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Nanotechnology - Toxicological Issues and Environmental Safety

Edited by
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Nanotechnology – Toxicological Issues and Environmental Safety

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Series C: Environmental Security

Nanotechnology – Toxicological Issues and Environmental Safety

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PREFACE

The North Atlantic Treaty Organization (NATO), through the NATO "Security Through Science Program," sponsored an Advanced Research Workshop (ARW) entitled "Nanotechnology – Toxicological Issues and Environmental Security" that was held August 12–17, 2006 in Varna, Bulgaria.

The purpose of the workshop was to bring to focus and discuss the toxicological, ecological, and environmental safety issues surrounding the development, manufacture and use of nanomaterials. This represented the first international workshop organized specifically to share concerns and discussions on these issues between scientists from NATO and Partner countries. Scientists, representing the fields of toxicology, risk assessment, molecular biology physics, nanotechnology, ecology, epidemiology, medicine, public health, scientific ethics, and environmental protection from Belgium, Bulgaria, Czech Republic, Hungary, Moldova, Republic of Macedonia, Romania, Russian Federation, and the United States participated. Their main goal was to exchange experience in nanotoxicology and risk assessment, identify the most important gaps in knowledge, and draw directions for future research and collaborations in the field of nanotechnology regarding safe application and development.

The ARW was opened with two introductory lectures that summarized the global directions and issues in nanotechnologies, as well as the status and perspectives of nanotechnologies in the Partner countries. This was followed by sessions on nanomaterials/nanoparticles – toxicological issues; risk assessment, and control measures; public participation and educational/ethical issues and lastly; institutional mechanisms and status reports from various countries. There were two spirited round table discussions. The first dealt with toxicological issues of nanomaterials and nanoparticles and the second with risk assessment and control measures. Some of the questions addressed in the discussions included the following: How do we determine/select which nanomaterials should be tested for toxicity (prevalence, potential use, properties)? What are the minimum physiochemical properties that should be established before animals or *in vitro* tests are undertaken? What types of equipments are required for this characterization (and their availability)? Are there any opportunities to employ *in vitro* screening approach or computational toxicology? How important and how feasible is it to do toxicokinetics/distribution studies? What are the main sources and routes of human exposure (occupational, environmental)? What exposure metrics are most predictive of biological effect (e.g., mass, number, surface area)? Are the current environmental fate and transport models applicable to nanomaterials? What methodologies should be used for detection and characterization of occupational and environmental exposures? Are the

current personal - protection equipment, including respirators, adequate for all nanomaterials? What are the major gaps in knowledge needed for risk assessment of nanomaterials? Do we have adequate guidelines for working safely with nanomaterials? Do we have enough information to initiate monitoring of health effects of nanomaterials in workers? What institutional mechanisms and approaches for risk management should be developed – international and/or specific dependent on the country infrastructure and economy? These intensive and provocative discussions held during the workshop are summarized in the section entitled "Conclusions and Recommendations." A shared hope of the workshop was that it will serve as a stepping-stone for future collaborations between countries in fostering the safe use of nanotechnology.

The ARW was funded by a NATO award provided by the Assistant Secretary General for Public Diplomacy upon consideration by the Advisory Panel on Chemistry/Biology/Physics and the Program Director Dr. F. Pedrazzini. The co-directors of the workshop were P. Simeonova (United States) and N. Opopol (Moldova). Other members of the organizing committee were F. Kaloyanova (Bulgaria) and D. Solodoukhina (Russian Federation). We appreciate the financial support and the organization which made the workshop possible.

We should like to express our particular thanks to A. Maynard and E. Kuempel (United States) for their help in organizing the discussion round tables and preparing the Conclusions and Recommendations of the workshop.

Finally we gratefully acknowledge the help of Springer and the publishing editor Annelies Kersbergen with this book.

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CONCLUSIONS AND RECOMMENDATIONS

NATO Advanced Research Workshop (ARW) "Nanotechnology – Toxicological Issues and Environmental Security," August 12–17, 2006, Varna, Bulgaria

Nanotechnology is one of the fastest growing technological fields of the 21st century. However, the success of the emerging nanotechnology applications will depend on dynamic development of nanomaterial toxicology, risk and exposure assessment. The objectives of the NATO ARW were to provide an exchange of experience in nanotoxicology and risk assessment between scientists from NATO and Partner countries, to identify the most important gaps in knowledge, and to identify directions for future research which will ensure safe application and development of nanotechnology.

The NATO ARW concluded that many NATO and Partner countries are involved in development of nanotechnologies. These emerging technologies, although only in the research stage in some countries, will impact numerous industries, including daily consumer products, health care, energy, and transportation. Little is known about the potential adverse health and ecological effects of exposure to engineered nanomaterials, the main components of many nanotechnologies. Concerns are coming from the initial toxicological studies as well as the research and epidemiological reports on ultrafine particle toxicity. The most attractive properties of nanomaterials for medical and technological applications, including their small size, large surface area, and high reactivity, may also lead to new and unusual toxicity. Both country-specific and global issues were identified at the workshop and detailed recommendations were made related to nanomaterial characterization, toxicity tests, exposure and risk assessment, development of protective and prevention stratagies, risk communications and managements in nanotechnologies. Concerns about nanomaterials' potential toxicity and impact on human health and the environment must be addressed while the field is still developing and exposure is limited. Corresponding political measures will provide equality of nanotechnology opportunities and sustainable development of NATO and Partner countries.

General Recommendations

1. *Potential risk:* Evaluation of potential risk must be an integral part of nanotechnology development in all countries.
2. *Product development:* Nanotechnology product development cycles should incorporate an evaluation of potential risk and risk reduction from the earliest stages.

3. *Strategic research:* Organizations investing in nanotechnology research should invest in strategic research to evaluate potential health and environmental impact, and to develop effective risk management and risk-reduction strategies. Governments need to fund basic and general research, while enabling industry to support relevant product and material-specific research. Mechanisms are needed to ensure the transparent release of information relevant to understanding and managing potential risk.
4. *Information exchange:* Countries investing in nanotechnology should partner to share information and resources when researching potential risk and developing risk-management policies. Information sharing is particularly encouraged between developed and developing economies. Regional networks should be initiated to share information, coordinate research, and establish research infrastructures.
5. *International harmonization:* International agreement is needed on strategic risk research needs and aims. International organizations, such as NATO should take a lead in ensuring a global response to potential nanotechnology risk management through international partnering, coordination, and information sharing.
6. *Multidisciplinary research:* Collaboration between diverse scientific disciplines should be encouraged and supported in order to develop effective risk assessment and management methods for nanotechnologies.
7. *Risk communication:* There must be dialogue (between government, industry, academics, nongovernmental organizations and the public) on the benefits and risks of nanotechnology based on relevant and high-quality science. Nanotechnology risk-research programs and publications should be subject to high standards of scientific peer review to ensure a high quality of published studies.
8. *Continuing education and training:* International issue-specific workshops should be held to support coordinated nanotechnology risk research and policy. Education and training, including participation of NATO and non-NATO country experts, is needed to ensure the safe handling and use of nanomaterials.

Recommendations for Research Needs

Characterization of nanomaterials

- Methods and tools should be developed to identify and characterize engineered nanomaterials in biological matrices (e.g., exploring the use of interactions between nanostructures and electromagnetic radiation could lead to new methodologies).
- Nano-specific tools for characterizing the physical and chemical properties of nanomaterials in risk research should be developed, through

collaborations and partnerships with researchers characterizing nanomaterial functionality and applicability. Centers of excellence should be developed that provide access to analytical tools.

- Research to define and characterize biologically active surface area of engineered nanomaterials should be a high priority.
- Novel methods of evaluating potential impact *in situ* should be developed, including the use of biomonitoring and the development of instruments that combine measurements of exposure with an analysis of potential hazard (such as reactive oxygen species, ROS, production). These should complement more conventional exposure-evaluation methods.
- Terminology and nomenclature standards should be developed for nano technologies and nanomaterials that are specific to addressing potential impact.

Exposure

- Universal personal aerosols samplers should be developed that measure particle mass, number, and surface-area concentration simultaneously.
- International guidance should be developed on the effective exposure control of engineered nanomaterials.

Hazard/Toxicity

- Well-characterized stable benchmark and reference materials should be developed and used for toxicology studies. The applicability of these materials should be assessed regularly against the properties and characteristics of newly developed nanomaterials.
- Rapid cellular assays should be agreed upon and used for screening and preliminary hazard ranking of engineered nanomaterials.
- Nanomaterials must be appropriately characterized in toxicity tests. International guidelines on minimum physical and chemical characterization requirements and toxicity screening tests for engineered nanomaterials should be developed, agreed upon, and applied (e.g., as criteria for peer-reviewed publication).
- Priorities for toxicity testing should include materials that are close to commercial use, or are already being used in substantial quantities.
- The relevance of all significant exposure routes should be investigated, including the main routes of oral, inhalation and dermal exposure.
- While *in vivo* tests will remain essential, alternatives should be developed that minimize reliance on animal testing for new engineered nanomaterials.

