, that households that are wealthy enough will buy silver wash machines and that 1 million pools use silver as a biocide. Silver discharges from silver socks in the two scenarios were estimated to be in the range of 6-930 kg and 180-2790 kg respectively depending on the silver contents in the socks, whereas the contribution from silver wash machines was found to be 2850 kg. The contribution from the swimming pools was by far the largest found; it was estimated to be 30 tons. In another scenario, Luoma estimated the total future discharges to be 457 tons assuming that there will 100, 10, and 5 products in the future that resemble the silver discharged from the socks, wash machines and the swimming pools, respectively. After waste treatment this could be reduced to 128 tons provided that 80% of the discharges are treated sufficiently to remove 90% of the silver.

Blaser *et al.* (2008) estimated the silver emission into wastewater by multiplying the amount of silver in biocidal plastics and textiles with the release rate of silver ions from these products and the period the products are in contact with water. Assuming that the removal in the STP was assumed to range between 99-85% wastewater removal Blaser *et al.* (2008) found that the predicted environmental concentration (PEC) for the STP would be $18 \mu\text{g}/\text{L}$ whereas $\text{PEC}_{\text{water}}$ and $\text{PEC}_{\text{sediment}}$ would be $320 \text{ ng}/\text{L}$ and $14 \text{ mg}/\text{kg}$, respectively.

Cosmetics and personal care products

Based on available information about the applied concentration of nanoparticles in cosmetics, personal care products and paints, Boxall *et al.* (2008) used a long series of algorithms (for among other pesticides, medicinal products, and ultrafine particles) to estimate the predicted environmental concentrations of nanoparticles in soil and water. Although anticipating that 10% market penetration probably provides a conservative estimate (with the exception of sunscreens), Boxall *et al.* calculated the PEC for three scenarios assuming that 10%, 50% and 100% of the products on the market contained nanoparticles. The highest total predicted concentrations in water was found to be for latex nanoparticles ($103\text{-}1025 \mu\text{g}/\text{L}$) stemming from laundry detergents followed by zinc oxide ($76.0\text{-}760 \mu\text{g}/\text{L}$) and titanium oxide ($24.5\text{-}245 \mu\text{g}/\text{L}$) used in among others paints and sunscreens. The use of hydroxyapatite in toothpaste and fullerene in cosmetics is estimated to lead to a environmental concentration of between $10.1\text{-}101$ and $0.31\text{-}3.13 \mu\text{g}/\text{L}$, respectively. Predicted concentrations in soil range from $4.3\text{-}43 \text{ mg}/\text{kg}$ for nanolatex to <0.01 for CeO_2 .

Mueller and Nowack (2008) modeled the predicted environmental concentration for nano-Ag, nano-TiO₂ and CNTs for air, water and soil in Switzerland. The calculations by Mueller and Nowack (2008) were based on a quantitative substance flow analysis of how nanoparticles from various products categories such as textiles, cosmetics, coatings, plastics, sports gear, electronics, etc. enter the environment through abrasion, outlet from the sewage treatment plants and/or waste incineration plants and landfills. Given the lack of information, numerous assumptions and estimates had to be made about worldwide production volumes, concentration of the nanoparticles in the products, levels of incombustible nanoparticles and behavior during wastewater treatment. For CNT, for example, it is estimated to be realistic that the worldwide production in 2007/2008 is 350 tons and that these are evenly incorporated into plastics and electronics. For the CNT that end up in the waste incinerator, it is furthermore assumed that 50% will be burned, 25% will end in the slag and 25% will become airborne. Of the 25% that become airborne, 99.9% will be caught in the filters of the waste incineration plant leaving 0.1% to enter the atmosphere. In a high exposure scenario, Mueller and Nowack (2008) assumed a worldwide production of 500 tons annually and that only 25% are burned and that only 99% of the airborne CNT are caught by the filters of the incineration plant.

Boxall *et al.* (2008) estimated PECs for nanoparticles in cosmetics and personal care products whereas the scope of the analysis of Muller and Nowacks include among others textiles, metal products and cosmetics. Still Muller and Nowack found PECs that were lower – although in the same order of magnitude – as Boxall *et al.* (2008) for Ag in water and soil, and for TiO₂ in air. This was the case even for the high exposure scenario, whereas estimates differ substantially for TiO₂ in soil with several orders of magnitude. Some of the difference between the PECs could be related to the fact that Boxall *et al.* (2008) assumed that no nanoparticles would be retained in the sewage treatment plants, whereas Mueller and Nowack (2008) assumed that 97% and 90% of the nanoparticles would be cleared in the realistic and the high exposure scenario, respectively.

5.4 Risk Characterization

Risk characterization is defined as “*estimation of the incidence and severity of the adverse effects likely to occur in a human population or environmental compartment due to actual or predicted exposure to a substance, and may include “risk estimation”, i.e. the quantification of that likelihood.*” (European Commission JRC 2003a).

Risk characterization is the final part of risk assessment where all the information gathered during the first three steps of risk assessment come together (CCA 2008). Often risk characterization boils down to the estimation of a risk quotient defined as PEC/PNEC. If the risk quotient is < 1 no further testing or risk reduction measures are

needed according to the European Commission JRC (2003b). If it is > 1 , further testing can be initiated to lower the PEC/PNEC ratio. If that is not possible risk reduction could be implemented.

Recently, a number of studies reported having completed risk assessments of the use of CeO₂-based diesel fuel additive in the UK (Park *et al.* 2008), and the use of nano-Ag, nano-TiO₂ and CNTs in Switzerland (Mueller and Nowack 2008). Park *et al.* assessed the risk of CeO₂ causing pulmonary inflammation. First, they estimated an internal dose of $3.8 \times 10^{-7} \text{ cm}^2/\text{cm}^2$ by converting the retained dose into surface area units and then dividing by the area of the proximal alveolar region of the lung. Then they compared this value to the highest no-observed-effect level (NOEL) found in a number of *in vitro* toxicity studies. This value was $26.75 \text{ cm}^2/\text{cm}^2$. Assuming that *in vitro* exposure data can be accurately projected to the *in vivo* situation, Park *et al.* (2008) concluded that “*it is highly unlikely that exposure to cerium oxide at the environmental levels (from both monitored and modeled experimental data) would elicit pulmonary inflammation*”.

Mueller and Nowack (2008) reported having completed the first quantitative risk assessment of nanoparticles in environment. In a first attempt to derive PEC values, Mueller and Nowack used threshold concentrations of 20 mg/L and 40 mg/L reported in the literature for nano-Ag on *B. subtilis* and *E. coli* and considered it to be equivalent to a NOEC. For nano-TiO₂ and CNT the lowest value found in the literature was $<1 \text{ mg/L}$ for algae, daphnia and fish (Mueller and Nowack 2008). Applying assessment factor of 1000, the Predicted No Effect Concentration (PNEC) in water was found to be 0.04, <0.001 and $<0.0001 \text{ mg/L}$.for nano-Ag, nano-TiO₂ and CNT, respectively. Combining these PNEC-values with the predicted exposure, Mueller and Nowack (2008) calculated the environmental concentrations in Switzerland for nano-Ag, nano-TiO₂ and CNTs stemming from textiles, cosmetics, coatings, plastics, sports gear, electronics, etc. Assuming worse-case exposure levels, Mueller and Nowack (2008) find that the risk quotient for nano-Ag and CNT is less than a one thousandth, and they state that their modeling suggests that currently little or no risk is to be expected from nano-Ag and CNT to organisms in water and air. Nano-TiO₂, on the other hand, might pose a risk to organism in water – according to Mueller and Nowack (2008) – with risk quotients ranging from > 0.7 and > 16 . PNEC for soil could not be determined due to lack of information.

Risk characterization involves critically reflection of the data behind each step and determining what the overall risk will be (CCA 2008).

In section 5.1-5.3 the current state of knowledge and most significant findings within each of the first three steps of risk assessment were presented, analyzed and discussed. Considering the results of this analysis along with the work done by Park *et al.* (2008), and Muller and Nowack (2008), it becomes clear that each element of risk assessment hold general as well as specific limitations and challenges. Risk

characterization being at the end of the line, the sum or maybe even the power all of these limitations are conveyed to calculating risk quotients for nanoparticles.

Toxicity has been reported on for multiple nanoparticles as demonstrated in section 5.1, but for most nanoparticles these need further confirmation before one can say that a hazard has been identified. Multiple studies relevant for hazard identification have been carried out on C₆₀, CNTs, quantum dots and nanomaterials, however, many of these studies are not meant to facilitate risk assessment in the sense that they use non-standardized tests, have no coherent endpoint, and differ substantially with regard to species tested, methods of administration, dose range, way of particle preparation, duration of exposure, and effects observed and reported. This hampers identification of hazard univocally for most nanoparticles. Preliminary results furthermore indicate that the diversity of nanomaterials and their properties makes it an overwhelming challenge to conduct *in vitro* and *in vivo* evaluation of their biological effects (CCA 2008, RCEP 2008). It is evident that the information provided is ‘all over the map’ making it impossible to systematically analyze the studies for properties of the nanoparticles which are important for the observed effects (Hansen *et al.* 2007, Warheit 2008).

Dose-response estimates assumes a no effect threshold can be established and although some studies have reported observing a dose-response relationship there is no evidence of a dose threshold below which nanoparticle instillation ceased to cause, for instance, inflammation (Wittmaack 2007, Hansen *et al.* 2007). A dose-response assessment is furthermore hindered by the fact that it is unclear what the best descriptors for dose is and which properties determine or influence the inherent hazards of nanoparticles. The current lack of characterization of the nanoparticles tested in various studies makes it impossible to identify causality between observed hazards and specific physical and chemical properties. There is furthermore substantial limitation in our ability to determine individual and multiple particle characteristics simultaneously and in a consistent manner – both prior and during tests – when using different characterization techniques and/or across laboratories (RCEP 2008).

Exposure assessment is hampered by difficulties in monitoring nanomaterial exposure in the workplace and the environment, and by the fact that the biological and environmental pathways of nanomaterials are still largely unexplored (CCA 2008, RCEP 2008). The assessment of worker exposure is hampered by both technical difficulties such as the lack of one consistent sampling method that can be used to characterize exposure in real-time (NIOSH 2006) and by lack of information and data, for example, about how many workers are potentially exposed, what kinds of nanomaterials workers are or might be exposed to, where and how they are exposed and at which concentrations, by dose or by particles number, and what kinds of protective measures there are used or available (ICON 2006). As with worker exposure, analytical methods to detect and quantify concentrations of nanoparticles in the environment have yet to become available (Muller and Nowack 2008, Luoma 2008). The total load to the

environment from current of nanomaterials is unclear. Several studies have tried to assess current and future consumer and environmental exposure for individual products, nanomaterials, and applications as well as product types. As elaborated on in section 5.3 many of these have been able to apply fairly simple mathematical equations and/or information available in the Technical Guidance Document to estimate the current and future exposure. However, in order to assess the consumer and environmental exposure to nanoparticles numerous assumptions had to be made about for instance: worldwide production volumes of nanoparticles, number of products produced entailing nanoparticles and at what concentrations, current and future market penetration, release from products throughout the life-cycle of the products by mass or other relevant metric(s), to what extent products become incinerated, end up in landfills or the sewage treatment plants, or end up directly in the environment, and release from waste incinerators and removal efficacy in the STPs, and their fate and distribution in surface water, soil and the air. These studies, no doubt, hold great value in regard to assessing the applicability of exposure assessment and should be seen as “proof of principle” rather than actual assessment of the exposure. Paucity of knowledge and lack of access to information hampers realistic exposure assessments.

Recently the European Commission’s Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR) delivered their opinion on the appropriateness of the risk assessment methodology in accordance with TGD for assessing the risks of nanomaterials. SCENIHR (2007) note that the TGD make “*very little reference to substances in particulate form*”. Although SCENIHR concluded that the TGD are “generally likely” to be able to identify the hazards associated with the use of nanoparticles, they found that the methodologies needed improvements on a number of areas, such as: 1) mass concentration might not be the most significant metrics in determination of exposure, 2) state of agglomerations of nanoparticles depends on the environment and affects their properties, and 3) biological processes involving nanoparticles are still largely unknown. Although SCENIHR note that these improvements are needed, they give no recommendations on how these improvements should be made in practice.

With respect to environmental exposure the SCENIHR expert panel is not even able to make a clear assessment of the validity and appropriateness of existing methodologies. They state that “*In the absence of sufficient data on the fate and effect of nanoparticles on the environment, it is neither feasible nor appropriate to propose firm rules on how substances in nanoparticle form should be evaluated. Instead the applicability of existing methods for risk assessment of nanoparticles should be evaluated*”. As noted by SCENIHR chemical risk assessment is a very complex process and the determination of risks associated with nanomaterials is an even more complex process than with conventional bulk materials (SCENIHR 2007). It is important to emphasize that the current uncertainties related to the risk assessment of nanoparticles

are “...not simply uncertainties in the values of some traditional parameters, but rather the uncertainties about the potentially unique or significantly modified causal mechanisms themselves” (SCENIHR 2007).

In order to apply uniform procedures for risk assessment of nanomaterials, calls for standards applicable to nanomaterials are frequently made. At present, there exist no standard procedures or widely accepted methods for assessing nanomaterials’ safety hazards (Environmental Defense and DuPont 2007). When looking at the individual steps in the risk assessment procedure, it becomes apparent that even at the starting point – hazard identification – there is no internationally agreed upon best practices. For example, the selection of a set of hazard relevant physico-chemical parameters is being discussed in the scientific community (SCENIHR 2006, Oberdörster *et al.* 2005a, b), and no standardized toxicity test guidelines exist. For exposure assessments, there are no standards for how to measure nanoparticle exposure in the body, the workplace (NIOSH 2006) and the environment (SCENIHR 2007), and effects assessments are hampered by the lack of basic toxicological test methods.

Although standardization work in the field of risk assessment of nanomaterials is currently underway on an international scale (OECD 2007), it is important to remember that standardization of chemical risk assessment procedures (including toxicity tests and related exposure/effect assessment protocols) were underway for more than twenty years. Much of this development was not just concerned with technical improvements of tests, but was closely connected to the development of environmental regulation to provide stakeholders with useful legitimate instruments rather than the scientific communities’ interest in the subject (Halffman 1998). Hopefully, we have learned from these past experiences, but a certain lag must be anticipated due to the deficits in scientific studies and procedures in the emerging field of nanotoxicology.

6. Discussion

The aim of this PhD thesis was threefold:

1. to investigate whether existing regulation is adequate in the short and the long term;
2. explore the feasibility of risk assessment for the purpose of dealing with the complex emerging risks of nanomaterials, and finally;
3. provide recommendations on how to govern nanotechnologies protecting human health and the environment.

In the following, the limitations of current regulation of nanomaterials and applications will be discussed and recommendations will be provided on how to address these limitations in the short term. The analysis of the applicability of the current regulation on nanomaterials showed that (eco)toxicological data and risk assessments are often necessary to support current regulation. However, risk assessment holds a number of limitations as well, and the short and long term feasibility of risk assessment of nanomaterials is discussed. Potential alternatives will be addressed shortly. Some of them have already been applied on nanomaterials.

6.1 Identified limitations of current regulation of nanomaterials

The analysis of the existing regulation showed that there are a number of potential gaps and each of them will be dependent on the specific nanomaterial and its specific application (Franco *et al.* 2007).