- Information should be developed on potentially confounding influences in toxicity studies on engineered nanomaterials (e.g., the role of photoactivity and differential adsorption of proteins).

Risk Assessment

- Research into assessing and managing the potential impacts of nanotechnologies in the workplace is a high priority, including measuring worker exposure, controlling nanomaterials release, and safe disposal of nanomaterials.
- Life-cycle analysis methodologies should be developed for evaluating the potential impact of engineered nanomaterials and products on human health and the environment, from production to disposal.
- The kinetics and dynamics of nanomaterials in the body and the environment should be studied (including material disposition, dispersion, transformation and accumulation).
- Data are needed on human exposure, biomonitoring, and health outcomes that might be related to exposure.

Recommendations for Risk Communication and Management

Risk Communication

- Education and training is needed for researchers, manufacturers, and users of nanomaterials regarding the safe development and use of nanomaterials.
- Open access to nanotechnology risk-relevant information within industries is needed, including toxicity data, exposure data, and best available working practices. Centralized web-based portals should be established, providing access to global resources for assessing and managing the potential risks of nanotechnologies. These should include international databases on nanomaterial risk, including published data, research, products and risk assessment and management methods.
- Clear and transparent communication with consumers is needed on the potential benefits and risks of products developed using or containing nanomaterials.
- Products that contain nanomaterials should clearly state on the ingredients list which components are present as nanomaterials.

Risk Management

- Existing regulations should be evaluated for their applicability to engineered nanomaterials, and where necessary new regulations should be developed.

- Chemical regulations should be extended and enforced so that MSDS for engineered nanomaterials contain accurate and relevant information on potential risks of engineered nanomaterials, and acknowledge where information is not currently available.
- International guidance should be developed and shared on the best available practices for working with engineered nanomaterials. Guidance should be categorized by process and use.
- Criteria should be developed for when and how medical screening is conducted when exposure to engineered nanomaterials potentially occurs.

The recommendations reflect the perspectives of meeting participants, and provide a valuable resource for developing further international collaborations and actions to ensure the potential risks of emerging nanotechnologies are assessed and managed appropriately.

Note: These are the individual views of the participants, and do not necessarily reflect the views of the countries and organizations represented at the meeting.

NANOTECHNOLOGIES: OVERVIEW AND ISSUES

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Abstract: Nanotechnology – the manipulation of matter at near-atomic scales to produce new materials and products – is a reality now, and our ability to produce evermore sophisticated materials, processes, and products by engineering at the nanoscale will only increase over the coming years. Yet our understanding of the potential health, safety, and environmental impacts of these emerging technologies is rudimentary at best. Current knowledge is sufficient to indicate that some nanotechnologies will present new risks. What we still lack is information on how to assess and manage these risks. The challenges to the scientific community are significant: Which nanotechnologies present a significant hazard? What are those hazards and how do they relate to risks to health, safety, and the environment? How can risks be identified and controlled effectively? These and similar questions will require the risk research community to devise new strategies, new thinking, and new funding if answers are to be found. Above all, new partnerships will be needed to address potential risks – between researchers, agencies, stakeholders, and governments. However, by finding ways to work together effectively within a strategic framework, there is every chance that beneficial, sustainable, and safe nanotechnologies will emerge.

Keywords: nanotechnology, risk, engineered nanomaterials, exposure, toxicity, control

1. Introduction

In 1959, the celebrated physicist and Nobel Laureate Richard Feynman challenged the scientific community to think small – not to restrict its vision,

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but to imagine what could be achieved if we were able to manipulate matter at the nanoscale (Feynman 1959). To many, this was the beginning of the dual concepts of nanoscience and nanotechnology. Now, more than 40 years later, nanotechnology is a multi-billion dollar research field that crosses many areas of research and development.

The rapid rise of nanotechnology – spurred on by an increasing ability to probe and control matter at the nanoscale – has led some to call it the next industrial revolution. Certainly, it has stimulated new research and innovative thinking throughout the scientific world. But it has also raised concerns. Within the scientific community, there are those who question the validity of this "new science" – citing centuries-old technologies based on nanostructured materials. Others have expressed concerns over the safety and societal impact of new, emerging technologies. Will these technologies lead to innovations that challenge the very basis of modern societies – such as artificially enhanced intelligence and longevity? And will the unique properties and behaviors of nanotechnology-enabled products lead to new risks to health and the environment?

In previous industrial revolutions, technologies were developed ahead of most thoughts about the possible consequences – and many would say we are still paying the price of a lack of forethought. However, the scientific and social climate within which nanotechnology is being developed is very different. High-profile technologies such as nuclear power and genetically modified organisms have demonstrated the need to balance risks versus benefits on a grand scale, if long-term rewards are to be reaped. But they have also highlighted the role of responsible science in society, and the power of social acceptance to change the course of a technology's development: Science and technology are now developed at the pleasure of those who will use them, benefit from them, and potentially suffer by them.

Just as the impact of nanotechnology has been surrounded by considerable hype, the potential risks have also been overemphasized at times. The idea of a "grey goo" – out-of-control self-replicating nano-bots overtaking the world – still captures the imagination of some. This is little more than fantasy at the moment. Yet, nanotechnology does raise the possibility of risks to health and the environment that do not occur with more conventional technologies. Fundamentally, nanotechnology is about developing products that behave differently through controlling their makeup at the nanoscale. These same properties will challenge the way we understand and address potential risk in some cases. We may not be facing a grey goo challenge in the foreseeable future, but managing the risk of products where nanostructure and chemistry conspire to create new properties is presenting sufficient challenges of its own.

Current research into the risks presented by engineered nanomaterials is rather limited. It is sufficient to alert us to the fact that some engineered

nanomaterials do indeed behave differently to their more conventional counterparts, and may present new and unusual risks. But we do not yet have the knowledge to understand fully the nature of these risks, and how to manage them.

This would perhaps not be of great concern if nanotechnology were a technology of the distant future. However, it is happening now, and answers on how to assess and manage risk are needed urgently. Lux Research estimates that US$32 billion worth of nano-enabled products were sold in 2005, and this will rise to US$180 billion by 2008 (Lux Research 2006). In the consumer marketplace, nearly 400 products are currently being sold that purportedly use nanotechnology – everything from computer chips to cosmetics to food supplements (PEN 2006). The reality is that people and the environment are being exposed to nano-enabled products on a daily basis, and information is urgently needed on how safe these products are.

A recent report by Lux Research stressed the importance of industry taking action on the environmental, health, and safety risks of nanotechnology, if these technologies are to succeed. The report concludes that, while there is ambiguity over the real risks presented by nanomaterials, "the hard data is simply too worrying for companies to gamble that nanomaterials won't cause harm" (Lux Research 2006). They also highlight the importance of perceived risk in determining the success or failure of products, and stress the need for companies to openly communicate what they are doing to ensure the safety of nanoproducts.

It is against this backdrop that the risk research community is struggling to understand what the key challenges are, who should be addressing them and how. In the remainder of this paper, I explore what nanotechnology is from the perspective of risk research, what the key risk challenges are, and how we might respond to them.

2. Nanotechnology – An Overview

Despite current interest in nanotechnology, there is no one definition of the field that has been universally accepted. Richard Feynman did not use the term specifically, but referred to "manipulating and controlling things at a small scale" (Feynman 1959). In his book *Engines of Creation: The Coming Era of Nanotechnology*, Eric Drexler explores the idea of manipulating matter at the nanoscale to build new materials and products atom by atom – molecular manufacturing (Drexler 1986).

This idea of having control over matter at the nanoscale is reflected in more recent definitions. The US National Nanotechnology Initiative (NNI) defines nanotechnology as "the understanding and control of matter at dimensions of roughly 1 to 100 nanometers, where unique phenomena enable novel applications" (NSET 2004). In 2004, a UK government report commissioned

from the Royal Society and Royal Academy of Engineering (2004) separately defined nanoscience and nanotechnology:

Nanoscience is the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at a larger scale.

Nanotechnologies is the design, characterization, production and application of structures, devices and systems by controlling shape and size at the nanometer scale.

Within these concepts and definitions, common ground emerges: an ability to control matter at the nanoscale, to visualize nanometer-scale structures, and the practical application of unique properties arising from these abilities and structures.

Within such boundaries, nanotechnology is more of a concept or philosophy than a discrete field of study: it is an idea that crosses traditional scientific boundaries, and is finding a home in research areas as diverse as solid state physics and medical diagnostics, and applications ranging from microprocessors to dietary supplements. A recent survey of nano-enabled consumer products identified early adoption of the technology in electronics goods, sports equipment, clothing, cosmetics, over-the-counter drugs, and food supplements (PEN 2006). Close to market applications are high-performance batteries, inexpensive, flexible solar cells, highly sensitive compact sensors, and water treatment/remediation technologies. More sophisticated applications including nano-engineered medical treatments, high-efficiency energy storage and transmission, faster and more powerful computers, and high-performance prosthetics are anticipated in the near future. This is not a technology that lends itself easily to sharply delineated categories, but rather represents a merging of ideas and abilities across many different areas.