Though there is no specific mentioning of nanomaterials in any of the EU legislation, in general it seems as if nanomaterials are covered by the broad scope of the various pieces of legislation. The question is whether nanomaterials are covered when it comes to the specifics. For REACH the main areas of concern seem to be that it is unclear when a nano-equivalent of a bulk substance should be registered under REACH, and that production thresholds for when (eco)toxicological information has to be submitted, are not currently met for many nanomaterials (although they might be in the near future). Furthermore, even though companies are urged to use already existing guidelines, both the European Commission (CEC 2008a) and its Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR 2007) as well as others have pointed out that current test guidelines supporting REACH are based on conventional methodologies for assessing chemical risks and may not be appropriate for the assessment of risks associated with nanomaterials. Somewhat similar issues have been raised for pharmaceuticals where the concern is that current product standards may not be suitably designed to address various aspects relating to novel applications of

nanotechnology in nanomedicine. Furthermore, if the estimated environmental concentration of medical products is below 0.01 ppb and “no other environmental concerns are apparent”, no further actions are to be taken for the medical product in terms of environmental risk assessment. Such pre-defined action limit could potentially be problematic since the new properties of nano-based products are expected to also affect their environmental profiles.

Chaundry *et al.* (2006) observed that potential gaps of regulation of nanomaterials seem to fall into two main categories. For one category, the key piece of regulation relating to a sector, application, product or substance fails to address an aspect of particular interest – for instance, if a piece of legislation is intended to address the human health impacts but fails to address possible environmental impacts of nanomaterials or nanoproducts. For the second category, a piece of legislation is intended to address a specific aspect of particular interest to a sector, application, product or substance but fails to address it due to exemptions (e.g. threshold, volume or tonnage related), lack of foresight, limitations in technical or scientific knowledge, etc. (Chaundry *et al.* 2006).

The identified gaps in this analysis fall into two, however somewhat different, categories. The first category concerns whether nanomaterials are covered by current legislation when it comes to 1) definitions of a substance, novel foods, hazardous waste, etc. and 2) thresholds values not tailored to the nanoscale, but based on bulk material, see e.g. REACH. The second category relates to the lack of metrological tools and toxicological data and the fact that occupational and environmental exposure limits cannot be established with existing methodologies – as required by some pieces of legislation e.g. pharmaceuticals regulation and the Safety at Workplace Directives.

6.2 Recommendations in regard to the “Incremental approach”

The Commission of the European Communities has adopted a so called incremental approach which focuses on adapting existing laws to regulate nanotechnologies and amending them in order to deal with nanomaterials. So far, the Commission has only found it appropriate to implement one amendment, i.e. carbon losing its exemption status under REACH. However, the other limitations need to be addressed as well sooner rather than later for the incremental approach to be successful, especially in view of the current pace of development of nanomaterials and applications.

In order to deal with the limitations of the Safety at Workplace Directives, it is recommended that occupational exposure of nanoparticles is limited as much as possible, while international standards for the safe handling of nanoparticles in laboratories and other workplaces is developed. Currently, a great deal of international attention is given to the identification of potential exposure scenarios at workplaces,

establishment of standard guidelines for workplaces and laboratories, identification of protection measures and development of efficient metrology infrastructures (NIOSH, 2006). For instance, the EU has funded a number of projects, such as ParticleRisk, NanoSAFE 1 and 2, with the main purpose of developing methods for detecting, tracking, and characterizing nanoparticles along with risk assessment and management procedures to secure industrial production of nanoparticles. Another project, NanoDERM, studies the quality of human skin as a barrier against nanoparticles, which is highly relevant and timely for both workers and consumers (European Commission 2007).

Many environmental laws (e.g. REACH, Directives on hazardous waste, etc.) are based on (eco)toxicological classification of substances. Terms like “toxic” or “persistent” are often used as triggering factors to establish specific regulations, set emission limits, prohibitions and other requirements. These rules acquire a consistent meaning only possible when (eco)toxicological data are available for a substance. Although work is underway in order to produce such (eco)toxicological data for nanoparticles, much of this work is only just getting started. For instance, the EU has funded a number of projects (e.g. IMPART-NanoTOX) attempting to increase the understanding of toxicological impact of nanoparticles on human and environmental health (European Commission 2007). A regulatory gap, on the other hand, emerges when there is insufficient scientific evidence or lack of reliable data. In this context REACH will play a crucial role because it should provide the necessary information to make consistent use of other laws. For this reason, it is recommended that all nanomaterials are treated as new substances under REACH until it is clarified whether all or only a few of them display unique (eco)toxicological properties at the nanoscale. Lowering the current 1 ton per annum threshold per producer or importer for engineered nanoparticles to different thresholds and units than mass is recommended (RS & RAE, 2004, European Commission, Health and Consumer Protection Directorate General 2004).

Besides providing the traditional physicochemical properties when registering a substance, producers and importers of nanomaterials should be obliged to provide additional information such as shape, crystal structure, surface- area, charge and chemistry paying respect to the specific properties of nanomaterials. The new European Chemical Agency should develop and provide guidance to primary manufacturers and down-stream users on safety assessment of nanomaterials and a semi-governmental institution should be established in order to help industry do nanomaterials' characterizations and to do (eco)toxicological testing.

Waste management regulations require stricter protective measures for handling, treatment and final disposal of wastes according to their (eco)toxicological characteristics. Since there are reasons to suspect that nanoparticles can display hazardous properties when released into the environment, it is recommended

introducing “free nanoparticles” in Annex II of the Directive on hazardous waste, which lists the constituents of a waste that render it hazardous.

Despite the many knowledge and regulatory gaps identified, one of the largest obstacles is the lack of access to key information along the life cycle of the products. Key information during production, extraction and refining, manufacturing, use and final disposal of the products is not available due to manufacturer’s secrecy and non-disclosure policies. It is impossible to obtain information on specific production volumes, the number of product units, concentration of nano-materials/particles in final products, or mass flows of nanoparticles from the raw material to the final product. Furthermore quantitative and qualitative characterizations of by-products, such as fullerene soot and carbon nanotube fibres, as well as their fate cannot be exhaustively described.

Without such information, public authorities will have a hard time monitoring and evaluating the effectiveness of the incremental approach. Some claims of confidential business information seem unreasonable (Hansen and Rejeski, 2008), and providing wider access to at least some information seems to be an important step in facilitating the availability of information up-and-down the supply chain and to other interested parties. In the case of emerging technologies, including nanomaterials, at a minimum information made publicly available for regular substances under REACH should also be made available i.e.: name, classification and labeling, physicochemical data, including information on pathways and environmental fate, results of each toxicological and ecotoxicological study, any derived no-effect level or predicted no-effect concentration, guidance on safe use, and, to the extent possible, analytical methods for detecting direct human exposures or discharge to the environment.

6.3 Recommendations in regard to voluntary environmental programs

As discussed in section 4 DEFRA has implemented a voluntary environmental program (VEP) in the UK so that industry can submit such information and help DEFRA “*develop a better understanding of what types of engineered nanoscale materials are likely to be produced in the UK, and to build up an understanding of their properties and characteristics so that the potential hazard, exposure and risk associated with these materials may be determined.*” (DEFRA 2006). However, so far, participation in the program has been limited and disappointing – perhaps due to the lack of incentives and obvious benefits for companies to participate.

In general these incentives and benefits can be categorized into two types:

- The carrot approach, defined as providing positive incentives such as cost-savings, technical assistance or other subsidies, and
- The stick approach defined as providing negative incentives such as threatening a harsher outcome such as legislation, for example, if a voluntary agreement is not reached.

Although DEFRA's VEP is completely voluntary, they do not include elements of these two types of incentives (particularly "stick" elements), since there are neither cost savings, technical assistance nor other positive incentives, nor is there more than a weak current threat of legislation, for the moment. In past VEPs, it seems that the "threat of regulatory intervention" used in combination with unbiased technical- and non-technical information support, progress reports, and favorable publicity has been outstanding incentives for participants (Hansen and Tickner 2007). A number of ways to provide these incentives have not yet been explored in, not only DEFRA's, but also other nanomaterial VEPs implemented around the world (Hansen and Tickner 2007). In the state of Massachusetts, for example, the 1989 Toxics Use Reduction Act includes mandatory materials accounting reporting for chemicals and bi-yearly pollution prevention planning. An industry fee on chemicals also funds both voluntary and confidential engineering technical assistance provided by the state government, as well as training, research and demonstration projects undertaken by the Toxics Use Reduction Institute at the University of Massachusetts Lowell. The training and technical support provide the "capacity" critical to innovation in safer materials and processes. This type of support is particularly important for small and medium sized firms that might pose the greatest risks where there is a general lack of expertise in health, safety, and environment. Non-technical support could be provided through the publishing of various newsletters, agency guidance documents on determining various nanomaterial characteristics, (eco)toxicological testing and monitoring, the establishment of a searchable nanomaterials database, the establishment of a website for participants to exchange information and firm-to-firm dialogues (Hansen and Tickner 2007). Guidance on determination of characteristics of nanomaterials would also help ensure consistency and reliability, as would guidance on health and safety testing. This guidance should offer clues how to report various problems experienced while gathering information (NPPTAC 2005). Hearing about and learning from the problems that participants experience when trying to do characterizations of their unique nanomaterials and trying to apply present environmental, health, and safety testing methods, is probably one of the most valuable assets of any program – given the fact that our understanding of the properties of nanomaterials, their application, and what constitutes their hazards and exposure pathways is still in its infancy. Guidelines would also help ensure the quality of the information being submitted. To further ensure the quality of information, periodic reporting should be made and feedback should be

provided back and forth between sponsors and participants. This includes getting external feedback from independent scientists or specialists and other stakeholders. Having signed agreements, which include a commitment to assure safe manufacturing and safe products, provide participants with a more stable regulatory environment. This can be an incentive for them to develop plans for information gathering, and safety and health management of nanomaterials. And having participants write progress reports could help ensure transparency to the public and to the regulating authorities (Beardsley *et al.* 1997). A key part of any effort to improve and ensure transparency is giving the public and other stakeholders access to information. Such access needs to be provided in terms of both raw data (so that data can be reanalyzed) and in a publicly accessible format. Ideally, representatives of the public should be involved in the design of the reporting format and resources should be provided so that organizations representing the public interest are able to adequately and competently review submissions, reviews, and reports. This is critical for ensuring the accountability of any program. A key element in ensuring participation in the VEP and increased likelihood of success is favorable publicity for participants. As already mentioned, this can be provided in a number of ways such as posting awards, giving press releases, etc. Additionally to such positive incentives, there should also be a number of disincentives for not participating in a VEP for nanomaterials. Disincentives could include the creation of a list of companies not participating in the VEPs on nanomaterials, but known to the DEFRA to develop and manufacture nanomaterials. The U.S. EPA has such a list of producers last known to produce orphan chemicals in their High Production Volume challenge program (OPPT 1999). Another disincentive could be informing non-participating firms every time one of its competitors decides to participate – an approach that was used effectively in the Energy Star Office Products program in the USA (Beardsley *et al.* 1997).

Disincentives for disclosure information such as potential future liability and supply chain dynamics are relevant with regard to nanomaterials. The question is what can be done to eliminate such disincentives? Davies (2006) suggested that the two be combined in the case of nanomaterials so that the insurance industry would refuse to insure any nanomanufacturer who did not adopt some oversight framework such as the one recently proposed by Environmental Defense and DuPont (2007), which urges companies to share not only information, but also insight into the basis of risk assessment and management decisions with other companies within the supply chain, including those involved in managing waste from the manufacture, use, or disposal of the material or product.

Implementation of these recommendations will only address some of the immediate concerns related to the application of the incremental approach to regulate current manufacturing and use of nanomaterials. However, they fail to address other concerns, especially the ones related to nanomaterial yet to be developed, i.e. the third and fourth generation of nanotechnological development.

Past experience with VEPs suggests that some kind of mandatory regulatory framework will be needed somewhere down the road, since participation to a considerable extent depends on the existence of a regulatory framework that would impose penalties on firms that do not undertake proactive measures for self-regulation. Indeed, VEPs are likely to be less effective without the backstop of mandatory regulation (Khanna and Damon 1999). As such any VEP on nanomaterials should be made mandatory after no more than three years in order to create a “threat of regulatory intervention”. The three-year leap period would give early participants time to adjust and provide a period of “trial and error” and to get something going while a more permanent regulatory program is developed. Having the VEP become mandatory after three years would also eliminate the problem of “orphan” nanomaterials. It would also ensure that first-moving companies get early benefits; and that there is a level regulatory playing field within a reasonable period of time, as competition gets tougher. One could fear that both, regulators and firms, will not be too keen on having a VEP turned into a mandatory regulatory framework since they might want to avoid the complex and costly conflicts that often are associated with regulatory reform (Baggott 1986). According to Lyon (2003), however, the initial VEP may reduce political resistance to future regulatory mandates because participants have less incentive to oppose new legislation when they receive government support, such as technical assistance, to implement risk management practices.

6.4 Limitations of risk assessment in regard to nanomaterials

Since early discussions about nanotechnology related risks, risk assessment has been put forward as the number one approach (along with LCA to some extent (Klöpffer *et al.* 2007)) in regard to understand the risks associated with the application of one kind of nanomaterials, namely nanoparticles, in our society. An official in charge of regulatory aspects of nanotechnology at the European Commission has even been cited for arguing that there is no regulatory void on nanotechnology because EU rules impose a risk assessment on all products and that nanomaterials were no exception to this obligation (Brekelmans cited in EurActiv 2008). Hence, the importance of risk assessment in providing the backbone in relation to current and future regulation of nanomaterials should not be underestimated.

What is worrying is that the present analysis of risk assessment identified a number of limitations and flaws in relation to each of the four elements of the risk assessment framework when applied on nanomaterials. It is currently impossible to systematically link reported nanoparticle properties to the observed effects for effective hazard identification. For dose-response assessment, it was unclear whether a no effect

threshold can be established and what the best hazard descriptor(s) of nanoparticles is and what the most relevant endpoints are.

There is a serious lack of characterization of the nanoparticles tested, which makes it difficult to identify which key characteristics – or combinations of key characteristics – determine the hazards documented in (eco)toxicological studies of nanoparticles. The nine inherent properties identified as possible hazard descriptors by Hansen *et al.* (2007) and others may possibly be reduced as knowledge advances in the field of nanotoxicology, and it is likely that the toxicity of nanomaterials is determined by combinations of these properties (Hansen *et al.* 2007, Wittmaack 2007). But perhaps properties not yet identified in the scientific literature may be relevant for the hazard identification of nanomaterials.

Although the lack of characterization is troublesome, it is hardly surprising as nanotoxicology is a very young field of research stemming from ultra fine particle research (Oberdörster *et al.* 2005a). A true understanding of the hazardous properties that materials begin to exhibit at the nanoscale requires a level of interdisciplinary research that has not yet been reached. In order to conduct and interpret scientific studies on the hazardous properties of nanomaterials, strong interdisciplinary collaboration is needed between nanoscientists, (eco)toxicologists and physicists, chemists, and material engineers.

The analysis of the limitations of risk assessment furthermore showed that the third element of risk assessment – exposure assessment – is hampered by difficulties in monitoring nanomaterial exposure in the workplace and the environment, partly due to the fact that the biological and environmental pathways of nanomaterials are still largely unexplored (CCA 2008) and partly due to the paucity of knowledge and lack of access to information which hampers realistic exposure assessments. Risk characterization, being at the end of the line, the sum or maybe even the power of all these limitations are conveyed to calculating risk quotients for nanoparticles. Considerable work is still required if future risk assessment of current nanomaterials and products is to be relevant and reliable.

Despite some moves to respond to the limitations of risk assessment and uncertainty rather than simply discuss them, coordinated action seems slow in emerging. In 2001, a report written by an expert panel commissioned by the European Environment Agency (EEA) on how to avoid repeating the mistakes of the technological development recommended looking out for “warning signs” such as materials exhibiting novelty, persistency, readily dispersed, bioaccumulative, and that lead to irreversible action (EEA 2001). These characteristics resonate with many nanomaterials (RCEP 2008), some of which have novel properties, are capable of being incorporated in highly diverse products, may be transported to places in new ways, and may be designed to be persistent. Too little is known to predict the environmental fate of nanomaterials and feasible documentation of environmental dispersion through

monitoring is not expected in the short term (RS & RAE 2004). The extent to which specific nanomaterials are bioaccumulative or lead to irreversible action is largely unknown, but the current state of knowledge suggests that the potential exists for such behavior under some circumstances (SCENIHR 2007). The global response to these warning signs has been patchy, at best. In general, government policy has been slow to respond, to gather essential data on production and to use patterns and personal protection equipment. Arguably, efforts have been better than those seen with many earlier technologies but they are still far from ideal. A number of reports make specific recommendations on developing responsive research strategies (Oberdörster *et al.* 2005b, Maynard 2006, Moore 2006, Tsuji *et al.* 2006). Calls for proposals in the European seventh framework program reflect some of these recommendations, while countries like Australia are beginning to develop integrated environment, safety and health research programs. In the USA, the nanotechnology risk-research portfolio looks impressive on paper, although it only accounts for between 1 to 4% of the total NNI nanotechnology R&D budget (Maynard 2006). Research strategies that target recognized areas of uncertainty and address many of the issues raised previously, should be relatively easy to develop, as the critical questions to be addressed are generally agreed upon (National Nanotechnology Initiative 2008, Hansen *et al.* 2008a).

Besides the dangers of missing important areas entirely, because the right questions have not yet been identified, there are a number of additional problems when it comes to risk assessment of future nanomaterials, their application and their variety – especially when we consider the pace of the technological development.

6.4.1 Case-by-case risk assessment of nanoparticles

The need to assess the risk of nanoparticles on a case-by-case basis is often mentioned in order to take the unique properties of nanomaterials into consideration (SCENIHR 2007, Environmental Defense and DuPont 2007) and an official in charge of regulatory aspects of nanotechnology at the European Commission has even been cited for stating that product authorization as well must be conducted “on a case-by-case basis” (Brekelmans cited in EurActiv 2008).

While chemical risk assessment is based on the fact that the chemical identity governs the fate and effects of a chemical, the situation for nanomaterials may be somewhat different. By definition, the properties of nanomaterials cannot be determined by their chemical composition alone, and hazard identification of nanomaterials – and specifically nanoparticles – has come under intense scrutiny in recent years. However, we are still in what one could term the “pre-hazard identification”-phase, meaning that we do not know which characteristics determine the hazards of nanoparticles. As noted by Kulinowski, executive director of the Center for Biological & Environmental Nanotechnology at Rice University “*We have to remember that so much of what needs to be done is still in the discovery stage*” (Hanson 2008). There seems to be a general

agreement that the hazards will depend on surface area, surface charge, surface chemistry, state of agglomeration as well as chemical composition (Hansen *et al.* 2007), and especially surface area/reactivity has been mentioned as new “nano-relevant” properties for inclusion in hazard identification. However, for the time being, all of the mentioned particle characteristics may impact the overall hazard and since the causal relationships still need to be discovered, further research is needed in this area before relevant data demands for hazard identification purposes can be defined. Even with well-defined data demands, the experiences from chemical risk assessments tell us that case-by-case risk assessment of nanoparticles will be very time- and resource intensive. For nanomaterials, this situation is further complicated by the fact that the hazard characteristics will not only be linked to the chemical identity and that a large number of combinations of characteristics may influence the overall hazard. For instance, there are 20 different structural types of single-walled carbon nanotubes alone and their length can vary from 5 to 300 nm. According to Schmidt (2007) four different processes exist for manufacturing them, five methods for purifying them, and ten surface coatings are typically applied – hence there are up to 50,000 potential combinations of single-walled carbon nanotubes – and each version may have different chemical, physical, and biological properties that determine their overall hazard. This example may serve to show the complexity and how demanding case-by-case evaluations are. However, not all of these single-walled nanotubes are expected to be of commercial relevance. On the other hand, there are numerous other kinds of nanoparticles such as fullerenes, quantum dots, metals and metaloxide nanoparticles.

6.4.2 The pace of development

While the completion of risk assessment of chemicals has been slow in the past, the pace of nanotechnological development and commercialization has not as illustrated in section 2. The number of products claiming the use of nanotechnology has doubled within the last two years (Project of Emerging Nanotechnologies 2007), and it is projected that 2 million workers will work in the nano-industry within 2014. In a recent commentary published in *Nature Nanotechnology*, a panel of experts listed the great challenges in the field of nanotoxicology over the next 15 years, ranging from the development of strategic research programs to the validated alternatives for *in vivo* nanomaterial toxicity tests (Maynard *et al.* 2006). They recommend that, for instance, strategic programs that enable relevant risk-focused research are developed within the next 12 months, models for predicting the potential impact of engineered nanomaterials on the environment and human health are developed within the next 10 years and methods to evaluate the toxicity of engineered nanomaterials are developed and validated within the next 5 – 15 years (Maynard *et al.* 2006). As the authors of the article note, meeting these challenges over the course of the next 15 years will depend on coordination, collaboration, resources and ingenuity, even if effort to meet these

challenges is assumed to be coordinated and fully funded. Even if the classical chemical risk assessment framework turns out to be adaptable for the assessment of the risks of nanoparticles, despite the limitations noted above, it is, however, important to remember that these simple “passive” nanoparticles only represent the “first-generation” of the nanotechnological development (Roco and Renn 2006). The second and third generation started in 2000 and 2005 whereas the fourth generation of nanotechnological development is expected to begin in the near future (i.e. 2010). This generation is projected to involve the development of heterogeneous molecular nanosystems where each molecule in the nanosystem has a specific structure and plays a different role (Roco and Renn 2006).

If the recommendations by Maynard *et al.* (2006) are implemented, it means that we might be able to assess the human health and environmental risks of passive nanoparticles around 2020 – at the time when the development of nanotechnology is about to end its four generation of development and enter a fifth according to Roco and Renn (2006).

6.5 Alternatives to risk assessment

It might be naïve to suggest that risk assessment should be abandoned due to the many and profound limitations identified and discussed here. It is, however, also naïve to suggest that risk assessment will be able to adequately inform decision makers on how to protect human health and the environment despite of these limitations any time soon.

Several government agencies, academic scholars, industrial as well as NGOs have argued that the basic principles of risk assessment – hazard identification, dose-response assessment, exposure assessment and risk characterization – can be applied effectively to nanoparticles as long as some adjustments are implemented (Nordan *et al.* 2006, Environmental Defense and DuPont 2007, CCA 2008). In general, it could seem as if several stakeholders, on the one hand, first advocate in favor of using the traditional risk assessment approach after which they, on the other hand, point to a lot of fundamental challenges that need to be addressed before risk assessment can actually be applied effectively to nanoparticles (SCENIHR 2007, U.S. EPA 2007, EFSA 2008, CCA 2008).

Given the limitations of risk assessment and given the future impact on every aspect of our lives and society that nanotechnology is expected to have, alternative decision making tools should be explored and new ways to govern and regulate nanomaterials should be sought.

One tool that has already been applied on nanomaterials is MultiCriteria Decision Analysis (MCDA) (Linkov *et al.* 2007). The common purpose of MultiCriteria

Decision Analysis methods is to evaluate and choose among different decision alternatives based on multiple criteria, using systematic and structured analysis in contrast to “ad hoc” decisions. A number of different MCDA-methods exist following various optimization algorithms, varying in both the types of value information needed and in the extent they are dependent on computer software. Some techniques rank options whereas others identify a single optimal alternative and again others differentiate between acceptable and unacceptable alternatives. Key issues in relation to MCDA are:

- 1) who defines what the initial criteria are;
- 2) what alternatives are available to the decision maker;
- 3) how the different criteria are translated into a numerical score in order to rank the different alternatives (Mayer and Stirling 1999).

Other available tools yet to be applied to nanomaterials are Adaptive management and Bayesian decision making. Adaptive management is probably the decision making tool that is most often mentioned as a vital component of management of complex and uncertain risks (Holling *et al.* 1978, Holling 2001), however, it has yet to be applied on nanomaterials. Adaptive management sees the management of a risk as a process consisting of many small decisions rather than a “one hit” decision. In Adaptive management, the decision maker takes a decision which is then interpreted as a hypothesis that needs to be tested and validated. Monitoring is implemented to see whether the hypothesis is to be confirmed or rejected. If the hypothesis is rejected a new decision is made and the process starts all over again. Bayesian decision making might be another option. In short, Bayesian statistics uses the knowledge from past experiences to tell something about the probability for future scenarios. In Bayesian decision making, a decision is made on the basis on the evidence available at one point in time. As new and more evidence becomes available, regulators and decision makers can find out how much they should adopt and maybe change their original decision in the light of the new evidence by using Bayesian decision making (Pascual, 2004). It is of vital importance that these methods are explored and utilized to the fullest and that their strengths and weaknesses are evaluated in the view of nanomaterials.

In the face of uncertainty, a frequent response is calling for more research before action is taken. Yet it is important to remember that “*Experts have often argued at an early stage that we ‘know enough’ to take protective action*” (EEA 2001). Deciding when to act and when to refrain from taking action is often a difficult call. Good policy depends on identifying the right balance between information and action while keeping the end-point (preventing harm) in mind, and incorporating review procedures for course corrections. It is over 15 years since first indications of nanomaterial harm were published (Oberdörster *et al.* 1992), and in the intervening time, an increasing body of literature has been developed on how nanomaterials interact with people and the environment (Hansen *et al.* 2007). Yet many governments still call for more information

as a substitute for action, and there are indications that understanding and managing the risks of engineered nanomaterials is being paralyzed by analysis. It is clear that more scientific information is needed, but we need to act on what we know now to enable industry to produce market nanotechnology-enabled products that are as safe as possible. Engineered nanomaterials are already on the market and in some cases, the risks are poorly understood and ineffectively regulated. Applying current knowledge to nanotechnology oversight will not solve every problem, but it will help prevent basic mistakes being made while the knowledge needed for more effective oversight is developed (Hansen *et al.* 2008a).

7. Conclusion

The development of nanotechnology has been rapid by almost any metric one can think of – governmental funding, industrial patents and number of research publications on nanotechnology in general and nanorisks in specific. Nanomaterials are currently used for various kinds of applications and the number of consumer products proclaimed to contain nanomaterials is increasing rapidly. Little is known, however, about how many products on the market actually contain nanomaterials, how many units are produced and sold of a given product or how much nanomaterial is used in each of the individual products. Very little is furthermore known about the production volumes at which nanomaterials are currently produced and how these materials are applied in various industrial applications. Besides application with regard to food packaging, processing, etc., projected applications include: remediation of contaminated soil and groundwater, fuel cells and batteries, medical applications, drinking water treatment, and weapons and explosives. The current production and use of nanomaterials is most likely not representative for the future use and production, but factual information is hard to obtain which hampers regulation and risk assessment of nanomaterials in the short and the long term.

At the moment, issuing a specific long-term regulation on nanotechnology seems technically problematic and politically improbable and hence the Commission of the European Communities has adopted an incremental approach making consistent use of existing regulation when this can be easily applied as such or suitably amended

Through an in-depth analysis of key pieces of regulation such as REACH, pharmaceutical regulation and the worker safety directives, a number of limitations of the current legislation were identified. Although nanomaterials might be covered by the general scope of many of the existing legislative frameworks, it is often unclear if nanoparticles actually are covered when it comes to specific nanomaterials and applications. The main problems seem to be that metrology tools are unavailable, that thresholds are not tailored to the nanoscale, but based on bulk material, profound lack of (eco)toxicological data, and that no risk thresholds and occupational exposure limits cannot be established with existing methodologies. So far, the only amendments that have been implemented in the EU is to take carbon and graphite from the list of substances exempted from registration under REACH which is deemed to be dissatisfactory to address the current regulatory uncertainty and the potential risks of nanomaterials.

It is recommended that all nanomaterials are treated as new substances under REACH and that nanomaterials are registered based on a threshold and units different than mass. Besides providing the traditional physicochemical properties producers and importers of nanomaterials should be obliged to provide (eco)toxicological data and

additional information on the specific properties of nanomaterials under the help and guidance of the new European Chemical Agency. Key information during production, extraction and refining, manufacturing, use and final disposal of the products should also be provided to the European Chemical Agency and disclosed to the public. Without such information, public authorities will have a hard time monitoring and evaluating the effectiveness of the incremental approach.

Although REACH is expected to provide the backbone of the “incremental” approach, other pieces of legislation need to be revised as well. In regard to the occupational exposure of nanoparticles, it is recommended that exposure is limited as much as possible, while international standards for the safe handling of nanoparticles is developed. For waste management, it is recommended to introduce “free nanoparticles” in Annex II of the Directive on hazardous waste whereas it is recommended that estimated environmental concentrations are not used as trigger values for further action in regard to regulation of nanomedical products.

Several governments have opted to implement voluntary environmental programs (VEPs), arguing that this is the only viable proportional option for the time being. It is generally known that key elements of any successful VEP are: incentives to participate for various stakeholders, agency guidance and technical assistance, signed commitments and periodical reporting, quality of information, and transparency both in design, reporting and evaluation. However, many of these elements have not been fully addressed in the VEP that are implemented currently on nanomaterials.

It is recommended that more incentives to participate in the program are provided. These include providing technical assistance, training and external feedback from independent scientists or specialists and other stakeholders. Non-technical support should be given as well through the publishing of various newsletters, agency guidance documents on determining various nanomaterial characteristics, (eco)toxicological testing and monitoring and favorable publicity. Disincentives should be implemented as well – including the creation of a list of companies not participating in the program, but known to develop and manufacture nanomaterials. Companies should be obligated to share information and insight into the basis of risk assessment and management decisions with other companies within the supply chain through liability laws. Finally, any voluntary program on nanomaterials should be made mandatory after no more than three years in order to create a “threat of regulatory intervention”

Implementation of these recommendations will only address some of the immediate concerns related to the application of the incremental approach and voluntary programs to regulate current manufacturing and use of nanomaterials. However, they fail to address other concerns, especially the ones related to nanomaterial yet to be developed, the third and fourth generation of nanotechnological development. This strongly indicates that a new regulatory framework will be needed (RCEP 2008).

Risk assessment plays a fundamental role of providing decision support for regulators and industry in the EU's incremental approach. The in-depth analysis of the current state of knowledge performed in the thesis identified a number of limitations in each of the four steps that together constitute the risk assessment framework.

Toxicity has been reported on for multiple nanoparticles, but for most nanoparticles these need further confirmation before one can say that a hazard has been identified. Multiple studies relevant for hazard identification have been carried out on C₆₀, CNTs, quantum dots and other nanomaterials, however, many of these studies are not meant to facilitate risk assessment in the sense that they use non-standardized tests, have no coherent endpoint, and differ substantially with regard to species tested, methods of administration, dose range, way of particle preparation, duration of exposure, and effects observed and reported. This hampers identification of hazard univocally for most nanoparticles. It is evident that the information provided is 'all over the map' making it impossible to systematically analyze the studies for properties of the nanoparticles which are important for the observed effects.

Dose-response estimates assumes a no effect threshold can be established and although some studies have reported observing a dose-response relationship there is no evidence of a dose threshold below which nanoparticle instillation ceased to cause inflammation. A dose-response assessment is furthermore hindered by the fact that it is unclear what the best descriptors for dose is and which properties determine or influence the inherent hazards of nanoparticles. The current lack of characterization of the nanoparticles tested in various studies makes it impossible to identify causality between observed hazards and specific physical and chemical properties. There is furthermore substantial limitation in our ability to determine individual and multiple particle characteristics simultaneously and in a consistent manner – both prior and during tests – when using different characterization techniques and/or across laboratories.