The Royal Society and Royal Academy of Engineering understood this when they coined the term "nanotechnologies" – representing the enormous diversity of possible technologies based on manipulating matter at the nanoscale (The Royal Society and The Royal Academy of Engineering 2004).

With an increasing ability to manipulate matter at the nanoscale comes the opportunity to utilize material properties and behavior that are specific to engineered nanostructures. Some of the most dramatic changes in going from the macroscale to the nanoscale occur in metals and metal oxides. Gold, for instance, loses its "gold" color when separated into nanometer-diameter particles, and becomes red. Although the chemistry was not understood at the time, colloidal gold nanoparticles were used to color medieval stained-glass windows red. Titanium dioxide and zinc oxide on the other hand shift from being opaque to visible light at the macroscale, to being transparent at the nanoscale. Semiconductors such as cadmium selenide exhibit a property known

as quantum confinement at the nanoscale – where the Bohr radius of an electron-hole pair exceeds the diameter of the particle. This leads to some very interesting behavior, in which particles fluoresce with a wavelength that is determined by their physical size, rather than their chemistry.

Other materials show unusual behavior that is only accessible by engineering at the nanoscale. Single-walled carbon nanotubes, for instance, are in essence a single sheet of graphite wrapped into a tube around 1.5 nm in diameter, but up to hundreds of nanometers long. Yet the properties of the nanotubes – whether they are highly conducting or semiconductors for instance – may depend on the precise offset of the graphite sheet as it is wrapped into a tube (chirality).

These are just a few examples of first-generation nanomaterials. As our ability to manipulate matter at the nanoscale becomes increasingly sophisticated, more complex structures, materials, and devices are anticipated (Roco 2004). For instance, artificial "transport molecules" – or nano-cars – have been made by attaching C60 molecules (the wheels) to rodlike molecules (Shirai et al. 2005). The resulting devices show directional motion across surfaces, and may form a basis for constructing inorganic systems to transport molecules in a manner analogous to biological systems. Indeed, the more advanced generations of nanotechnology are anticipated to lead to ways of mimicking biological systems and functions.

These may seem trivial examples, but nanotechnology is all about discovering something that is different, and using it to develop a competitive edge. So gold nano-shells are used as the basis for targeted cancer treatments; TiO_2 nanoparticles are used widely in sunscreens that are transparent to visible light but opaque to UV; CdSe "quantum dots" are developed into medical diagnostics and treatments, and various photoelectronic applications; and single-walled carbon nanotubes are considered in applications as diverse as electronic displays to high-strength composite materials to hydrogen storage. In each case, it is the unique material behavior that comes from engineering at the nanoscale that is being exploited.

3. Identifying Nano-Specific Risks

By its very nature, nanotechnology challenges how we understand and manage risk to human health and the environment. Commercial nano-enabled products rely on what is different and unique about engineered nanomaterials, raising the question: Are there also different and unique risks associated with these same properties? This is a valid question, but is insufficiently bounded to lead to specific solutions. For instance, it makes little sense to compare the risk to health of an electron microscope (a nanotechnology-based tool) with the risk

to health from free single-walled carbon nanotubes (a nanotechnology material); or the environmental impact of nano-electronics printing equipment (a nano-technology-based process) with unbound TiO_2 nanoparticles. Clearly, different nanotechnologies will entail different risks.

If the potential risks of nanotechnologies are to be addressed systematically, we need to understand what is unique about these risks, where conventional understanding and approaches might fail, and how to discern between technologies presenting a greater or lesser potential risk.

Nano-specific risks will depend on individual technologies, and how they are implemented. There is one common element – and that is the role of structure, as well as chemistry – in determining the behavior of many nanomaterials. This immediately places evaluations of engineered nanomaterials outside the realms of conventional chemical-based regulations and oversight. To address the potential impact of an engineered nanomaterial, we must understand how its structure will determine behavior within the environment or the body, as well as its chemistry. This brings with it additional challenges: while chemistry might remain the same through the life of a product, structure may change, and this in turn may alter the potential of a nanomaterial to cause harm. For instance, airborne nanoparticles agglomerate into larger particles with complex structures; discrete nanoparticles may be encapsulated into a composite material; machining and grinding nanomaterials might lead to the release of respirable particles with unusual nanostructures; the degradation of nanoproducts at the end of their life may lead to previously encapsulated nanostructured materials being released into the environment. At each stage, changes in material structure may enhance, or even suppress, the potential to cause harm.

If the structure of nanomaterials plays a role in determining impact, and yet conventional approaches to addressing risk rely on chemistry alone, the possibility of misassessing risk arises. For instance, the Manufacturers Safety Data Sheets for some carbon nanotubes have listed the material as synthetic graphite, and referenced safety precautions for use with materials having this chemistry. Yet, the desirable properties of carbon nanotubes are far removed from graphite, and it is likely that the health impact will be also. Likewise, there has been a tendency to assess the potential risks associated with TiO_2 on a chemical basis alone, even though some TiO_2 nanoparticles exhibit a distinctly different physicochemical behavior to larger particles (Oberdörster et al. 1994).

Structure can also affect exposure and dose in very fundamental ways. A given nanoparticle may not have a greater intrinsic hazard potential than a larger particle, but if it is more able to enter the body or be dispersed in the environment, it may have a greater real hazard potential. For instance, diameter affects where airborne particles deposit in the lungs if inhaled (Maynard 2006), and there is evidence that the small size of nanoparticles enables them to

penetrate cells, migrate along olfactory nerves (Elder et al. 2006), and cross from the lungs to the blood and subsequently to other organs (Kreyling et al. 2002). There are also indications that some nanoparticles might be able to penetrate to the dermis if applied to the skin (Tinkle et al. 2003; Ryman-Rasmussen et al. 2006), although this seems to depend on particle type and size, and the carrier medium.

The physical shape and structure of nanostructured materials might also be responsible for specific impacts. Asbestos is known to cause disease because of its structure as well as its chemistry: although the analogy is applied to engineered nanomaterials with caution, it is likely that some materials will show a similar dependency. Studies on carbon nanotubes in the lungs have already demonstrated unusual responses that appear to be associated with material structure (Shvedova et al. 2003, 2005; Lam et al. 2004; Warheit et al. 2004). There is also evidence that nanometer-scale particles can lead to oxidative stress by virtue of their size, as well as their chemistry (Brown et al. 2001).

The challenge we face is how to discern between nanotechnologies and nano-enabled products that present a significant risk to the environment and human health, and those that present a lesser risk. Here, it is useful to start from the classical risk assessment framework – describing risk in terms of both exposure and hazard. To present a significant risk, a nanotechnology must lead to a material with the potential to cause harm, and a route for significant exposure to occur in a form where the *hazard potential will be realized*. This qualifier is necessary to distinguish between different contexts that might apply to a nanomaterial throughout its life. For example, consider a hypothetical use of single-walled carbon nanotubes in an epoxy-based composite material. Assume that exposure potential is high during handling the unprocessed carbon nanotube material, and finishing components made from the nano-composite by grinding. Both processes may lead to nanotubes being inhaled, but the full hazard potential of the material is more likely to be realized when inhaling agglomerates of unprocessed nanotubes, rather than respirable particles of resin-encapsulated nanotubes.

Maynard and Kuempel (2005) address the question of identifying nano-materials which may present a unique risk to human health through the following criteria:

1. The material must be able to interact with the body in such a way that its nanostructure is biologically available.
2. The material should have the potential to elicit a biological response that is associated with its nanostructure.

These provide a useful working framework for distinguishing between materials and products that are more or less likely to present a health risk, as well as separating materials that present a unique risk from those that behave in a conventional way. Although Maynard and Kuempel were considering human exposure, the criteria also work well when considering environmental impact.

When linked to human exposure via the skin, respiratory system and gastrointestinal system, categories of materials and sources begin to emerge that may present a greater risk under some circumstances. These include unbound nanometer-diameter particles (in powders, aerosols, and liquid suspensions); inhalable agglomerates and aggregates of nanometer-diameter particles – where nanoscale structure-based functionality is retained; aerosolized liquid suspensions of nanomaterials; and the attrition of nanomaterial composites through various mechanisms (Maynard 2006).

The same categories of nanomaterials will be relevant when considering potential environmental impact. In addition, the release of what might be called active nanomaterials – for want of a better phrase – into the environment over a product's lifetime must be considered. These will include nanomaterials that result from the transformation of nano-enabled products through wear and tear, as well as chemical and biological actions.

4. Responding to the Challenge

Managing the risks presented by some emerging nanotechnologies will require new ways of addressing risk. These will ultimately need to be based on new knowledge – "safe" nanotechnologies will first and foremost be built on sound science. The challenge to the science community – including policy-makers and research managers, as well as the researchers themselves – is how to develop an understanding of the potential health, safety, and environmental risks presented by emerging nanotechnologies in parallel with the technologies' development and implementation.

This is not an easy challenge. It will require collaborations and partnerships that cross traditionally rigid boundaries. It will depend on realistic funding levels for risk-based research. And it will need a strategic steer to ensure that right research is pursued at the appropriate time.

The challenge is real, and it is urgent. Nano-based products are a reality now, as demonstrated by nearly 400 products identified by a public web-based inventory published by the Project on Emerging Nanotechnologies (PEN 2006). Many researchers and nanotechnology industry workers are already producing and handling engineered nanomaterials on a day-by-day basis, with little information on assessing and managing risk (Maynard and Kuempel 2005). These products and materials are being released into the

environment (intentionally or unintentionally) with little understanding, at least in some cases, about what the long-term impacts might be. Even if the term "nanotechnology" goes out of vogue, our increasing ability to manipulate matter at the nanoscale will lead to ever more sophisticated technologies over the coming decades, that demonstrate new and unusual behaviors.

So how do we respond to the challenge? First and foremost, strategic, prioritized research is needed to address immediate issues – including the safe use of nanomaterials under development and already in commercial use. Research is also needed to build capacity and understanding that will enable future challenges to be met, including an ability to predict the impact of emerging nanotechnologies. Finally, global coordination, cooperation and partnering between researchers, producers and users will be essential if relevant solutions are to be developed and adopted around the world.

In recent years, a number of groups have published lists of the research needed to address potential risks of engineered nanomaterials. These include government agencies, independent reviews, industry groups, user groups and academics (The Royal Society and The Royal Academy of Engineering 2004; Chemical Industry Vision 2020 technology Partnership and SRC 2005; Dennison 2005; EC 2005; EPA 2005; HM Government 2005; Maynard and Kuempel 2005; NIOSH 2005; Oberdörster et al. 2005). A review of research recommendations in nine such publications by Maynard showed considerable consensus over what still needs to be done (Maynard 2006). Thirteen overarching categories of research needed to better understand and manage potential risks associated with nanotechnology were identified:

- **Human health hazard:** how nanomaterials get into and behave within the body, and how toxicity can be tested for and predicted.
- **Health outcomes:** disease resulting from exposure to engineered nanomaterials within the workforce, the general population, and sensitive groups such as children and the elderly.
- **Environment:** how engineered nanomaterials enter the environment, where they go and how they behave once there, the impact they have, and how they might be controlled.
- **Exposure:** sources of engineered nanomaterials exposure, exposure measurement, and how changes in nanomaterials with time might affect exposure.
- **Characterization:** significant characteristics of engineered nanomaterials, such as size, shape, surface area and surface chemistry, to be measured when evaluating risk.

- **Control:** identifying where engineered nanomaterials might potentially escape into the environment or workplace and ways of preventing such escapes, and research into the efficacy of personal protective equipment (including respirators).
- **Risk reduction:** new ways of assessing risk, and new ways of working safely with engineered nanomaterials.
- **Standards:** the development of appropriate nanotechnology standards – and in particular, standards that develop an appropriate language for describing nanomaterials, standards for measuring exposure and standard materials for toxicity evaluation.
- **Safety:** the potential for engineered nanomaterials and nano-products to cause physical harm, such as through explosion and fire hazards.
- **Informatics:** how to collect, sort, and use the vast and diverse amount of data being generated on engineered nanomaterials that is relevant to understanding risk.
- **Research approaches:** how to plan and carry out risk-based research effectively.
- **Transportation:** containment requirements, labeling, the potential for release, and the exposure hazard for different types of engineered nanomaterials, as they are moved from one place to another – in raw, intermediate, or highly processed forms.
- **Emergency responders:** how to respond effectively and safely – and in particular how procedures and protocols may differ from incidents involving conventional materials – to spills and other accidental releases of engineered nanomaterials.

These categories are useful for pointing to where progress needs to be made in support of safe nanotechnologies. But outside the context of a strategic research framework, they offer little guidance on what needs to be done when.

Strategic research requires prioritization, which is never easy, and always contentious. However, the danger of not prioritizing is that limited resources are spread too thin, irrelevant research is pursued at the expense of necessary research, and answers to specific questions on risk are not forthcoming. One possible framework within which risk-focused research can be prioritized is to consider criteria for immediate, medium-term, and long-term needs:

- ***Immediate needs*** – ensuring that current nanotechnologies are as safe as possible, appropriate workplace practices for handling engineered nanomaterials exist, and appropriate ways of using and disposing of nano-based products are understood.

- ***Medium-term needs*** – establishing associations between nanomaterial exposure and disease or environmental impact, and developing an understanding how to minimize impact. This research would include human health outcomes, ecotoxicity, toxicity screening, risk management systems, control methods, lifecycle assessment, and exposure methods.
- ***Long-term needs*** – developing ways of predicting and preemptively managing the potential risk of emerging nanotechnologies, including mechanistic toxicology; predictive risk assessment and management of later generation nanotechnologies; and emergent behavior and convergence between different technologies.

These criteria were used in (Maynard 2006) to identify critical research priorities for the next 2 years. Identified short-term goals fell into five categories:

- **Risk assessment:** this includes research methodologies, risk assessment tools, and information management.
- **Environmental impact:** high-priority research goals include identifying routes of release and exposure, and measurement methods.
- **Human health impact:** high-priority research goals include exposure measurement methods, controlling release of material and preventing exposure, and developing toxicity screening tests.
- **Predicting hazard:** the ability to predict the hazard of a new engineered nanomaterial, and even to reduce its toxicity through careful engineering, is a long-term goal than needs an initial research investment now.
- **Materials characterization:** an ability to characterize nanomaterials appropriately when evaluating potential risk will require investment in basic research now.

These goals recognize that medium- and long-term research needs will only be successfully addressed if necessary capacity is developed in the short-term. This includes developing a solid science foundation on which to build new knowledge, and developing an expertise and facilities base that is capable of offering new knowledge. Investment needed now in medium- to long-term research areas includes addressing environmental impacts, developing a systematic approach to understanding and managing risks from nanotechnologies, and developing the capabilities to better predict risks posed by new nanomaterials. In addition, the global research community needs to be energized and challenged to tackle the potential dangers of some nanotechnologies – and to this end, Maynard et al. have issued five grand challenges to developing "safe nanotechnologies" (Maynard et al. 2006).

Effective partnerships will be essential if these goals are to be achieved. At a fundamental level, the complexity of nanotechnologies demands interdisciplinary collaboration, if risks are to be assessed and managed effectively. Oberdörster et al. (2005) have emphasized the need for toxicologists to work with physicists, chemists, and engineers in characterizing engineered nanomaterials. Similar cross-disciplinary collaborations will be needed when addressing other aspects of risk.

At a higher level, organizations overseeing risk-focused research will need to cooperate and coordinate if holistic research strategies are to be developed, that respond to oversight needs. Just as risk-based research cannot be compartmentalized for nanotechnologies, management of research and oversight will be ineffective if there is no overarching plan for integrating areas such as human health and environmental impact. This degree of coordination is essential within government. Yet, it must also extend out into the private sector, and to organizations representing the interests of end users and the environment. By ensuring tripartite oversight of nanotechnology risk-focused research, there is a greater chance of ensuring that research is relevant and responsive to real needs.

Finally, global partnerships will be essential to the long-term success of emerging nanotechnologies – whether these are between individual researchers, industries, or governments. Nanotechnology presents global challenges to addressing risk, and information will need to be shared openly if global solutions are to be found. Certainly, an ability to develop safe nanotechnologies in one country should not be used to develop a competitive edge. Conversely, we need to avoid a situation where a lack of concern over risk in some sectors provides an (albeit short-term) economic advantage. In this sense, a global nanoeconomy will benefit from a global-level playing field when it comes to understanding and managing risks.

Developing such global solutions will require globally coordinated research. Resources for risk-focused research are always likely to be limited. However, by ensuring that international research programs are complementary rather than duplicative, these limited resources can be leveraged to maximum effect. This in turn will require combined efforts from regions around the world to develop partnerships and act in concert.

5. Summary

Nanotechnology is a reality now, and our ability to produce ever-more sophisticated materials, processes, and products by engineering at the nanoscale will only increase over the coming years. Yet our understanding of the potential health, safety, and environmental impacts of these emerging technologies is

rudimentary at best. While it is by no means certain that emerging nanotechnologies will present a significant risk, we can be sure that inaction in addressing risk will pave the way to public distrust and the potential for serious harm to occur.

Current knowledge is sufficient to indicate that some nanotechnologies will present new risks. What we still lack is information on how to assess and manage potential risks. The challenges to the scientific community are significant: Which nanotechnologies present a significant hazard? What are those hazards and how do they relate to risks to health, safety, and the environment? How can risks be identified and controlled effectively? These and similar questions will require new strategies, new thinking, and new funding within organizations and groups addressing the risks of emerging nanotechnologies.

Above all, new partnerships will be needed to address potential risks – between researchers, agencies, stakeholders, and governments. By finding ways to work together effectively within a strategic framework, there is every chance that beneficial, sustainable, and safe nanotechnologies will emerge.