Exposure assessment is hampered by difficulties in monitoring nanomaterial exposure in the workplace and the environment, and by the fact that the biological and environmental pathways of nanomaterials are still largely unexplored. The assessment of worker exposure is hampered by both technical difficulties such as the lack of one consistent sampling method that can be used to characterize exposure in real-time and by lack of information and data, for example, about how many workers are potentially exposed, what kinds of nanomaterials workers are or might be exposed to, where and how they are exposed and at which concentrations, by dose or by particles number, and what kinds of protective measures there are used or available. As with worker exposure, analytical methods to detect and quantify concentrations of nanoparticles in the environment have yet to become available. The total load to the environment from current use of nanomaterials is unclear. Several studies have tried to assess current and future consumer and environmental exposure for individual products, nanomaterials,

and applications as well as product types. These studies, no doubt, hold great value in regard to assessing the applicability of exposure assessment and should be seen as “proof of principle” rather than actual assessment of the exposure. Paucity of knowledge and lack of access to information hampers realistic exposure assessments.

Risk characterization being at the end of the line, the sum or maybe even the power all of these limitations are conveyed to calculating risk quotients for nanoparticles.

It is concluded that that we do not know enough to say that nanomaterials are safe, but that there is evidence that some nanomaterials are hazardous depending on how they are applied and how humans and the environment are exposed to them. It is furthermore concluded that the existing regulation is not adequate to deal with nanomaterials in the short and the long term and that too little is being done currently to amend existing regulation through the incremental approach adopted by the EU and voluntary environmental program implemented in the UK. Although recognizing the adaptations are needed, risk assessment have repeatedly been proposed by expert committees, policy-makers, members of industry and non-governmental organization as means to inform decision makers about the risks of nanomaterials. However, in this thesis, risk assessment is found to be inadequate to timely inform policy-makers about the health and environmental risks of nanomaterials, if not in the short term, then most definitely, in the long term. Risk assessment is deemed not feasible for the purpose of dealing with the complex emerging risks of nanomaterials and will not be adequate to ensure a decision making process that enables us to make informed decisions within a reasonable period of time.

It is recommended that current regulation is adapted immediately to reflect the challenges posed by current nanomaterials and their applications and that risk assessment is abandoned as the primary decision making tool and that alternative tools are pursued to support transparent and informed decision making processes.

8. List of references

Adelman, P., Baierl, T., Drosselmeyer, E., Politis, C., Polzer, G., Seidel, A., Steinleitner C. 1994. Effect of fullerenes on alveolar macrophages in vitro. In Toxic and Carcinogenic Effect of Solid Particles in the Respiratory Tract. Mohr, U., Dungworth, D.L., Mauderly, J., Oberdoester, G., Eds., Washington, DC: ILSI Press, pp 405-407.

Alessandrini, F., Ziesenis, A., Takenaka, S., Karg, E., Heyder, J., Ring, J., Behrendt, H. 2003. Effects of inhaled CdO particles on the sphingolipid synthesis of rat lungs. *Inhal Toxicol* 15(4): 343-356.

Alt, V., Bechert, T., Steinrucke, P., Wagener, M., Seidel, P., Dingeldein, E., Domann, E., Schnettler, R. 2004. An in vitro assessment of the antibacterial properties and cytotoxicity of nanoparticulate silver bone cement. *Biomaterials* 25(18): 4383-4391.

ASTM Int'l. 2006. E 2456-06 Terminology for Nanotechnology. Pennsylvania: ASTM International.

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., Bottero, J. Y. 2006. In vitro interactions between DMSA-coated maghemite nanoparticles and human fibroblasts: A physicochemical and cyto-genotoxic study. *Environ Sci Technol* 40(14): 4367-4373.

Babynin, E., Nuretdinov, I., Gubskaya, V., Barabanshchikov, B. 2002. Study of mutagenic activity of fullerene and some of its derivatives using His⁺ reversions of *Salmonella typhimurium* as an example. *Genetica* 38(4): 453-457.

Baggott, R. 1986. By Voluntary Agreement - the Politics of Instrument Selection *Pub Admin* 64(1): 51-67.

Baggs, R. B., Ferin, J., Oberdörster, G. 1997. Regression of pulmonary lesions produced by inhaled titanium dioxide in rats. *Veterinary Pathol* 34: 592-597.

Baierl, T., Drosselmeyer, E., Seidel, A., Hippeli, S. 1996. Comparison of immunological effects of Fullerene C-60 and raw soot from Fullerene production on alveolar macrophages and macrophage like cells in vitro. *Exp Toxicol Pathol* 48(6): 508-511.

Baker, C., Pradhan, A., Pakstis, L., Pochan, D. J., Shah S.I. 2005. Synthesis and antibacterial properties of silver nanoparticles. *J Nanosci Nanotechnol* 5:244-249.

Balbus, J.M., Florini, k., Denison, R.A. Walsh, S.A. 2007. Protecting workers and the environment: An environmental NGO's perspective on nanotechnology. *J Nanopart Res* 9:11-22.

Ballou, B., Lagerholm, B.C., Ernst, L.A., Bruchez, M.P., Waggoner, A.S. 2004. Noninvasive imaging of quantum dots in mice. *Bioconjug Chem* 15(1):79-86.

Baun, A., Hartmann, N. B., Grieger, K., Kusk, K. O. 2008. Ecotoxicity of engineered nanoparticles to aquatic invertebrates – a brief review and recommendations for future toxicity testing. *Ecotoxicology* 17(5):387-395.

Baun, A., Sørensen, S. N., Rasmussen, R.F., Hartmann, N. B., Koch, C. B.2008. Toxicity and bioaccumulation of xenobiotic organic compounds in the presence of aqueous suspensions of aggregates of nano-C60. *Aquatic Toxicol* 86:379-387.

Baun, A., Hansen, S. F. 2008. Environmental challenges for nanomedicine. *Nanomedicine* 2(5): 605-608.

Becker, C. W., Balogh, L., Khan, M. K. 2004. H-3 dendrimer nanoparticle organ/tumor distribution. *Pharmaceut Res* 21:476-483.

Beckett, W. S., Chalupa, D. F., Pauly-Brown, A., Speers, D. M., Stewart, J. C., Frampton, M. W., Utell, M. J., Huang, L. S., Cox, C., Zareba, W., Oberdorster, G. 2005. Comparing Inhaled Ultrafine versus Fine Zinc Oxide Particles in Healthy Adults: A Human Inhalation Study. *Am. J. Respir. Crit. Care Med.* 171(10): 1129-1135.

Beck-Speier, I., Dayal, N., Karg, E., Maier, K. L., Roth, C., Ziesenis, A., Heyder, J. 2001. Agglomerates of ultrafine particles of elemental carbon and TiO₂ induce generation of lipid mediators in alveolar macrophages. *Environ Health Perspect* 109: 613-618.

Bello., D., Hart, A.J., Ahn, K., Hallock, M., Yamamoto, M., Garcia, E.J., Ellenbecker, M.J., Wardle, B.L.. 2008. Particle exposure levels during CVD growth and subsequent handling of vertically-aligned carbon nanotube films. *Carbon* 46: 974-981.

Berry, C. C., Wells, S., Charles, S., Aitchison, G., Curtis, A. S. G. 2004. Cell response to dextran-derivatised iron oxide nanoparticles post internalisation. *Biomaterials* 25(23): 5405-5413.

Berry, C. C., Wells, S., Charles, S., Curtis, A. S. G. 2003. Dextran and albumin derivatised iron oxide nanoparticles: influence on fibroblasts in vitro. *Biomaterials* 24(25): 4551-4557.

Berry, C. C. Wells, S. Charles, S. Aitchison, G. Curtis, A. S. G. 2004. Cell response to dextran-derivatised iron oxide nanoparticles post internalisation. *Biomaterials* 25 (23), 5405-5413.

Bertazza, L., Celotti, L., Fabbrini, G., Loi, M. A., Maggini, M., Mancin, F., Marcuz, S., Menna, E., Muccini, M., Tonellato, U. 2006. Cell penetrating silica nanoparticles doped with two-photon absorbing fluorophores. *Tetrahedron* 62(44): 10434-10440.

Bhattacharya, R., Mukherjee, P., Xiong, Z., Atala, A., Soker, S., Mukhopadhyay, D. 2004. Gold Nanoparticles Inhibit VEGF165-Induced Proliferation of HUVEC Cells. *Nano Lett* 4(12): 2479-2481.

Bhattacharya, R., Patra, C. R., Verma, R., Kumar, S., Greipp, P. R., Mukherjee, P. 2007. Gold nanoparticles inhibit the proliferation of multiple myeloma cells. *Advanced Materials* 19(5): 711-716.

Binnig, G., Quate, C.F., Gerber, C.H. 1986. Atomic Force Microscope., *Phys Rev Lett* 56(9): 930-933.

Binnig, G., Rohrer, H., Gerber, C.H., Weibel, E. 1982. Surface Studies by Scanning Tunneling Microscopy., *Phys Rev Lett* 49(1): 57-61.

Biswas, P., and C.Y. Wu, 2005. Critical review: Nanoparticles and the environment. *J. Air Waste Manage Assoc* 55: 708–746.

Blaser, S. A., Scheringer, M., MacLeod, M., Hungerbühler, K. 2008. Estimation of cumulative aquatic exposure and risk due to silver: Contribution of nano functionalized plastics and textiles. *Sci Total Environ* 390: 396 – 409.

Bogdanovic, G., Kojic, V., Dordevic, A., Canadanovic-Brunet, J., Vojinovic-Miloradov, M., Baltic, V. V. 2004. Modulating activity of fullerol C-60(OH)(22) on doxorubicin-induced cytotoxicity. *Toxicol in Vitro* 18(5): 629-637.

Bottini, M., Bruckner, S., Nika, K., Bottini, N., Bellucci, S., Magrini, A., Bergamaschi, A., Mustelin, T. 2006. Multi-walled carbon nanotubes induce T lymphocyte apoptosis. *Toxicology Letters* 160(2): 121-126.

Bourrinet, P., Bengéle, H. H., Bonnemain, B., Dencausse, A., Idee, J. M., Jacobs, P. M., Lewis, J. M. 2006. Preclinical safety and pharmacokinetic profile of ferumoxtran-10, an ultrasmall superparamagnetic iron oxide magnetic resonance contrast agent. *Invest radiol* 41(3): 313-324.

Boxall, A.B. A., Chaudhry, Q., Sinclair, C., Jones, A., Aitken, R., Jefferson, B., Watts, C. 2008. *Current And Future Predicted Environmental Exposure To Engineered Nanoparticles*. York: Central Science Laboratory.

Brayner, R., Ferrari-Iliou, R., Brivois, N., Djediat, S., Benedetti, M. F., Fievet, F. 2006. Toxicological Impact Studies Based on Escherichia Coli Bacteria in Ultrafine ZnO Nanoparticles Colloidal Medium. *Nano Lett* 6(4): 866-870.

Bruinink, A., Stark, W. J. 2006. In vitro cytotoxicity of oxide nanoparticles: Comparison to asbestos, silica, and the effect of particle solubility. *Environ Sci Technol* 40: 4374-4381.

Brunner, T.J., Wick, P., Manser, P., Spohn, P., Grass, R.N., Limbach, L.K. 2006. In vitro cytotoxicity of oxide nanoparticles: Comparison to asbestos, silica, and the effect of particle solubility. *Environ Sci Technol* 40(14): 4374-4381

Brusentsov, N. A., Kuznetsov, V. D., Brusentsova, T. N., Gendler, T. S., Novakova, A. A., Volter, E. R., Haliulina, E. A., Danilkin, M. I. 2004. Magnetisation of ferrifluids and effects of intracellular deposition of ferrite nanoparticles. *J Magn Magn Mater* 272-276, 2350-2351.

BSI. 2007a. *Guidance on the labelling of manufactured nanoparticles and products containing manufactured nanoparticles*. PAS 130:2007. London: British Standards Institution.

BSI 2007b. *Terminology for Nanofabrication*. PAS 135:2007. London: British Standards Institution.

BSI 2007c. *Terminology for Nanomaterials*. PAS 136:2007. London: British Standards Institution.

BSI 2007d. Terminology for carbon Nanostructures. PAS 134:2007. London: British Standards Institution.

Burlaka, A. P., Sidorik, Y. P., Prylutska, S. V., Matyshevskaya, O. P., Golub, O. A., Prolutskyy, Y. I., Scharff, P. 2004. Catalytic system of the reactive oxygen species on the C60 fullerene basis. *Exp Oncol* 26(4): 326-327.

C & EN. 2008. Carbon Losses its Exemption Statues under REACH. *Chemical & Engineering News*, June 23, 2008 p. 9.

Cai, R., Hashimoto, K., Itoh, K., Kubota, Y., Fujishima, A. 1991. Photokilling of Malignant cells with Ultrafine TiO₂ Powder. *B Chem Soc Jpn* 64(4): 1268-1273.

Cai, R., Kubota, Y., Shuin, T., Sakai, H., Hashimoto, K., Fujishima, A. 1992. Induction of Cytotoxicity by Photoexcited TiO₂ Particles. *Cancer Res* 52(8): 2346-2348.

Carrero-Sanchez, J. C., Elias, A. L., Mancilla, R., Arrellin, G., Terrones, H., Laclette, J. P., Terrones, M. 2006. Biocompatibility and Toxicological Studies of Carbon Nanotubes Doped with Nitrogen. *Nano Lett* 6(8): 1609-1616.

Cassee, F. R., Muijsers, H., Duistermaat, E., Freijer, J.J. Geerse, K.B., Marijnissen, J.C.M. Arts, J.H.E. 2002. Particle size-dependent total mass deposition in lungs determines inhalation toxicity of cadmium chloride aerosols in rats. Application of a multiple path dosimetry model. *Arch Toxicol* 76: 277–286.

Cataldo, F., 2002. A study on the thermal stability to 1000 °C of various carbon allotropes and carbonaceous matter both under nitrogen and in air. *Fuller Nanotub Car N* 20(4): 293–311.

CCA. 2008. Small Is Different: A Science Perspective On The Regulatory Challenges of the Nanoscale. Ottawa: The Council of Canadian Academies.

CEC. 2008. Communication From The Commission To The European Parliament, The Council And The European Economic And Social Committee Regulatory Aspects Of Nanomaterials [Sec(2008) 2036] Com(2008) 366 final. Brussels: Commission of the European Communities.

CEC. 2008b. Proposal for a Regulation of the European Parliament and of the Council on novel foods and amending Regulation (EC) No XXX/XXXX# [common procedure] (presented by the Commission) [SEC(2008) 12] [SEC(2008) 13]. COM(2007) 872 final

2008/0002 (COD). 14-1 2008. Brussels, Commission of the European Communities.

Center for Responsible Nanotechnologies. 2008. What is Nanotechnology?. Center for Responsible Nanotechnology. Available: <http://www.crnano.org/whatis.htm> (Accessed December 3 2008).

Chan, W. H., Shiao, N. H., Lu, P. Z. 2006. CdSe quantum dots induce apoptosis in human neuroblastoma cells via mitochondrial-dependent pathways and inhibition of survival signals. *Toxicol Lett* 167(3): 191-200.

Chang, E., Thekkek, N., Yu, W. W., Colvin, V. L., Drezek, R. 2006. Evaluation of quantum dot cytotoxicity based on intracellular uptake. *Small* 2(12): 1412-1417.

Chang, J. S., Chang, K. L. B., Hwang, D. F., Kong, Z. L. 2007. In vitro cytotoxicity of silica nanoparticles at high concentrations strongly depends on the metabolic activity type of the cell line. *Environ Sci Technol* 41(6): 2064-2068.

Chaundry, Q., Blackburn, J., Floyd, P., George, C., Nwaogu, T., Boxall, A., Aitken, R. 2006. A scoping study to identify gaps in environmental regulation for the products and applications of nanotechnologies. London: Department for Environment, Food and Rural Affairs.