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BIOKINETICS AND EFFECTS OF NANOPARTICLES[1]

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Abstract: Exposures to airborne nanosized particles (<100 nm) have been experienced by humans throughout their evolutionary stages. Recently, the rapidly developing field of nanotechnology is likely to become yet another source for human exposures to nanosized particles – engineered nanoparticles (NPs) – by different routes, i.e., inhalation, ingestion, dermal, or even injection. Nanotechnology is defined as research and technology development at the atomic, molecular, or macromolecular levels, in the length scale of ~1–100 nm range. One of the many promising applications of engineered NPs is in the area of medicine, for example, targeted drug delivery as aerosols and to tissues which are difficult to reach. The discipline of nanomedicine has arisen to develop, test, and optimize these applications. However, the same properties that makes NP attractive for development in nanomedicine and for specific industrial processes could also prove deleterious when NP interact with cells. An emerging discipline – nanotoxicology, which can be defined as safety evaluation of engineered nanostructures and nanodevices – is gaining increased attention. Nanotoxicology research will not only provide information for risk assessment of NP based on data for hazard identification, dose–response relationships, and biokinetics, but will also help to advance further the field of nanomedicine by providing information to alter undesirable NP properties. Although potential adverse effects of engineered NP have not been systematically investigated, there are a number of studies in the area of inhalation toxicology and also human epidemiology from which some preliminary conclusions about effects of nanosized particles can be drawn. There are also some decades-old – mostly forgotten – studies with nanosized particles which shed light on the biokinetics of such particles once introduced into the organism. This presentation summarizes results of studies with nanosized particles with a focus on the respiratory tract and skin as portals of entry. Examples of translocation and effects of nanosized particles and presumed

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[1] This presentation consists for the most part of updated excerpts of a review by Oberdörster et al., 2005, *Environ. Health Perspect*. 113: 823–839.

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mechanisms will be highlighted. They illustrate, on the one hand, that we need to be aware of possible acute adverse effects and potential long-term consequences; on the other hand, the findings also give us ideas about the intriguing possibilities that NP offer for potential use as diagnostic tools or as therapeutic delivery systems. A thorough evaluation of desirable versus adverse effects is required for the safe use of engineered NP, and major challenges lie ahead to answer key questions of nanotoxicology, foremost being the assessment of human and environmental exposure, the identification of potential hazards (toxicity *vs*. benefit), and the biopersistence in cells and subcellular structures. Results so far demonstrate that the highly desirable properties of nanoparticles, which makes them attractive as medicinal aerosols, as well as their potential to induce toxicity, depend not only on their size but on a variety of surface properties. To establish the principles which govern NP-cell interactions will be a major challenge for the field of Nanotoxicology.

Keywords: nanoparticle, toxicity, risk, respiratory tract, skin, translocation

1. Introduction

Exposures to airborne ultrafine particles (UFPs, <100 nm) have been experienced by humans throughout their evolutionary stages, but it is only with the advent of the industrial revolution that such exposures have increased dramatically because of anthropogenic sources such as internal combustion engines, power plants, and many other sources of thermodegradation. The rapidly developing field of nanotechnology is likely to become yet another source for human exposures to engineered nanoparticles (NPs) by different routes: inhalation (respiratory tract), ingestion gastrointestinal GI tract, dermal (skin), and injection (blood circulation). Table 1 summarizes some of the natural and anthropogenic sources of NPs, the latter divided into unintentional and intentional sources.

Obvious differences between unintentional and intentional anthropogenic UFP are the polydispersed and chemically complex nature (elemental, soluble, and volatile carbon compounds; soluble and poorly soluble inorganics) (Cyrys et al. 2003; Hughes et al. 1998) of the former, in contrast to the monodisperse and precise chemically engineered characteristics and solid form of the latter, generated in gas or liquid phase (National Nanotechnology Initiative [NNI] 2004). However, despite these differences, the same toxicologic principles are likely to apply for NPs, because not only size but also a number of other particle parameters determine their biologic activity (Table 2).

TABLE 1. Ultrafine/nano Particles (<100nm): natural and anthropogenic sources.

Natural	**Anthropogenic**	
	Unintentional	***Intentional***
Gas to particle conversions Forest fires Volcanoes *(hot lava)* Viruses Biogenic magnetite: *magnetotactic bacteria;* *protictists, mollusks,* *arthropods, fish,* *birds, human brain,* *meteorite?* Ferritin (12.5 nm) Microparticles *(<100nm)* *(activated cells)*	Internal combustion engines Power plants Incinerators Airplane jets Metal fumes *(smelting, welding, etc.)* Polymer fumes Other fumes Heated surfaces Frying, boiling, grilling Electric motors	Engineered nanoparticles: *(controlled size and shape,* *designed for functionality)* *metals, semiconductors,* *metal oxides* *quantum dots/rods* *fullerenes, nanotubes* *nanowires* *nanoshells* *nanorings…etc.* *(nanotechnology applied to* *many products: cosmetics,* *medical, fabrics, electronics,* *optics, displays, etc.)*
	Human Exposure Routes **Ultrafine particles:** *Inhalation* **Nanoparticles:** *Inhalation* *Ingestion Dermal Injection*	

Figure 1 depicts the range of sizes of airborne ambient particulate matter, including the nucleation-mode, Aitken-mode, accumulation- mode, and coarse-mode particles. Ambient particles <0.1 μm, defined as UFPs in the toxicologic literature, consist of transient nuclei or Aitken nuclei (NRC 1983). More recently, even smaller particles in the nucleation mode with peak diameters around 4 nm have been observed (McMurry and Woo 2002).

TABLE 2. Ultrafine Particles and Engineered Nanoparticles (non-fibrous)- Interchangeable Terms?

Both Consist of nanosized particles:	Ultrafine Particles	Engineered NP
Primary particles	<100 nm	<100 nm
– Size	Polydispersed	Monodispersed
– Size distribution		
Aggregations when generated	Yes	No
Agglomeration in air	Yes	Yes
Chemistry	variable to well defined	well defined
Toxicological significance	small size, high surface areas per mass, chemistry	

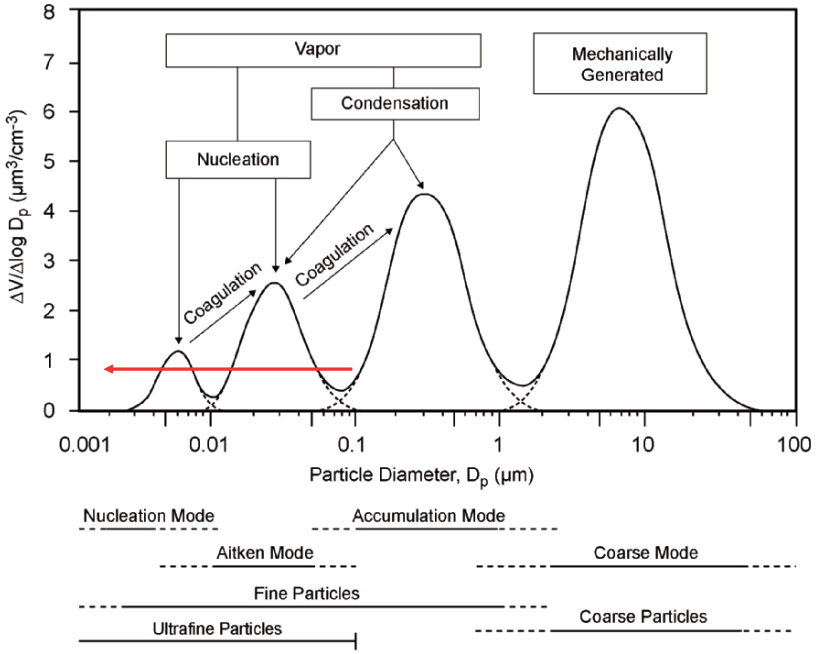


Figure 1. Idealized size distribution of traffic-related particulate matter. The four polydisperse modes of traffic-related ambient particulate matter span approximately 4 orders of magnitude from below 1 nm to above 10 μm. Nucleation and Aitken mode particles are defined as ultrafine particles (less than ~100 nm). Source-dependent chemical composition is not well controlled and varies considerably. In contrast engineered nanoparticles (1–100 nm) have well-controlled chemistry and are generally monodispersed (From US EPA 2004.)

Table 3 shows the tremendous differences in particle number concentrations and particle surface areas for particles of the four ambient modes, assuming an airborne concentration of 10 pg/cm^3 of unit density particles of each size. The extraordinarily high number concentrations of NPs per given mass will likely be of toxicologic significance when these particles interact with cells and subcellular components. Likewise, their increased surface area per unit mass can be toxicologically important if other characteristics such as surface chemistry and bulk chemistry are the same. Although the mass of UFPs in ambient air is very low, approaching only 0.5–2 μg/m^3 at background levels (Hughes et al. 1998), it can increase several fold during high-pollution episodes or on highways (Brand et al. 1991; Shi et al. 2001; Zhu et al. 2002).

TABLE 3. Particle Number and Particle Surface Area per 10 pg/cm^3 Airborne Particles of Unit Density.

Particle diameter (nm)	Particle number (N/cm^3)	Particle surface area (μm^3)
5	153,000,000	12,000
20	2,400,000	3,016
250	1,200	240
5,000	0.15	12

Small size, high number per mass, and surface chemistry confer both desirable and undesirable properties.

Detailed Physico-chemical characterization of NP is essential.