Chen, F. Q., Gerion, D. 2004. Fluorescent CdSe/ZnS nanocrystal-peptide conjugates for long-term, nontoxic imaging and nuclear targeting in living cells. *Nano Lett* 4(10): 1827-1832.

Chen, H. H. C., Yu, C., Ueng, T. H., Chen, S. D., Chen, B. J., Huang, K. J., Chiang, L. Y. 1998. Acute and subacute toxicity study of water-soluble polyalkylsulfonated C-60 in rats. *Toxicol Pathol* 26(1): 143-151.

Chen, H. H. C., Yu, C., Ueng, T. H., Liang, C. T., Chen, B. J., Hong, C. C., Chiang, L. Y. 1997. Renal effects of water-soluble polyarylsulfonated C-60 in rats with an acute toxicity study. *Fullerene Sci Techn* 5(7): 1387-1396.

Chen, M., von Mikecz, A. Formation of nucleoplasmic protein aggregates impairs nuclear function in response to SiO₂ nanoparticles. 2005. *Exp Cell Res* 305(1): 51-62.

Chen, Y. W., Hwang, K. C., Yen, C. C., Lai, Y. L. 2004. Fullerene derivatives protect against oxidative stress in RAW 264.7 cells and ischemia-reperfused lungs. *Am J Physiol-Reg I* 287(1): R21-R26.

Cheng, F. Y., Su, C. H., Yang, Y. S., Yeh, C. S., Tsai, C. Y., Wu, C. L., Wu, M. T., Shieh, D. B. 2005. Characterization of aqueous dispersions of Fe₃O₄ nanoparticles and their biomedical applications. *Biomaterials* 26(7): 729-738.

Cheng, J. P., Flahaut, E., Cheng, S. H. 2007. Effect of carbon nanotubes on developing zebrafish (*Danio rerio*) embryos. *Environ Toxicol Chem* 26(4): 708-716.

Cheng, M. D. 2004. Effects of nanophase materials (<= 20 nm) on biological responses. *J Environ Sci Heal A* 39(10): 2691-2705.

Chiron, J., Lamande J, Moussa F, Trivin, F., Ceolin, R. 2000. Effect of "micronized" C60 fullerene on the microbial growth in vitro. *Ann Pharm Fr* 58(3): 170-175.

Chlopek, J., Czajkowska, B., Szaraniec, B., Frackowiak, E., Szostak, K., Beguin, F. 2006. In vitro studies of carbon nanotubes biocompatibility. *Carbon* 44(6): 1106-1111.

Chorkendorff I, Niemantsverdriet JW. 2003. Concepts of modern catalysis and kinetics. New York: Wiley-VCH.

Cientifica 2006. Nanotube Production Survey. Cientifica

Connor, E. E., Mwamuka, J., Gole, A., Murphy, C. J., Wyatt, M. D. 2005. Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. *Small* 1 (3): 325-327.

Council of the European Communities. 1990. Council Directive 90/385/EEC of 20 June 1990 on the approximation of the laws of the Member States relating to active implantable medical devices. *Official Journal L* 189 of 20 July 1990, pp. 17 - 36.

Council of the European Communities. 1993. Council Directive 93/42/EEC of 14 June 1993 concerning medical devices. *Official Journal L* 169 of 12 July 1993, pp. 1 - 43.

Council of the European Communities. 1991. Council Directive 91/689/EEC of 12 December 1991 on hazardous waste (91/689/EEC). *Official Journal L* 377:P.0020-0027.

Council of the European Communities. 1998. Council Directive 98/24/EC of 7 April 1998 on the protection of the health and safety of workers from the risks related to chemical agents at work (fourteenth individual Directive within the meaning of Article 16(1) of Directive 89/391/EEC). *Official Journal L* 131:23.

Council of the European Communities.1989. Council Directive 89/391/EEC of 12 June 1989 on the introduction of measures to encourage improvements in the safety and health of workers at work. Official Journal L 183:0008.

Cui, D. X., Tian, F. R., Ozkan, C. S., Wang, M., Gao, H. J. 2005. Effect of single wall carbon nanotubes on human HEK293 cells. *Toxicol Lett* 155(1): 73-85.

Beardsley, D., Davies, T., Hersh, R. 1997. Improving Environmental Management: What Works, What Doesn't. *Environment* 39(7): 6.

Davies, J.C. 2006. *Managing The Effects Of Nanotechnology*. Washington, D.C.: Project on Emerging Nanotechnologies, Woodrow Wilson International Center For Scholars.

DEFRA. 2006a. UK Voluntary Reporting Scheme for engineered nanoscale materials. London: Department of Environment, Food and Rural Affairs.

DEFRA. 2006b. Voluntary Reporting Scheme for engineered nanoscale materials First quarterly report covering the period 22/09/06 - 22/12/06. London: Department for Environment, Food and Rural Affairs.

Department of Environment Food and Rural Affairs, Consultation on a Proposed Voluntary Reporting Scheme for Engineered Nanoscale Materials. Available: <http://www.defra.gov.uk/environment/nanotech/policy/index.htm#voluntary> (Accessed May 17 2007).

DEFRA 2008. The UK Voluntary Reporting Scheme for Engineered Nanoscale materials: Seventh Quarterly Report. London: Department for Environment, Food and Rural Affairs (<http://www.defra.gov.uk/environment/nanotech/pdf/vrs-seventh-progress-report.pdf>)

Delehanty, J. B., Medintz, I. L., Pons, T., Brunel, F. M., Dawson, P. E., Mattoussi, H. 2006. Self-Assembled Quantum Dot-Peptide Bioconjugates for Selective Intracellular Delivery. *Bioconjugate Chem.* 17(4): 920-927.

Derfus, A. M., Chan, W. C. W., Bhatia, S. N. 2004. Probing the cytotoxicity of semiconductor quantum dots. *Nano Lett* 4(1): 11-18.

Donaldson, K., Brown, D., Clouter, A., Duffin, R., MacNee, W., Renwick, L., Tran, L. and V. Stone. 2002. The pulmonary toxicology of ultrafine particles. *J Aerosol Med* 15(2): 213-220.

D'Silva, J., Van Calster, G. 2008. Regulating Nanomedicine: A European Perspective. Available <http://ssrn.com/abstract=1286215> (Accessed November 15 2008).

Dubertret, B., Skourides, P., Norris, D. J., Noireaux, V., Brivanlou, A. H., Libchaber, A. 2002. In vivo imaging of quantum dots encapsulated in phospholipid micelles. *Science* 298(5599): 1759-1762.

Dufour, E. K., Kumaravel, T., Nohynek, G. J., Kirkland, D., Toutain, H. 2006. Clastogenicity, photo-clastogenicity or pseudo-photo-clastogenicity: Genotoxic effects of zinc oxide in the dark, in pre-irradiated or simultaneously irradiated Chinese hamster ovary cells. *Mutat Res-Gen Tox En* 607(2): 215-224.

Dugan, L. L., Gabrielsen, J. K., Yu, S. P., Lin, T. S., Choi, D. W. 1996. Buckminsterfullerenol free radical scavengers reduce excitotoxic and apoptotic death of cultured cortical neurons. *Neurobiol Dis* 3(2): 129-135.

Editorial. 2007. Regulating nanomedicine. *Nature Materials* 6: 249.

EEA. 2001. Late Lessons from Early Warnings: The Precautionary Principle 1896–2000. Copenhagen: European Environmental Agency.

EFSA. 2008. Draft Opinion of the Scientific Committee on the Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety (Question No EFSA-Q-2007-124). European Food Safety Authority.

EGE 2007. Opinion 21 - On the ethical aspects of nanomedicine, The European Group on Ethics in Science and New Technologies (EGE), Retrieved 15 March 2008 http://ec.europa.eu/european_group_ethics/publications/docs/final_publication_%20op21_en.pdf

EMA 2006. European Medicines Agency: reflection paper on nanotechnology-based medicinal products for human use (EMA/CHMP/70769/2006). London: European Medicines Agency.

Environmental Defense and DuPont. 2007. Nano Risk Framework. Environmental Defense and DuPont.

EP & CEU. 2002. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal of the European Communities L 31-1-L 31/24.

EP & CEU. 2004. Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency.

EP & CEU. 2006. Regulation (EC) No. 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 99/45/EC and repealing Council Regulation (EEC) No. 793/93 and Commission.

ETC Group 2003. The Big Down: From Genomes to Atoms. Winnipeg: ETC Group. Available: www.etcgroup.org (Accessed February 14 2005).

EurActiv. 2008. No regulatory void on nanotech, says Commission. Tuesday 7 October Available: <http://www.euractiv.com/en/science/regulatory-void-nanotech-commission/article-176050> (Accessed 28 October 2008).

European Commission JRC. 2003a Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market Part I. EUR 20418 EN/1. European Communities.

European Commission JRC. 2003b. Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market Part II. EUR 20418 EN/2. European Communities.

European Commission JRC. 2003c. Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified

substances Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market Part III. EUR 20418 EN/3. 2003. European Communities.

European Commission JRC. 2003d. Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market Part IV. EUR 20418 EN/4. 2003. European Communities.

European Commission, Health and Consumer Protection Directorate General, 2004. Nanotechnologies: a Preliminary Risk Analysis on the Basis of a Workshop. European Commission, Brussels.

European Commission. 2007. Nanotechnology Emerging Needs. Available: http://cordis.europa.eu/nanotechnology/src/pressroom_projects_nmp6.htm (Accessed January 27 2007).

European Medicines Agency: Guideline on the environmental risk assessment of medicinal products for human use EMEA/CHMP/SWP/4447/00. European Medicines Agency, London, UK (2006).

Fang, J., Lyon, D. Y., Wiesner, M. R., Dong, J., Alvarez, P. J. J. 2007. Effect of a Fullerene Water Suspension on Bacterial Phospholipids and Membrane Phase Behavior. *Environ Sci Technol* 41(7): 2636-2642.

Fent, K., Weston, A. A., Caminada, D. 2006. Ecotoxicology of human pharmaceuticals. *Aqua Toxicol* 76(2): 122-159 (2006).

Ferin, J., Oberdorster, G., Penney, D. P. 1992. Pulmonary Retention of Ultrafine and Fine Particles in Rats. *Am J Respir Cell Mol Biol* 6(5): 535-542.

Ferin, J., Oberdorster, G., Soderholm, S. C., Gelein, R. 1991. Pulmonary Tissue Access of Ultrafine Particles. *J Aerosol Med* 4(1): 57-68.

Ferrari, M. 2005. Acute toxicity of intravenously administered microfabricated silicon dioxide drug delivery particles in mice. *Drugs Res Develop* 6: 71-81.

FOE. 2008. Out of the Laboratory and on to Our Plates Nanotechnology in Food & Agriculture. Friends of the Earth Australia, Europe & U. S. A.

Fortner, J. D., Lyon, D. Y., Sayes, C. M., Boyd, A. M., Falkner, J. C., Hotze, E. M., Alemany, L. B., Tao, Y. J., Guo, W., Ausman, K. D., Colvin, V. L., Hughes, J. B. 2005. C-60 in water: Nanocrystal formation and microbial response. *Environ Sci Technol* 39(11): 4307-4316.

Franco, A., Hansen, S.F., Olsen, S.I., Butti, L. 2007. Limits and Prospects of the “Incremental Approach” and the European Legislation on the Management of Risks related to Nanomaterials. *Regul Toxicol Pharm* 48: 171-183.

Fujitani, Y., Kobayashi, T., Arashidani, K., Kunugita, N., Suemura, K. 2008. Measurement of the Physical Properties of Aerosols in a Fullerene Factory for Inhalation Exposure Assessment. *J Occup Environ Hyg* 5(6):380-9.

Fumelli, C., Marconi, A., Salvioli, S., Straface, E., Malorni, W., Offidani, A. M., Pellicciari, R., Schettini, G., Giannetti, A., Monti, D., Franceschi, C., Pincelli, C. 2000. Carboxyfullerenes protect human keratinocytes from ultraviolet-B-induced apoptosis. *J Invest Dermatol* 115(5): 835-841.

Funtowicz S, Jerome R. 1992. Three types of risk assessment and the emergence of Post-Normal Science in Social Theories of Risk (Krimsky S, Golding D, eds). Praeger, 251-274.

Gaspar, R. 2007. Regulatory issues surrounding nanomedicines: setting the scene for the next generation of nanopharmaceuticals. *Nanomed* 2(2):143-147.

Gavelin, K., Wilson, R., and Donaldson, R. 2007. Democratic technologies? The final report of the Nanotechnology Engagement Group (NEG), London: Involve

Gharbi, N., Pressac, M., Hadchouel, M., Szwarc, H., Wilson, S. R., Moussa, F. 2005. [60]Fullerene is a powerful antioxidant in vivo with no acute or subacute toxicity. *Nano Lett* 5(12): 2578-2585.

Gojova, A., Guo, B., Kota, R. S., Rutledge, J. C., Kennedy, I. M., Barakat, A. I. 2007. Induction of inflammation in vascular endothelial cells by metal oxide nanoparticles: Effect of particle composition. *Environ Health Perspect* 115(3): 403-409.

Goodman, C. M., McCusker, C. D., Yilmaz, T., Rotello, V. M. 2004. Toxicity of gold nanoparticles functionalized with cationic and anionic side chains. *Bioconjugate Chem* 15(4): 897-900.

Gordon, T., Chen, L. C., Fine, J. M., Schlesinger, R. B., Su, W. Y., Kimmel, T. A., Amdur, M. O. 1992. Pulmonary Effects of Inhaled Zinc-Oxide in Human-Subjects, Guinea-Pigs, Rats, and Rabbits. *Am Ind Hyg Assoc J* 53(8): 503-509.

Green, M., Howman, E. 2005. Semiconductor quantum dots and free radical induced DNA nicking. *Chem Commun* (1): 121-123.

Gupta, A. K., Curtis, A. S. G. 2004. Surface modified superparamagnetic nanoparticles for drug delivery: Interaction studies with human fibroblasts in culture. *J Mater Sci-Mater M* 15(4): 493-496.

Gupta, A. K., Gupta, M. 2005. Cytotoxicity suppression and cellular uptake enhancement of surface modified magnetic nanoparticles. *Biomaterials* 26(13): 1565-1573.

Gurr, J. R., Wang, A. S. S., Chen, C. H., Jan, K. Y. 2005. Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. *Toxicology* 213(1-2): 66-73.

Halffman, W. 1998. Standardisation as a trust device. In: *The politics of chemical risk. Scenarios for a regulatory future* (Bal R, Halffman W, eds.). Dordrecht, The Netherlands: Kluwer Academic Publishers, pp 265-292.

Halliday, J. EU Parliament votes for tougher additives regulation. *Foodnavigator.com* 12 July 2008. Available: <http://www.foodnavigator.com/Legislation/EU-Parliament-votes-for-tougher-additives-regulation> (Accessed November 15 2008).

Han, J. H., Lee, E. J., Lee, J. H., So, K. P., Lee, Y. H., Bae, G. N., Lee, S-B., Ji, J. H., Cho, M. H., Yu, I. J. 2008. Monitoring Multiwalled Carbon Nanotube Exposure in Carbon Nanotube Research Facility. *Inhal Toxicol* 20(8): 741-749.

Handy, R. D., Owen, R., Valsami-Jones, E. 2008. The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs. *Ecotoxicology* 17(5): 315-25.

Hansen, S. F., Larsen, B. H., Olsen, S. I., Baun, A. 2007. Categorization framework to aid Hazard Identification of Nanomaterials. *Nanotoxicology* 1:243-250.

Hansen, S. F., Tickner, J. A. 2007. The Challenges of Adopting Voluntary Health, Safety and Environment Measures for Manufactured Nanomaterials. Lessons from the past for more effective adoption in the future. *Nanotechnology Law & Business* 4 (3): 341-359.

Hansen, S. F., Maynard, A., Baun, A., Tickner, J. A. 2008a. Late lessons from early warnings for nanotechnology. *Nat Nanotechnol* 3(8): 444-447.

Hansen, S. F., Michelson, E., Kamper, A., Borling, P., Stuer-Lauridsen, F. & Baun, A. 2008b. Categorization framework to aid exposure assessment of nanomaterials in consumer products. *Ecotoxicology* 17(5):438-447.

Hansen, S.F., Baun, A., Michelson, E.S, Kamper, A., Borling, P., Stuer-Lauridsen, F. 2008c. Nanomaterials in Consumer Products: Categorization and Exposure assessment. In I. Linkov, J. Steevens (eds.), *Nanotechnology Risks and Benefits*. Springer: Dordrecht. 363-372.

Hansen, S.F., Rejeski, D. 2008. Applying the Chemical Policy Options to Emerging Technologies and Materials: Adaptations and Challenges. In J.A. Tickner and Y. Torrie (eds.). *Options for State Chemicals Policy Reform A Resource Guide*. Lowell Center for Sustainable Production, University of Massachusetts, Lowell. Available: <http://sustainableproduction.org/downloads/OptionsforStateChemicalsPolicyReform.pdf> (Accessed February 18 2008).

Hanson, D. 2008. Nanotech Strategy Prioritizing federal efforts for studying health and safety of nanomaterials evolves. *Chem Eng News* 86:29-30.

Hardman 2006. A Toxicologic Review of Quantum Dots: Toxicity Depends on Physicochemical and Environmental Factors. *Environ Health Perspect.* 114:165–172.

Harremoës P. 2003. Ethical aspects of scientific uncertainty in environmental analysis and decision making. *J Clean Prod* 11:705-712.

Hilger, I., Fruhauf, S., Lin, W., Hiergeist, R., Andra, W., Hergt, R., Kaiser, W. A. 2003. Cytotoxicity of selected magnetic fluids on human adenocarcinoma cells. *J Magn Magn Mater* 261(1-2): 7-12.

HM Government, Department of Trade and Industry. 2005. Response to the Royal Society and Royal Academy of Engineering Report: 'Nanoscience and Nanotechnologies: Opportunities and Uncertainties'. Available:<http://www.berr.gov.uk/files/file14873.pdf>. (Accessed December 5 2008).

Hohr, D., Steinfartz, Y., Schins, R. P. F., Knaapen, A. M., Martra, G., Fubini, B., Borm, P. J. A. 2002. The surface area rather than the surface coating determines the acute inflammatory response after instillation of fine and ultrafine TiO₂ in the rat. *Int J Hyg Envir Heal* 205(3): 239-244.

Holling, C.S. (ed.). 1978. Adaptive Environmental Assessment and Management. John Wiley & Sons., New York, USA.

Holling, C.S. 2001. Understanding the complexity of economic, ecological, and social systems. *Ecosystems* 4:390-405.

Hoshino, A., Hanaki, K., Suzuki, K., Yamamoto, K. 2004. Applications of T-lymphoma labeled with fluorescent quantum dots to cell tracing markers in mouse body. *Biochem Bioph Res Co* 314(1): 46-53.

Huang, S. S., Tsai, S. K., Chih, C. L., Chiang, L. Y., Hsieh, H. M., Teng, C. M., Tsai, M. C. 2001. Neuroprotective effect of hexasulfobutylated C-60 on rats subjected to focal cerebral ischemia. *Free Radical Bio Med* 30(6): 643-649.

Huang, Y. L., Shen, C. K. F., Luh, T. Y., Yang, H. C., Hwang, K. C., Chou, C. K. 1998. Blockage of apoptotic signaling of transforming growth factor-beta in human hepatoma cells by carboxyfullerene. *Eur J Biochem* 254(1): 38-43.

Huff, T. B., Hansen, M. N., Zhao, Y., Cheng, J. X., Wei, A. 2007. Controlling the Cellular Uptake of Gold Nanorods. *Langmuir* 23(4): 1596-1599.

Hund-Rinke, K., Simon, M. 2006. Ecotoxic effect of photocatalytic active nanoparticles TiO₂ on algae and daphnids. *Environ Sci Pollut R* 13(4): 225-232.

Igami, M., Saka, A. 2007. Capturing the Evolving Nature of Science, Development of New Scientific Indicators and Mapping of Science., STI Working Paper 2007/1, OECD Directorate for Science, Technology and Industry.

Iijima, S. 1991. Helical Micro-tubules of Graphitic Carbon. *Nature* 345: 56-58.

Isakovic, A., Markovic, Z., Nikolic, N., Todorovic-Markovic, B., Vranjes-Djuric, S., Harhaji, L., Raicevic, N., Romcevic, N., Vasiljevic-Radovic, D., Dramicanin, M., Trajkovic, V. 2006. Inactivation of nanocrystalline C60 cytotoxicity by [gamma]-irradiation. *Biomaterials* 27(29): 5049-5058.

Isakovic, A., Markovic, Z., Todorovic-Markovic, B., Nikolic, N., Vranjes-Djuric, S., Mirkovic, M., Dramicanin, M., Harhaji, L., Raicevic, N., Nikolic, Z., Trajkovic, V. 2006. Distinct Cytotoxic Mechanisms of Pristine versus Hydroxylated Fullerene. *Toxicol. Sci.* 91(1): 173-183.

ISO 2008. Nanotechnologies - Terminology and definitions for nano-objects - Nanoparticle, nanofibre and nanoplate. ISO/TS 27687:2008. Geneva: International Organization for Standardization.

Ivankovic, S., Gotic, M., Jurin, M., Music, S. 2003. Photokilling squamous carcinoma cells SCCVII with ultrafine particles of selected metal oxides. *J Sol-Gel Sci Techn* 27(2): 225-233.

Han, J. H., Cho, M. H., Yu, I. J. 2008. Monitoring Multiwalled Carbon Nanotube Exposure in Carbon Nanotube Research Facility. *Inhal Toxicol* 20(8): 741-749.

Jaiswal, J. K., Mattoussi, H., Mauro, J. M., Simon, S. M. 2003. Long-term multiple color imaging of live cells using quantum dot bioconjugates. *Nat Biotechnol* 21(1): 47-51.

Jia, X., Li, N., Chen, J. 2005. A subchronic toxicity study of elemental Nano-Se in Sprague-Dawley rats. *Life Sci* 76(17): 1989-2003.

Jordan, A., Wust, P., Scholz, R., Tesche, B., Fahling, H., Mitrovics, T., Vogl, T., CervosNavarro, J., Felix, R. 1996. Cellular uptake of magnetic fluid particles and their effects on human adenocarcinoma cells exposed to AC magnetic fields in vitro. *Int J Hyperther* 12(6): 705-722.

Jovanovic, A. V., Flint, J. A., Varshney, M., Morey, T. E., Dennis, D. M., Duran, R. S. 2006. Surface Modification of Silica Core-Shell Nanocapsules: Biomedical Implications. *Biomacromolecules* 7(3): 945-949.

Kagan, V. E., Tyurina, Y. Y., Tyurin, V. A., Konduru, N. V., Potapovich, A. I., Osipov, A. N., Kisin, E. R., Schwegler-Berry, D., Mercer, R., Castranova, V., Shvedova, A. A.

2006. Direct and indirect effects of single walled carbon nanotubes on RAW 264.7 macrophages: Role of iron. *Toxicol Lett* 165(1): 88-100.

Kamat, J. P., Devasagayam, T. P. A., Priyadarsini, K. I., Mohan, H. 2000. Reactive oxygen species mediated membrane damage induced by fullerene derivatives and its possible biological implications. *Toxicology* 155(1-3): 55-61.

Khanna, M., Damon, L.A. 1999. EPA's Voluntary 33/50 Program: Impact on Toxic Releases and Economic Performance of Firms. *J. Evtl Econ & Man* 37(1):1-25.

Kirchner, C., Javier, A. M., Susha, A. S., Rogach, A. L., Kreft, O., Sukhorukov, G. B., Parak, W. J. 2005. Cytotoxicity of nanoparticle-loaded polymer capsules. *Talanta* 67(3): 486-491.

Kleiner, K., Hogan, J. 2003. How safe is nanotech. *New Scientist* 2388:15

Klöpffer, W., Curan, M.A., Frankl, P., Heijungs, R., Köhler, A. Olsen, S.I. 2006. Nanotechnology and Life Cycle Assessment: A Systems Approach to Nanotechnology and the Environment Synthesis of results obtained at a workshop in Washington, DC, October 2006. Washington, DC: Project on Emerging Nanotechnologies, Woodrow Wilson International Center for Scholars.

Kroto, H.W., Heath, J.R., O'Brien, S.C., Curl, R.F. and Smalley, R.E. 1985. C₆₀ : Buckminsterfullerene. *Nature* 318: 162–163.

Lai, H.-S., Chen, W.-J., Chiang, L.-Y. 2000. Free Radical Scavenging Activity of Fullerenol on the Ischemia-reperfusion Intestine in Dogs. *World J Surg* 24: 450-454.

Lam, C. W, James, J. T., McCluskey, R., Hunter, R. L. 2004. Pulmonary Toxicity of Single-Wall Carbon Nanotubes in Mice 7 and 90 Days After Intratracheal Instillation. *Toxicol Sci* 77: 126-134.

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(3):189-217.

Larson, D. R., Zipfel, W. R., Williams, R. M., Clark, S. W., Bruchez, M. P., Wise, F. W., Webb, W. W. 2003. Water-soluble quantum dots for multiphoton fluorescence imaging in vivo. *Science* 300(5624): 1434-1436.

Levi, N., Hantgan, R. R., Lively, M. O., Carroll, D. L., Prasad, G. 2006. C60-Fullerenes: Detection of intracellular photoluminescence and lack of cytotoxic effects. *J Nanobiotech* (14): 1-11.

Limbach, L. K., Wick, P., Manser, P., Grass, R. N., Bruinink, A., Stark, W. J. 2007. Exposure of engineered nanoparticles to human lung epithelial cells: Influence of chemical composition and catalytic activity on oxidative stress. *Environ Sci Technol* 41(11): 4158-4163.

Lin, A. M. Y., Chyi, B. Y., Wang, S. D., Yu, H.-H., Kanakamma, P. P., Luh, T.-Y., Chou, C. K., Ho, L. T. 1999. Carboxyfullerene Prevents Iron-Induced Oxidative Stress in Rat Brain. *J Neurochem* 72: 1634-1640.

Lin, W., Huang, Y. w., Zhou, X. D., Ma, Y. 2006. In vitro toxicity of silica nanoparticles in human lung cancer cells. *Toxicol Appl Pharm* 217(3): 252-259.

Linkov, I., Satterstrom, K.F., Steevens, J., Ferguson, E., Pleus, R.C., 2007. Multi-criteria decision analysis and environmental risk assessment for nanomaterials *Journal of Nanoparticle Research* 9:543 - 554.

Linkov, I., Satterstrom, F.K., Monica, J., Hansen, S.F., Davis, T. 2008a. Nano Risk Governance: Current Developments and Future Perspectives. *Risk Analysis* Submitted.

Linkov I., Satterstrom F.K., Corey, L.M. 2008b. Nanotoxicology and nanomedicine: making hard decisions. *Nanomed Nanotechnol Biol Med* 4: 167-171

Lockman, P. R., Koziara, J. M., Mumper, R. J., Allen, D. D. 2004. Nanoparticle surface charges alter blood-brain barrier integrity and permeability. *J Drug Target* 12: 635-641.

Lovern, S. B., Klaper, R. 2006. Daphnia Magna mortality when exposed to titanium dioxide and fullerene (C60) nanoparticles. *Environ Toxicol Chem* 25(4): 1132-1137.

Lovern, S. B., Strickler, J. R., Klaper, R. 2007. Behavioral and Physiological Changes in Daphnia magna when Exposed to Nanoparticle Suspensions (Titanium Dioxide, Nano-C60, and C60HxC70Hx). *Environ Sci Technol* 41(12):4465-4470.

Lovric, J., Bazzi, H. S., Cuie, Y., Fortin, G. R. A., Winnik, F. M., Maysinger, D. 2005. Differences in subcellular distribution and toxicity of green and red emitting CdTe quantum dots. *J Mol Med* 83(5): 377-385.

Lu L.H., Lee Y.T., Chen, H. W., Chiang, L. Y., Huang, H. C. 1998. The possible mechanisms of the antiproliferative effect of fulleranol, polyhydroxylated C₆₀, on vascular smooth muscle cells. *Brit J Pharmacol* 123(6): 1097-1102.

Luoma, S. N. 2008. *Silver Nanotechnologies and the Environment Old Problems or New Challenges?* PEN 15. Washington, DC: Project on Emerging Nanotechnologies, Woodrow Wilson International Center for Scholars.

Lux Research. 2004. *The Nanotech Report 2004*. New York: Lux Research.

Lyon, D. Y., Adams, L. K., Falkner, J. C., Alvaraz, P. J. J. 2006. Antibacterial Activity of Fullerene Water Suspensions: Effects of Preparation Method and Particle Size . *Environ Sci Technol* 40: 4360-4366.

Lyon, D. Y., Fortner, J. D., Sayes, C. M., Colvin, V. L., Hughes, J. B. 2005. Bacterial cell association and antimicrobial activity of a C₆₀ water suspension. *Environ Toxicol Chem* 24(11): 2757-2762.

Lyon, T.P. 2003. *Voluntary Versus Mandatory Approaches to Climate Change Mitigation*. Washington D.C.: Resources for the Future.

Macnee, W., Donaldson, K. 2008. Carbon Nanotubes Introduced Into The Abdominal Cavity Of mice Show Asbestoslike Pathogenicity In A Pilot Study. *Nat Nanotechnol* 3: 423 – 428.

Macoubrie, J. 2005. *Informed Public Perceptions of Nanotechnology and Trust in Government*. PEN 1. Washington, D.C., Woodrow Wilson International Center for Scholars Project for Emerging Nanotechnologies.

Maness, P. C., Smolinski, S., Blake, D. M., Huang, Z., Wolfrum, E. J., Jacoby, W. A. 1999. Bactericidal activity of photocatalytic TiO₂ reaction: Toward an understanding of its killing mechanism. *Appl Environ Microb* 65(9): 4094-4098.

Martin, F.J., Melnik, K., West, T., Shapiro, J., Cohen, M., Boiarski, A.A., Ferrari, M.. 2005. Acute toxicity of intravenously administered microfabricated silicon dioxide drug delivery particles in mice. *Drugs Res Develop* 6:71-81.

Mashino, T., Nishikawa, D., Usui, N., Yamori, T., Seki, M., Endo, T., Mochizuki, M. 2003. Antibacterial and Antiproliferative Activity of Cationic Fullerene Derivatives. *Bioorg Med Chem Lett* 13: 4395-4397.