1.1. PHYSICOCHEMICAL CHARACTERISTICS AS DETERMINANTS OF BIOLOGIC ACTIVITY

The small size and corresponding large specific surface area of solid NPs confer specific properties to them, for example, making them desirable as catalysts for chemical reactions. The importance of surface area becomes evident when considering that surface atoms or molecules play a dominant role in determining bulk properties (Amato 1989); the ratio of surface to total atoms or molecules increases exponentially with decreasing particle size. Increased surface reactivity predicts that NPs exhibit greater biologic activity per given mass compared with larger particles, should they be taken up into living organisms and provided they are solid rather than solute particles. This increased biologic activity can be either positive and desirable (e.g., antioxidant activity, carrier capacity for therapeutics, penetration of cellular barriers for drug delivery) or negative and undesirable (e.g., toxicity, induction of oxidative stress or of cellular dysfunction), or a mix of both.

The characteristic biokinetic behaviors of NPs are attractive qualities for promising applications in medicine as diagnostic and therapeutic devices and as tools to investigate and understand molecular processes and structures in living cells (Akerman et al. 2002; Foley et al. 2002; Kreuter 2001; Li et al. 2003). For example, targeted drug delivery to tissues that are difficult to reach (e.g., central nervous system [CNS]), NPs for the fight against cancer, intravascular nanosensor, and nanorobotic devices, and diagnostic and imaging procedures are presently under development. The discipline of nanomedicine – defined as medical application of nanotechnology and related research – has arisen to design, test, and optimize these applications so that they can eventually be used routinely by physicians. There are also many promising applications in different industrial fields in addition to nanomedicine, promising a bright future for nanotechnology (Fig. 2).

Figure 2. The many promising uses of nanotechnology may be compromised by the potential to cause adverse effects. Appropriate toxicological testing is necessary to determine a potential risk.

However, in apparent stark contrast to the many efforts aimed at exploiting desirable properties of NPs for multiple industrial applications and for improving human health are the limited attempts to evaluate potential undesirable effects of NPs when administered intentionally for medicinal purposes, or after unintentional exposure during manufacture or processing for industrial applications. The same properties that make NPs so attractive for development in nanomedicine and for specific industrial processes could also prove deleterious when NPs interact with cells, reflecting a potentially dark side of nanotechnology (Fig. 2). Thus, evaluating the safety of NPs should be of highest priority given their expected worldwide distribution for industrial applications and the likelihood of human exposure, directly or through release into the environment (air, water, soil). Nanotoxicology – "science of engineered nanodevices and nanostructures that deals with their effects in living organisms" – is gaining increased attention. Nanotoxicology research not only will provide data for safety evaluation of engineered nanostructures and devices

but also will help to advance the field of nanomedicine by providing information about their undesirable properties and means to avoid them.

1.1.1. Concepts of Nanotoxicology

Studies with ultrafine and fine titanium dioxide (TiO_2) particles showed that ultrafine anatase TiO_2 (20 nm), when instilled intratracheally into rats and mice, induced a much greater pulmonary-inflammatory neutrophil response (determined by lung lavage 24 h after dosing) than did fine anatase TiO_2 (250 nm) nm) when both types of particles were instilled at the same mass dose (Fig. 3a). Also, expressed as particle number showed significant differences in the inflammatory response. However, when the instilled dose was expressed as particle surface area, it became obvious that the neutrophil response in the lung for both ultrafine and fine TiO_2 fitted the same dose response curve (Fig. 3b), suggesting that particle surface area for particles of different sizes but of the same chemistry and crystallinity, is a better dosemetric than is particle mass or

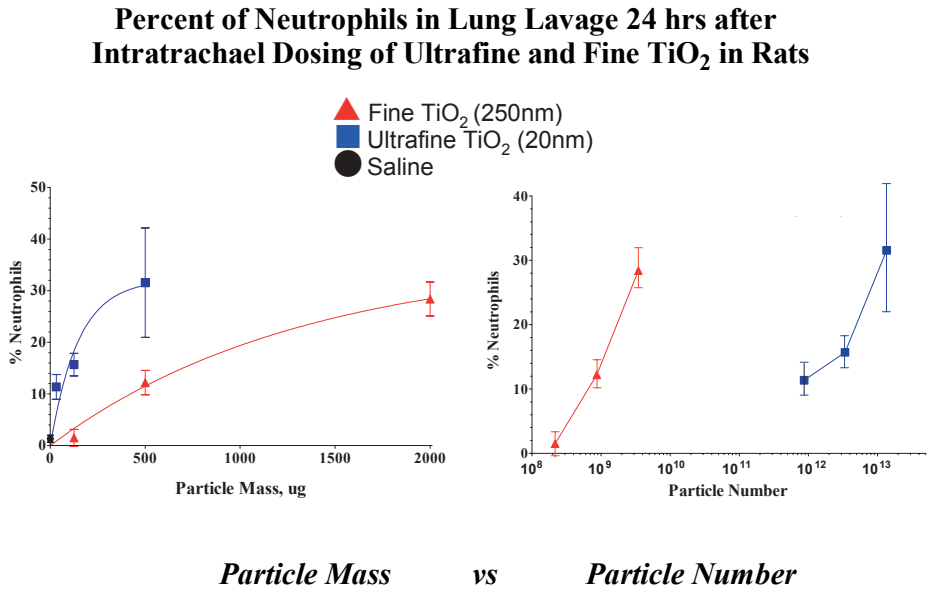


Figure 3a. Percent of neutrophils in lung lavage of rats as indicators of inflammation 24 h after intratracheal instillation of different mass doses of 20 and 250 nm TiO_2 particles in rats and mice. The steeper dose–response of nanosized TiO_2 is obvious when dose is expressed as mass or particle number.

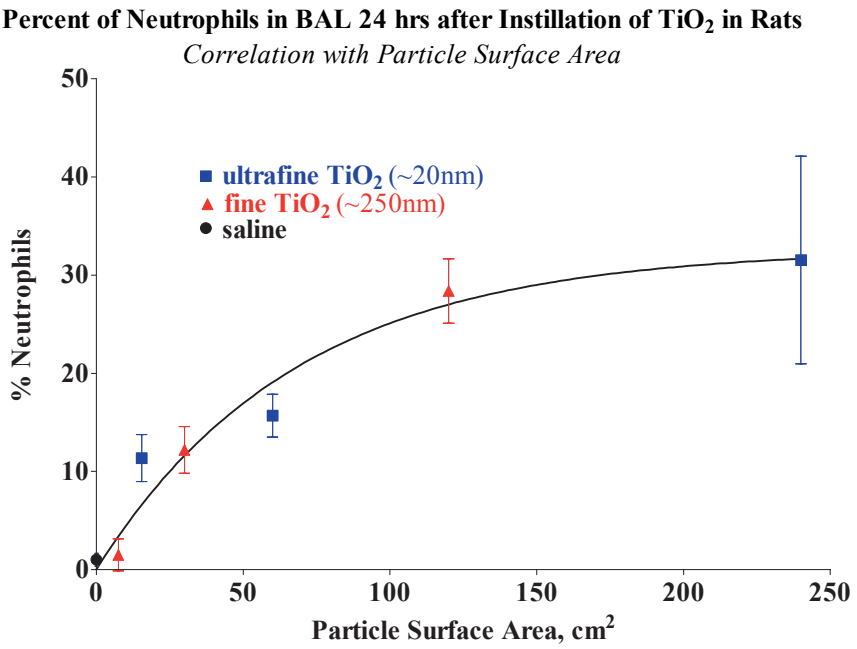


Figure 3b. The same dose–response relationship but with dose expressed as particle surface area, indicating that particle surface area seems to be a more appropriate dosemetric for comparing effects of different sized particles provided they are of the same chemical structure (anatase TiO_2 in this case).

particle number (Oberdörster, 2000). Moreover, normalizing the particle surface dose to lung weight shows excellent agreement of the inflammatory response in both rats and mice (Fig. 3c). The better fit of dose–response relationships by expressing the dose as surface area rather than mass when describing toxicologic effects of inhaled solid particles of different sizes has been pointed out repeatedly, especially when particles of different size ranges – nano to fine – were studied (Brown et al. 2001; Donaldson et al. 1998, 2002; Driscoll 1996; Oberdörster and Yu 1990; Oberdörster et al. 1992a; Tran et al. 1998, 2000) However, it needs to be considered that other particle parameters, such as shape, surface chemistry, surface charge, agglomeration state, etc., influence effects aswell. In the case of TiO_2, photocatalytic activity also plays a role, as pointed out recently (Sayes et al. 2006). Thus, particle chemistry, and specifically surface chemistry plays a decisive role in addition to particle size.

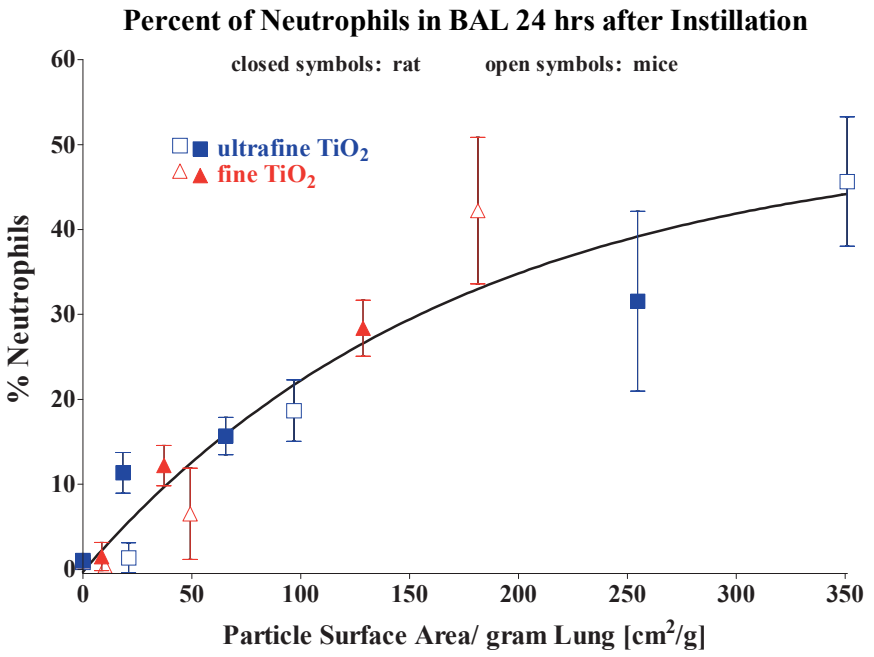


Figure 3c. Dose–response relationship of TiO_2 in rats and mice, normalized by particle surface area and lung weight. (20 nm TiO_2; ■ 250 nm TiO_2, ▲ saline control ●)

Engineered nanomaterials can have very different shapes, for example, spheres, fibers, tubes, rings, and planes. Toxicologic studies of spherical and fibrous particles have well established that natural (e.g., asbestos) and manmade (e.g., biopersistent vitreous) fibers are associated with increased risks of pulmonary fibrosis and cancer after prolonged exposures (Greim et al. 2001). Critical parameters are the three Ds: dose, dimension, and durability of the fibers. Fibers are defined as elongated structures with a diameter-tolength ratio (aspect ratio) of 1:3 or greater and with a length of >5 μm and diameter ≤3 μm (World Health Organization [WHO] 1985). Carbon nanotubes have aspect ratios of up to ≥100, and length can exceed 5 μm with diameters ranging from 0.7 to 1.5 nm for singlewalled nanotubes, and 2–50 nm for multiwalled nanotubes. Results from three studies using intratracheal dosing of carbon nanotubes in rodents indicate significant acute inflammatory pulmonary effects that either subsided in rats (Warheit et al. 2004) or were more persistent in mice (Lam et al. 2004; Shvedova et al. 2005). Administered doses were very high, ranging from 1 to 5 mg/kg in rats; in mice doses ranged from 3.3 to 16.6 mg/kg (Lam et al. 2004) or somewhat lower, from 0.3 to 1.3 mg/kg (Shvedova et al.

2004). Granuloma formation as a normal foreign body response of the lung to high doses of a persistent particulate material was a consistent finding in these studies. Metal impurities (e.g., iron) from the nanotube generation process may also have contributed to the observed effects. Although these *in vivo* first studies revealed high acute effects, including mortality, this was explained by the large doses of the instilled highly aggregated nanotubes that caused death by obstructing the airways and should not be considered a nanotubes effect per se (Warheit et al. 2004).

Future studies should be designed to investigate both effects and also the fate of nanotubes after deposition in the respiratory tract, preferentially by inhalation using well dispersed airborne nanotubes reflecting conditions at the workplace. In order to design the studies using appropriate dosing, it is necessary to assess the likelihood and degree of human exposure. It is of utmost importance to characterize human exposures in terms of the physicochemical nature, the aggregation state, and concentration (number, mass, surface area) of engineered nanomaterials and perform animal and *in vitro* studies accordingly. If using direct instillation into the lower respiratory tract, a large range of doses, which include expected realistic exposures of appropriately prepared samples, needs to be considered.

1.1.2. Portals of Entry and Target Tissues

Most of the toxicity research on NPs *in vivo* has been carried out in mammalian systems, with a focus on respiratory system exposures for testing the hypothesis that airborne UFPs cause significant health effects. With respect to NPs, other exposure routes, such as skin and GI tract, also need to be considered as potential portals of entry. Portal-of-entry–specific defense mechanisms protect the mammalian organism from harmful materials. However, these defenses may not always be as effective for NPs, as is discussed below.

2. Respiratory Tract

In order to appreciate what dose the organism receives when airborne particles are inhaled, information about their deposition as well as their subsequent fate is needed. Here we focus on the fate of inhaled nanosized materials both within the respiratory tract itself and translocated out of the respiratory tract. There are significant differences between NPs and larger particles regarding their behavior during deposition and clearance in the respiratory tract.

2.1. EFFICIENT DEPOSITION OF INHALED NPs

The main mechanism for deposition of inhaled NPs in the respiratory tract is diffusion due to displacement when they collide with air molecules. Other deposition mechanisms of importance for larger particles, such as inertial impaction, gravitational settling, and interception, do not contribute to NP deposition, and electrostatic precipitation occurs only in cases where NPs carry significant electric charges. Figure 4 shows the fractional deposition of inhaled particles in the nasopharyngeal, tracheobronchial, and alveolar regions of the human respiratory tract under conditions of nose breathing during rest, based on a predictive mathematical model (International Commission on Radiological Protection [ICRP] 1994). These predictions apply to particles that are inhaled as singlet particles of a given size and not as aggregates; the latter obviously will have larger particle size and different deposition site. In each of the three

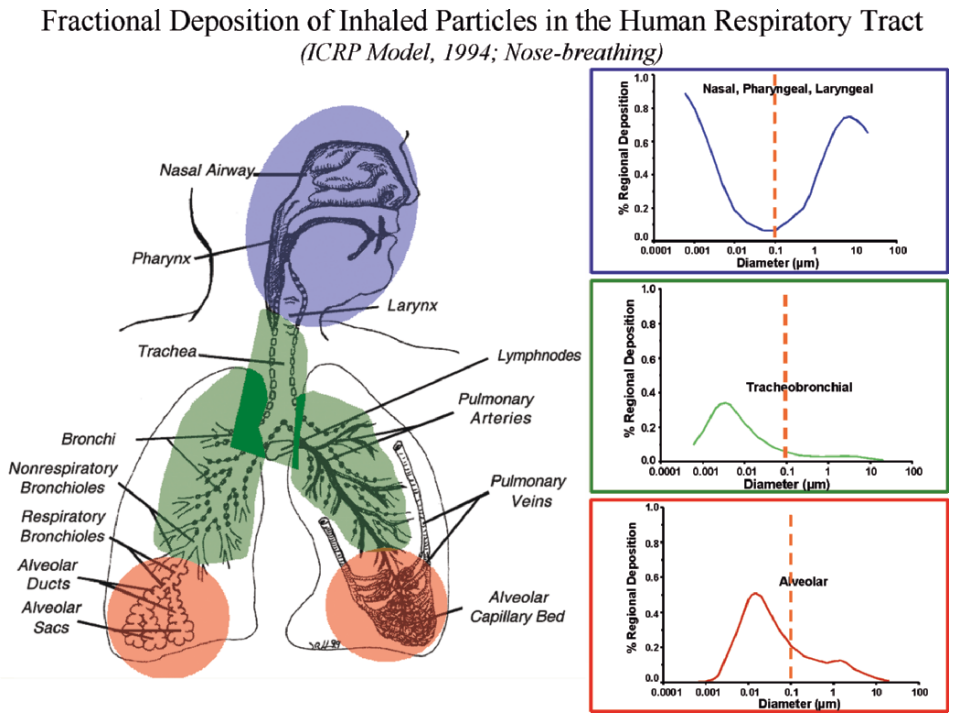


Figure 4. Predicted fractional deposition of inhaled particles in the nasopharyngeal, tracheobronchial, and alveolar region of the human respiratory tract during nose-breathing (based on data from ICRP 1994. (Drawing courtesy of J. Harkema.)

regions of the respiratory tract, significant amounts of a certain size of NPs (1–100 nm) are deposited. For example, 90% of inhaled 1 nm particles are deposited in the nasopharyngeal compartment, only approximately 10% in the tracheobronchial region, and essentially none in the alveolar region. On the other hand, 5 nm particles show about equal deposition of approximately 30% of the inhaled particles in all three regions; 20 nm particles have the highest deposition efficiency in the alveolar region (~50%), whereas in tracheobronchial and nasopharyngeal regions this particle size deposits with approximately 15% efficiency. These different deposition efficiencies should have consequences for potential effects induced by inhaled NPs of different sizes as well as for their disposition to extrapulmonary organs, as discussed further below.