- Mashino, T., Usui, N., Okuda, K., Hirota, T., Mochizuki, M. 2003. Respiratory Chain Inhibition by Fullerene Derivatives: Hydrogen Peroxide Production Caused by Fullerene Derivatives and a Respiratory Chain System. *Bioorgan Med Chem* 11: 1433-1438.
- Mayer, S., Stirling, A. 1999. Rethinking Risk: A Pilot Multicriteria Mapping of a Genetically Modified Crop in Agricultural Systems in the UK. Sussex: SPRU.
- Maynard, A. 2006. Nanotechnology: A Research Strategy for Addressing Risk. Washington, D.C.: Project on Emerging Nanotechnologies, Woodrow Wilson International Center for Scholars.
- Maynard, A.D., Aitken, R.J. 2007. Assessing exposure to airborne nanomaterials: Current abilities and future requirements. *Nanotoxicology* 1(1): 26-41.
- Maynard, A.D., Aitken, R.J., Butz, T., Colvin, V.L., Donaldson, K., Oberdörster, G., Philbert, M.A., Ryan, J., Seaton, A., Stone, V., Tinkle, S.S., Tran, L., Walker, N., Warheit, D.B. 2006. Safe Handling of Nanomaterials. *Nature* 444: 267-269.
- Maynard, A.D., Baron, P.A., Foley, M., Shvedova, A.A., Kisin, E.R., Castranova, V. 2004. Exposure To Carbon Nanotube Material: Aerosol Release During The Handling Of Unrefined Singlewalled Carbon Nanotube Material. *J Toxicol Env Heal A* 67: 87-107.
- Monica, J. C. 2008. ISO Publishes Nanotechnology Definition. Nanotechnology Law Report - September 2008. Washington, D.C.: PorterWright.
- Monteiro-Riviere, N. A., Nemanich, R. J., Inman, A. O., Wang, Y. Y. Y., Riviere, J. E. 2005. Multi-walled carbon nanotube interactions with human epidermal keratinocytes. *Toxicol Lett* 155(3): 377-384.
- Moore, M. N. 2006. Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? *Environ Int* 32:967-976.
- Mori, T., Takada, H., Ito, S., Matsubayashi, K., Miwa, N., Sawaguchi, T. 2006. Preclinical studies on safety of fullerene upon acute oral administration and evaluation for no mutagenesis. *Toxicology* 225(1): 48-54.

Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramirez, J. T., Yacaman, M. J. 2005. The bactericidal effect of silver nanoparticles. *Nanotech* 16(10): 2346-2353.

Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramirez, J. T., Yacaman, M. J. 2005. The bactericidal effect of silver nanoparticles. *Nanotech* 16(10): 2346-2353.

Mueller, N., Nowack, B. 2008. Exposure Modeling of Engineered Nanoparticles in the Environment. *Eviron Sci Technol* 42: 4447–4453.

Muller, J., Huaux, F., Moreau, N., Misson, P., Heilier, J. F., Delos, M., Arras, M., Fonseca, A., Nagy, J. B., Lison, D. 2005. Respiratory toxicity of multi-wall carbon nanotubes. *Toxicol Appl Pharm* 207(3): 221-231.

Muller, K., Skepper, J. N., Posfai, M., Trivedi, R., Howarth, S., Corot, C., Lancelot, E., Thompson, P. W., Brown, A. P., Gillard, J. H. 2007. Effect of ultrasmall superparamagnetic iron oxide nanoparticles (Ferumoxtran-10) on human monocyte-macrophages in vitro. *Biomaterials* 28(9): 1629-1642.

N&ET Working Group. 2007. Report on nanotechnology to the medical devices expert group findings and Recommendations. Available: http://ec.europa.eu/enterprise/medical_devices/net/entr-2007-net-wg-report-nano-final.pdf (Accessed December 5 2008).

Nakagawa, Y., Wakuri, S., Sakamoto, K., Tanaka, N. 1997. The photogenotoxicity of titanium dioxide particles. *Mutat Res-Gen Tox En* 394(1-3): 125-132.

NanoRoad SME: Roadmap Report Concerning the Use of Nanomaterials in the Medical & Health Sector. European Commission (2006). Available: http://www.nanoroad.net/download/roadmap_mh.pdf (Accessed December 5 2008).

Nanoscale Science Engineering and Technology Subcommittee, The Committee on Technology, National Science and Technology Committee. The National Nanotechnology Initiative Strategy Plan December 2004. Arlington, VA: National Nanotechnology Coordination Office.

National Nanotechnology Initiative. 2008. Strategy for Nanotechnology-Related Environmental, Health and Safety Research. Washington, D.C.: National Nanotechnology Initiative.

National Pollution Prevention And Toxics Advisory Committee. 2005. Overview Document On Nanoscale Materials. U.S. Environmental Protection Agency.

Nigavekar, S.S., Sung, L.Y., Llanes, M., El-Jawahri, A., Lawrence, T.S., Becker, C.W., Balogh, L., Khan, M.K. 2004. H-3 dendrimer nanoparticle organ/tumor distribution. *Pharmaceut Res* 21: 476-483.

Niidome, T., Yamagata, M., Okamoto, Y., Akiyama, Y., Takahashi, H., Kawano, T., Katayama, Y., Niidome, Y. 2006. PEG-modified gold nanorods with a stealth character for in vivo applications. *J Controlled Release* 114(3): 343-347.

NIOSH. 2006. Approaches to Safe Nanotechnology: An Information Exchange with NIOSH. Washington, D.C.: Department Of Health And Human Services Centers for Disease Control and Prevention National Institute for Occupational Safety and Health.

NMT Network. 2005. Industrial map of UK. Department of Trade and Industry , UK (1st ed. 2005).

Nordan, M.M., Holman, M.W., Bünger, M. 2006. Nanotech Commercialization Has Advanced, but Government Action to Address Risk Has Not. House Committee on Science. 21-9-2006.

Nowack, B., Bucheli, T.D. 2007. Occurrence, behavior and effects of nanoparticles in the environment. *Environ Pollut* 150: 5-22.

Oberdörster E. 2004. Manufactured Nanomaterials (fullerenes, C₆₀) Induce Oxidative Stress in Juvenile Largemouth Bass. *Environ Health Perspect* 112: 1058-1062

Oberdörster, G. 2000. Toxicology of ultrafine particles: In vivo studies. *Phil Trans. R Soc Lond A* 358: 2719-2740.

Oberdorster G, Oberdorster E, Oberdorster J. 2005a. Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect* 113: 823-839.

Oberdörster, 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. 2005b. Principles for characterizing the potential human health effects from exposure to nanomaterials: Elements of a screening strategy. *Partic Fibre Toxicol* 2:8

Oberdorster G, Oberdorster E, Oberdorster J. 2007. Concepts of Nanoparticle Dose Metric and Response Metric. *Environ Health Perspect* 115(6): A 290.

Oberdorster, E., Zhu, S. Q., Blickley, T. M., Clellan-Green, P., Haasch, M. L. 2006. Ecotoxicology of carbon-based engineered nanoparticles: Effects of fullerene (C-60) on aquatic organisms. *Carbon* 44(6): 1112-1120.

Oberdörster, G., Ferin, J., Gelein, R., Soderholm, S. C., Finkelstein, G. 1992. Role of the alveolar macrophage in lung injury: studies with ultrafine particles. *Environ Health Perspect* 97:193-199.

Oberdorster, G., Ferin, J., Lehnert, B. E. 1994. Correlation Between Particle-Size, In-Vivo Particle Persistence, and Lung Injury. *Environ Health Perspect* 1994a, 102, 173-179.

Oberdorster, G., Oberdorster, E., Oberdorster, J. 2005. Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect* 113:823-839.

Oberdörster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W., Cox, C. 2004. Translocation of inhaled ultrafine particles to the brain. *Inhal Toxicol* 16: 437-445.

Oberdörster, G., Stone, V., Donaldson, K. 2007. Toxicology of nanoparticles: A historical perspective. *Nanotoxicology* 1(1): 2-25.

Oberdorster, G. 1996. Significance of particle parameters in the evaluation of exposure-dose-response relationships of inhaled particles. *Particu Sci Technol* 14: 135–151.

OECD. 2007. Current Developments/Activities on the Safety of Manufactured Nanomaterials/Nanotechnologies Tour de Table at the 2nd Meeting of the Working Party on Manufactured Nanomaterials Berlin, Germany, 25-27 April 2007. ENV/JM/MONO(2007)16. Paris: Organisation for Economic Co-operation and Development.

Office of Pollution Prevention and Toxics. 1999. 33/50 Program The Final Record U.S. Environmental Protection Agency. Available: www.Epa.Gov/Opptintr/3350 (Accessed December 5 2008).

Osier, M., Oberdorster, G. 1997. Intratracheal inhalation vs intratracheal instillation: Differences in particle effects. *Fund Appl Toxicol* 40(2): 220-227.

Osier, M., Baggs, R.B., Oberdörster, G. 1997. Intratracheal Instillation versus Intratracheal Inhalation: Influence of Cytokines on Inflammatory Response. *Environ Health Perspect* 105(Suppl 5): 1265-1271.

Pal, S., Tak, Y. K., Song, J. M. 2007. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. *Appl Environ Microbiol* 73(6): 1712-1720.

Park, B., Donaldson, K., Duffin, R., Tran, L., Kelly, F., Mudway, I., Morin, J-P., Guest, R., Jenkinson, P., Samaras, Z., Giannouli, M., Kouridis, H., Martin, P. 2008. Hazard and Risk Assessment of a Nanoparticulate Cerium Oxide-Based Diesel Fuel Additive - A Case Study. *Inhal Toxicol* 20(6):547-566.

Pascual, P. 2004 Wrestling Environmental Decisions From an Uncertain World. *Environ Law Rep* 35: 10539-10549.

Pernodet, N., Fang, X. H., Sun, Y., Bakhtina, A., Ramakrishnan, A., Sokolov, J., Ulman, A., Rafailovich, M. 2006. Adverse effects of citrate/gold nanoparticles on human dermal fibroblasts. *Small* 2(6): 766-773.

Pisanic II, T. R., Blackwell, J. D., Shubayev, V. I., Finones, R. R., Jin, S. 2007. Nanotoxicity of iron oxide nanoparticle internalization in growing neurons. *Biomaterials* 28(16): 2572-2581.

Poland, C. A., Duffin, R., Kinloch, I., Maynard, A., Wallace, W. A. H., Seaton, A., Stone, V., Brown, S., Macnee, W., Donaldson, K.. 2008. Carbon Nanotubes Introduced Into The Abdominal Cavity Of mice Show Asbestoslike Pathogenicity In A Pilot Study. *Nat Nanotechnol* 3, 423 – 428.

Porter, A. E., Muller, K., Skepper, J., Midgley, P., Welland, M. 2006. Uptake of C60 by human monocyte macrophages, its localization and implications for toxicity: Studied by high resolution electron microscopy and electron tomography. *Acta Biomater* 2(4): 409-419.

Powers, K.W., Brown, S.C., Krishna, V.B., Wasdo, S.C., Moudgil, B.M. Production and application. Environmental Project No. 1206 2007. 2007. Copenhagen: Danish Ministry of the Environment Danish Environmental Protection Agency.

Project of Emerging Nanotechnologies. 2007. A Nanotechnology Consumer Product Inventory. Project of Emerging Nanotechnologies, Woodrow Wilson International Center for Scholars. Available: <http://www.nanotechproject.org/44> (Accessed May 26 2007).

Rahman, Q., Lohani, M., Dopp, E., Pemsel, H., Jonas, L., Weiss, D. G., Schiffmann, D. 2002. Evidence that ultrafine titanium dioxide induces micronuclei and apoptosis in Syrian hamster embryo fibroblasts. *Environ Health Perspect* 110(8): 797-800.

RCEP. 2008. Twenty-seventh Report Novel Materials in the Environment: The case of nanotechnology Royal Commission on Environmental Pollution. Norwich: The Stationery Office

Renn, O., Roco, M. C. 2006. Nanotechnology and the need for risk governance. *J Nanopart Res* 8: 153–191

Rincon, A. G., Pulgarin, C. 2003. Photocatalytical inactivation of *E. coli*: effect of (continuous-intermittent) light intensity and of (suspended-fixed) TiO₂ concentration. *Appl Catal B-Environ* 44(3): 263-284.

Roberts, A. P., Mount, A. S., Seda, B., Souther, J., Qiao, R., Lin, S., Ke, P. C., Rao, A. M., Klaine, S. J. 2007. In vivo Biomodification of Lipid-Coated Carbon Nanotubes by *Daphnia magna*. *Environ Sci Technol* 41(8): 3025-3029.

Roberts, S.M. 2006. Research strategies for safety evaluation of nanomaterials. Part VI. Characterization of nanoscale particles for toxicological evaluation. *Toxicol Sci* 90: 296-303.

Roco, M. C., Bainbridge, W.S. 2005. Societal implications of nanoscience and nanotechnology: Maximizing human benefit. *J Nanopart Res* 7(1):1-13.

Rouse, J. G., Yang, J., Barron, A. R., Monteiro-Riviere, N. A. 2006. Fullerene-based amino acid nanoparticle interactions with human epidermal keratinocytes. *Toxicol in Vitro* 20(8): 1313-1320.

Royal Society & the Royal Academy of Engineering. 2004. Nanoscience and Nanotechnologies: Opportunities and Uncertainties. London: Royal Society.

Rubins, J.B. 2003. Alveolar macrophages: wielding the double-edged sword of inflammation. *Am J Respir Crit Care Med* 167(2):103-104.

Ruckelshaus, W. D. 1985. Risk, science and democracy. *Issues in science and technology*. 19-38.

Ryan, J. J., Bateman, H. R., Stover, A., Gomez, G., Norton, S. K., Zhao, W., Schwartz, L. B., Lenk, R., Kepley, C. L. 2007. Fullerene Nanomaterials Inhibit the Allergic Response. *J Immunol* 179(1): 665-672.

Sayes, C. M., Fortner, J. D., Guo, W., Lyon, D., Boyd, A. M., Ausman, K. D., Tao, Y. J., Sitharaman, B., Wilson, L. J., Hughes, J. B., West, J. L., Colvin, V. L. 2004. The differential cytotoxicity of water-soluble fullerenes. *Nano Lett* 4(10): 1881-1887.

Sayes, C. M., Gobin, A.M., Ausman, K. D., Mendez, J., West, J. L., Colvin, V. L. 2005. Nano-C-60 cytotoxicity is due to lipid peroxidation. *Biomaterials* 26: 7587-7595.

Sayes, C. M., Liang, F., Hudson, J. L., Mendez, J., Guo, W. H., Beach, J. M., Moore, V. C., Doyle, C. D., West, J. L., Billups, W. E., Ausman, K. D., Colvin, V. L. 2006a. Functionalization density dependence of single-walled carbon nanotubes cytotoxicity in vitro. *Toxicol Lett* 161(2): 135-142.

Sayes, C. M., Wahi, R., Kurian, P. A., Liu, Y., West, J. L., Ausman, K. D., Warheit, D. B., Colvin, V. L. 2006b. Correlating Nanoscale Titania Structure with Toxicity: A Cytotoxicity and Inflammatory Response Study with Human Dermal Fibroblasts and Human Lung Epithelial Cells. *Toxicol Sci* 92(1):174-185.

Sayes, C. M., Marchione, A. A., Reed, K. L., Warheit, D. B. 2007a. Comparative Pulmonary Toxicity Assessments of C60 Water Suspensions in Rats: Few Differences in Fullerene Toxicity in Vivo in Contrast to in Vitro Profiles. *Nano Lett* 7(8): 2399-2406.

Sayes, C. M., Reed, K. L., Warheit, D. B. 2007b. Assessing Toxicity of Fine and Nanoparticles: Comparing In Vitro Measurements to In Vivo Pulmonary Toxicity Profiles. *Toxicol Sci* 97(1): 163-180.

SCENIHR. 2006. Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR modified Opinion (after public consultation) on The appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies. Brussels: European Commission Health & Consumer Protection Directorate-General.

SCENIHR. 2007. Scientific Committee for Emerging and Newly-Identified Health Risks (SCENIHR) The appropriateness of the risk assessment methodology in accordance with the Technical Guidance Documents for new and existing substances for assessing the risks of nanomaterials. Brussels: European Commission Health & Consumer Protection Directorate-General.

Schmidt, K. 2007. Nanofrontiers Visions for the future of Nanotechnology. PEN 6. Washington, D.C.: Project for Emerging Nanotechnologies, Woodrow Wilson International Center for Scholars.

Scientific Committee on Consumer Products. 2007. Opinion on the Safety of Nanomaterials in Cosmetic Products Adopted by the SCCP after the public consultation on the 14th plenary of 18 December 2007 SCCP/1147/07. Brussels: European Commission.

Seeger, E.M., Baun, A., Kästner, M., Trapp, S. 2008. Insignificant acute toxicity of TiO₂ nanoparticles to willow trees. *J Soils Sed* DOI 10.1007/s11368-008-0034-0).

Serita, F., Kyono, H., Seki, Y. 1999. Pulmonary clearance and lesions in rats after a single inhalation of ultrafine metallic nickel at dose levels comparable to the threshold limit value. *Ind Health* 37(4): 353-363.

Shvedova, A. A., Kisin, E. R., Mercer, R., Murray, A. R., Johnson, V. J., Potapovich, A. I., Tyurina, Y. Y., Gorelik, O., Arepalli, S., Schwegler-Berry, D., Hubbs, A. F., Antonini, J., Evans, D. E., Ku, B. K., Ramsey, D., Maynard, A., Kagan, V. E., Castranova, V., Baron, P. 2005. Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice. *Am J Physiol Lung Cell Mol Physiol* 289: 698–708

Shvedova, A. A., Castranova, V., Kisin, E. R., Schwegler-Berry, D., Murray, A. R., Gandelsman, V. Z., Maynard, A., Baron, P. 2003. Exposure to carbon nanotube material: Assessment of nanotube cytotoxicity using human keratinocyte cells. *J Toxicol Env Heal A* 66(20): 1909-1926.

Smith, C. J., Shaw, B. J., Handy, R. D. 2007. Toxicity of Single Walled Carbon Nanotubes on Rainbow Trout, (*Oncorhynchus mykiss*): Respiratory Toxicity, Organ Pathologies, and Other Physiological Effects. *Aquat Toxicol* 82(2): 94-109.

Sondi, I., Salopek-Sondi, B. 2004. Silver nanoparticles as antimicrobial agent: a case study on E-coli as a model for Gram-negative bacteria. *J Colloid Interf Sci* 275(1): 177-182.

Stoeger, T., Schmid, O., Takenaka, S., Schulz, H. 2007. Inflammatory Response to TiO₂ and Carbonaceous Particles Scales Best with BET Surface Area. *Environ Health Perspect* 115(6): A290–A291.

Stoeger, T., Reinhard, C., Takenaka, S., Schroepfel, A., Karg, E., Ritter, B., Heyder, J., Schultz, H. 2006. Instillation of six different ultrafine carbon particles indicates surface area threshold dose for acute lung inflammation in mice. *Environ Health Perspect* 114(3): 328-333.

Tang, Y. J., Ashcroft, J. M., Chen, D., Min, G., Kim, C. H., Murkhejee, B., Larabell, C., Keasling, J. D., Chen, F. F. 2007. Charge-Associated Effects of Fullerene Derivatives on Microbial Structural Integrity and Central Metabolism. *Nano Lett* 7 (3): 754-760.

Technology Transfer Center. 2007. Government Funding, Companies And Applications In Nanotechnology Worldwide 2007. Technology Transfer Center. Available: <http://www.Euronanotrade.Com/News/nanocommerc.doc>. (Accessed November 17 2007).

Thayer, A.M. 2007. Carbon Nanotubes by the Metric Ton. *Chemical & Engineering News*, November 12, 2007 p. 29-35.

Tegos, G. P., Demidova, T. N., rcila-Lopez, D., Lee, H., Wharton, T., Gali, H., Hamblin, M. R. 2005. Cationic Fullerenes Are Effective and Selective Antimicrobial Photosensitizers. *Chem Biol* 12(10): 1127-1135.

Templeton, R. C., Ferguson, P. L., Washburn, K. M., Scrivens, W. A., Chandler, G. T. 2006. Life-cycle effects of single-walled carbon nanotubes (swnts) on an estuarine meiobenthic copepod. *Environ. Sci. Technol.* 40 (23): 7387-7393.

Thill, A., Zeyons, O., Spalla, O., Chauvat, F., Rose, J., Auffan, M., Flank, A. M. 2006. Cytotoxicity of ceo₂ nanoparticles for escherichia coli physico-chemical insight of the cytotoxicity mechanism. *Environ Sci Technol* 40(19): 6151-6156.

Tønning, K., Poulsen, M. 2007. Nanotechnology in the Danish industry - survey on production and application. Environmental Project No. 1206 2007. 2007. Copenhagen: Danish Ministry of the Environment Danish Environmental Protection Agency.

Tsao, N., Luh, T. Y., Chou, C. K., Chang, T. Y., Wu, J. J., Liu, C. C., Lei, H. Y. 2002. In vitro action of carboxyfullerene. *J Antimicrob Chemother* 49(4): 641-649.

Tsuchiya, T., Yamakoshi, Y. N., Miyata, N. A Novel Promoting Action of Fullerene C60 on the Chondrogenesis in Rat Embryonic Limb Bud Cell-Culture System. *Biochemical and Biophysical Research Communications* 1995, 206 (3), 885-894.

Tsuji, J.S., Maynard, A.D., Howard, P.C., James, J.T., Lam, C.W., Warheit, D.B., Santamaria, A.B. 2006. Research strategies for safety evaluation of nanomaterials, Part IV: Risk assessment of nanoparticles. *Toxicol Sci* 89:42-50.

Uchino, T., Tokunaga, H., Ando, M., Utsumi, H. 2002. Quantitative determination of OH radical generation and its cytotoxicity induced by TiO₂-UVA treatment. *Toxicol in Vitro* 16(5): 629-635.

U.S. EPA. 2007. U.S. Environmental Protection Agency Nanotechnology White Paper. EPA 100/B-07/001. Washington, D.C.: Science Policy Council U.S. Environmental Protection Agency.

U.S. EPA. 2007. Nanotechnology under the Toxic Substances Control Act. Available: <http://www.epa.gov/oppt/nano/> (Accessed April 16 2007).

Sayes, C.M., Fortner, J.D., Guo, W., Lyon, D., Boyd, A.M., Ausman, K.D., Tao, Y.J., Sitharaman, B., Wilson, L.J. Hughes, J.B., West, J.L., Colvin, V.L. 2004. The differential cytotoxicity of water-soluble fullerenes. *Nano Lett* 4:1881-1887.

Vileno, B., Lekka, M., Sienkiewicz, A., Jeney, S., Stoessel, G., Lekki, J., Forro, L., Stachura, Z. 2007. Stiffness alterations of single cells induced by uv in the presence of nanotio₂. *Environ Sci Technol* 41(14): 5149-5153.

Wagner, A. J., Bleckmann, C. A., Murdock, R. C., Schrand, A. M., Schlager, J. J., Hussain, S. M. 2007. Cellular interaction of different forms of aluminum nanoparticles in rat alveolar macrophages. *J Phys Chem B* 111(25): 7353-7359.

Wang, B., Feng, W. Y., Wang, T. C., Jia, G., Wang, M., Shi, J. W., Zhang, F., Zhao, Y. L., Chai, Z. F. 2006. Acute toxicity of nano- and micro-scale zinc powder in healthy adult mice. *Toxicol Lett* 161(2): 115-123.

Wang, J., Zhou, G., Chen, C., Yu, H., Wang, T., Ma, Y., Jia, G., Gao, Y., Li, B., Sun, J., Li, Y., Jiao, F., Zhao, Y., Chai, Z. 2007a. Acute toxicity and biodistribution of different

sized titanium dioxide particles in mice after oral administration. *Toxicol Lett* 168(2): 176-185.

Wang, J. J., Sanderson, B. J. S., Wang, H. 2007b. Cyto- and genotoxicity of ultrafine TiO₂ particles in cultured human lymphoblastoid cells. *Mutat Res-Gen Tox En* 628(2): 99-106.

Warheit, D. B., Laurence, B. R., Reed, K. L., Roach, D. H., Reynolds, G. A. M., Webb, T. R. 2004. Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats. *Toxicol Sci* 77(1): 117-125.

Warheit, D. B., Webb, T. R., Sayes, C. M., Colvin, V. L., Reed, K. L. 2006. Pulmonary instillation studies with nanoscale TiO₂ rods and dots in rats: toxicity is not dependent upon particle size and surface area. *Toxicol Sci* 91(1): 227-236.

Warheit, D. B., Webb, T. R., Colvin, V. L., Reed, K. L., Sayes, C. R. 2007a. Pulmonary bioassay studies with nanoscale and fine-quartz particles in rats: Toxicity is not dependent upon particle size but on surface characteristics. *Toxicol Sci* 95 (1): 270-280.

Warheit, D. B., Webb, T. R., Reed, K. L., Frerichs, S., and Sayes, C. M. 2007b. Pulmonary toxicity study in rats with three forms of ultrafine-TiO₂ particles: Differential responses related to surface properties. *Toxicology* 230: 90–104.

Warheit, D.B. 2008. How meaningful are the results of nanotoxicity studies in the absence of adequate material characterization? *Toxicol Sci* 101(2): 183–185.

Weinberg, A. 1985. Science and its limits: the regulator's dilemma. *Issues Sci Technol* 59-72.

Weiss, R. 2005. Nanotechnology regulation needed, critics say. *Washington Post* 5 December A08. Available: <http://www.washingtonpost.com/wp-dyn/content/article/2005/12/04/AR2005120400729.html> (Accessed December 3 2008).

Wittmaack, K. 2007a. In search of the most relevant parameter for quantifying lung inflammatory response to nanoparticle exposure: Particle number, surface area, or what? *Environ Health Perspect* 115:187–194.

Wittmaack, K. 2007b. Dose and Response Metrics in Nanotoxicology: Wittmaack Responds to Oberdoerster *et al.* and Stoeger *et al.* *Environ Health Perspect* 115(6): A290-291.

Xiao, L., Takada, H., Maeda, K., Haramoto, M., Miwa, N. 2005. Antioxidant effects of water-soluble fullerene derivatives against ultraviolet ray or peroxy lipid through their action of scavenging the reactive oxygen species in human skin keratinocytes. *Biomed Pharmacother* 59(7): 351-358.

Yamago, S., Tokuyama, H., Nakamura, E., Kikuchi, K., Kananishi, S., Sueki, K., Nakahara, H., Enomoto, S., Ambe, F. 2005. In-Vivo Biological Behavior of A Water-Miscible Fullerene - C-14 Labeling, Absorption, Distribution, Excretion and Acute Toxicity. *Chem Biol* 2(6): 385-389.

Yamawaki, H., Iwai, N. 2006. Cytotoxicity of water-soluble fullerene in vascular endothelial cells. *Am J Cell Physiol* 290(6): C1495-C1502.

Yang, L., Watts, D. J. 2005. Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. *Toxicol Lett* 158 (2): 122-132.

Yang, X. L., Fan, C. H., Zhu, H. S. 2002. Photo-induced cytotoxicity of malonic acid [C-60]fullerene derivatives and its mechanism. *Toxicol in Vitro* 16 (1): 41-46.

Yu, W. W., Chang, E., Sayes, C. M., Drezek, R., Colvin, V. L. 2006. Aqueous dispersion of monodisperse magnetic iron oxide nanocrystals through phase transfer. *Nanotechnology* 17(17): 4483-4487.

Zhang, X., Sun, H., Zhang, Z., Niu, Q., Chen, Y., Crittenden, J.C. 2007. Enhanced bioaccumulation of cadmium in carp in the presence of titanium dioxide nanoparticles. *Chemosphere* 67: 160-166 (2007).

Zhang, E. S., Wang, H. L., Yan, X. X., Zhang, L. D. 2005. Comparison of short-term toxicity between Nano-Se and selenite in mice. *Life Sci* 76(10): 1099-1109.

Zhang, J. S., Gao, X. Y., Zhang, L. D., Bao, Y. P. 2001. Biological effects of a nano red elemental selenium. *Biofactors* 15(1): 27-38.

Zhang, J. S., Wang, H. L., Bao, Y. P., Zhang, L. 2004. Nano red elemental selenium has no size effect in the induction of seleno-enzymes in both cultured cells and mice. *Life Sci.* 75(2): 237-244.

Zhang, X., Sun, H., Zhang, Z., Niu, Q., Chen, Y. and Crittenden, J.C. 2007. Enhanced bioaccumulation of cadmium in carp in the presence of titanium dioxide nanoparticles. *Chemosphere* 67: 160-166.

Zhang, Y. B., Chen, W., Zhang, J., Liu, J., Chen, G. P., Pope, C. In vitro and in vivo toxicity of CdTe nanoparticles. *J Nanosci Nanotech* 7(2): 497-503.

Zhou, Y. M., Zhong, C. Y., Kennedy, I. M., Pinkerton, K. E. 2003. Pulmonary responses of acute exposure to ultrafine iron particles in healthy adult rats. *Environ Toxicol* 18(4): 227-235.

Zhu, R. R., Wang, S. L., Chen, X. P., Sun, X. Y., Zang, R., Yao, S. D. 2006. Selective apoptosis inducing effect of nano-TiO₂ on CHO cells. *Acta Chim Sinica* 64 (21): 2161-2164.

Zhu, S. Q., Oberdorster, E., Haasch, M. L. 2006. Toxicity of an engineered nanoparticle (fullerene, C-60) in two aquatic species, *Daphnia* and fathead minnow. *Mar Environ Res* 62: S5-S9.

Appendix

1. **Hansen, S.F.**, Larsen, B.H., Olsen, S.I., Baun, A. 2007. Categorization framework to aid Hazard Identification of Nanomaterials. *Nanotoxicology* 1: 243-250.
2. **Hansen, S.F.**, Michelson, E., Kamper, A., Borling, P., Stuer-Lauridsen, F. & Baun, A. 2008, Categorization Framework to Aid Exposure Assessment of Nanomaterials in Consumer Products. *Ecotoxicology* 17(5): 438-447.
3. Franco, A., **Hansen, S.F.**, Olsen, S.I., Butti, L. 2007. Limits and Prospects of the “Incremental Approach” and the European Legislation on the Management of Risks related to Nanomaterials. *Regulatory Toxicology and Pharmacology* 48: 171-183.
4. Baun, A., **Hansen, S.F.** 2008. Environmental Challenges for Nanomedicine. *Nanomedicine* 2(5): 605-608.
5. **Hansen, S.F.**, Tickner, J.A. 2007. The Challenges of Adopting Voluntary Health, Safety and Environment Measures for Manufactured Nanomaterials. Lessons from the past for more effective adoption in the future. *Nanotechnology Law & Business* 4(3): 341-359.
6. **Hansen, S.F.**, Maynard, A., Baun, A., Tickner, J.A. 2008. Late Lessons from Early Warnings for Nanotechnology. *Nature Nanotechnology* 3(8): 444-447.

Please note that the papers are not included in this web version but can be obtained from the library at Department of Environmental Engineering, Technical University of Denmark, Miljoevej, building 115, DK-2800 Kgs. Lyngby (library@env.dtu.dk).

The Department of Environmental Engineering (DTU Environment) conducts science-based engineering research within four themes: Water Resource Engineering, Urban Water Engineering, Residual Resource Engineering and Environmental Chemistry & Microbiology. Each theme hosts two to five research groups.

The department dates back to 1865, when Ludvig August Colding, the founder of the department, gave the first lecture on sanitary engineering as response to the cholera epidemics in Copenhagen in the late 1800s.

DTU Environment
Department of Environmental Engineering
Technical University of Denmark

Miljøvej, building 113
DK-2800 Kgs. Lyngby

Phone: +45 4525 1600
Fax: +45 4593 2850
e-mail: reception@env.dtu.dk
www.env.dtu.dk

ISBN 978-87-91855-58-0