2.1.1. Disposition of NPs in the Respiratory Tract

The preceding section summarized data demonstrating that inhaled NPs of different sizes can target all three regions of the respiratory tract. Several defense mechanisms exist throughout the respiratory tract aimed at keeping the mucosal surfaces free from cell debris and particles deposited by inhalation. Several reviews describe the well-known classic clearance mechanisms and pathways for deposited particles (Kreyling and Scheuch 2000; Schlesinger et al. 1997; U.S. EPA 2004), so here we only briefly mention those mechanisms and point out specific differences that exist with respect to inhaled NPs.

Once deposited, NPs – in contrast to larger-sized particles – appear to translocate readily to extrapulmonary sites and reach other target organs by different transfer routes and mechanisms. One involves transcytosis across epithelia of the respiratory tract into the interstitium and access to the blood circulation directly or *via* lymphatics, resulting in distribution throughout the body. The other is a not generally recognized mechanism that appears to be distinct for NPs and that involves their uptake by sensory nerve endings embedded in airway epithelia, followed by axonal translocation to ganglionic and CNS structures.

The most prevalent mechanism for clearance of solid fine and larger particles in the alveolar region is mediated by alveolar macrophages, through phagocytosis of deposited particles. In contrast, deposited particles of less than 100 nm are not efficiently cleared by this mechanism (Fig. 5). The success of macrophage–particle encounter appears to be facilitated by chemotactic attraction of alveolar macrophages to the site of particle deposition (Warheit et al. 1988). The chemotactic signal is most likely complement protein 5a (C5a), derived from activation of the complement cascade from serum proteins present on the alveolar surface (Warheit et al. 1986; Warheit and Hartsky 1993). This is followed by gradual movement of the macrophages with internalized particles toward the mucociliary escalator. The retention half-time of solid particles in

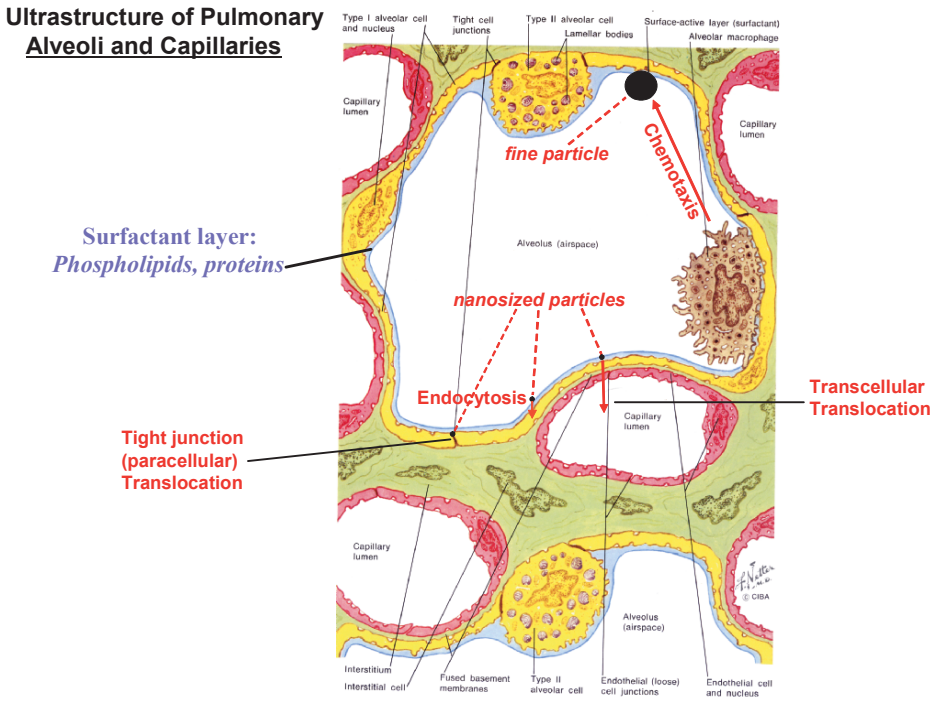


Figure 5. Phagocytosis and clearance of particles by alveolar macrophages is effective for fine particle, but inefficient for nanoparticles (<100 nm).

the alveolar region based on this clearance mechanism is about 70 days in rats and up to 700 days in humans. The efficacy of this clearance mechanism depends highly on the efficiency of alveolar macrophages to "sense" deposited particles, move to the site of their deposition, and then phagocytize them. This process of phagocytosis of deposited particles takes place within a few hours, so by 6–12 h after deposition essentially all of the particles are phagocytized by alveolar macrophages, to be cleared subsequently by the slow alveolar clearance mentioned above. However, it appears that there are significant particle size–dependent differences in the cascade of events leading to effective alveolar macrophage-mediated clearance. Figure 6 displays results of several studies in which rats were exposed to different-sized particles (for the 3 and 10 μm particles, 10 μg and 40 μg polystyrene beads, respectively, were instilled intratracheally) (Kreyling et al. 2002; Oberdörster et al. 1992b, 2000; Semmler et al. 2004). After 24 h, the lungs of the animals were lavaged repeatedly, retrieving about 80% of the total macrophages as determined in earlier lavage

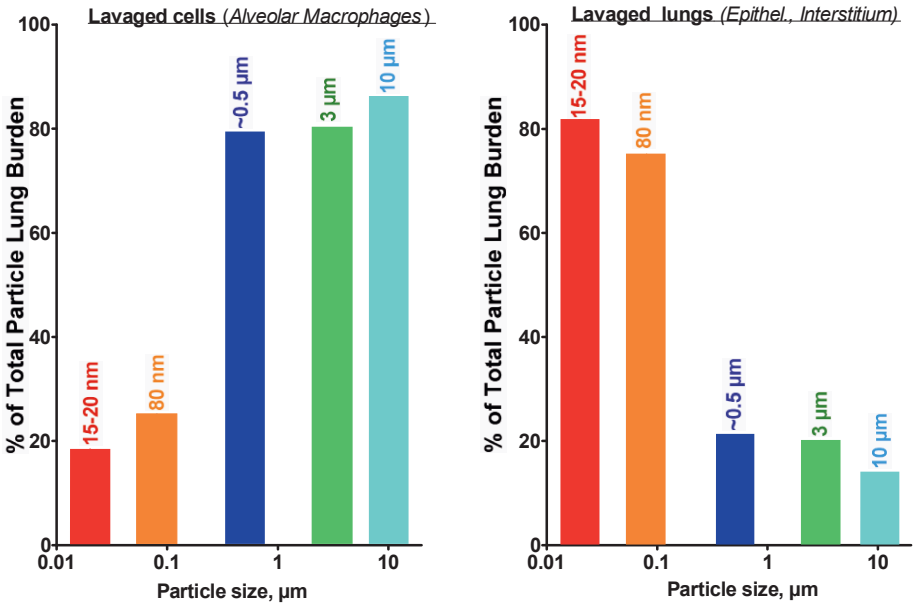


Figure 6. In vivo retention of inhaled nanosized and larger particles in alveolar macrophages (left side) and in exhaustively lavaged lungs (epithelial and interstitial retention, right side) 24 h post exposure. The alveolar macrophage is a most important defense mechanism in the alveolar region for fine and coarse particles, yet inhaled singlet NP are not efficiently phagocytized by alveolar macrophages.

experiments (Ferin et al. 1991). As shown in Fig. 6, approximately 80% of 0.5, 3, and 10 µm particles could be retrieved with the macrophages, whereas only approximately 20% of nanosized 15–20 nm and 80 nm particles could be lavaged with the macrophages. In effect, approximately 80% of the UFPs were retained in the lavaged lung after exhaustive lavage, whereas approximately 20% of the larger particles >0.5 µm remained in the lavaged lung. This indicates that NPs either were in epithelial cells or had further translocated to the interstitium.

2.1.2. Epithelial Translocation

Because of the apparent inefficiency of alveolar macrophage phagocytosis of NPs, one might expect that these particles interact instead with epithelial cells. Indeed, results from several studies show that NPs deposited in the respiratory tract readily gain access to epithelial and interstitial sites. This was also shown in studies with ultrafine PTFE fumes: shortly after a 15 min exposure, the

fluorine-containing particles could be found in interstitial and submucosal sites of the conducting airways as well as in the interstitium of the lung periphery close to the pleura (Oberdörster 2000). Such interstitial translocation represents a shift in target site away from the alveolar space to the interstitium and to the lymph and blood circulation, potentially causing direct particle induced effects there.

2.1.3. Translocation to the Circulatory System

Once the particles have reached pulmonary interstitial sites, uptake into the blood circulation, in addition to lymphatic pathways, can occur; again, this pathway is dependent on particle size, favoring NPs. Berry et al. (1977) were the first to describe translocation of NPs across the alveolar epithelium using intratracheal instillations of 30 nm gold particles in rats. Within 30 min postexposure, they found large amounts of these particles in platelets of pulmonary capillaries; the researchers suggested that this is an elimination pathway for inhaled particles that is significant for transporting the smallest air-pollutant particles – in particular, particles of tobacco smoke – to distant organs. They also hypothesized that this "might predispose to platelet aggregation with formation of microthrombi atheromatous plaques" (Berry et al. 1977). Since then, a number of studies with different particle types have confirmed the existence of this translocation pathway, as summarized in Table 4.

Collectively, these studies indicate that particle size and surface chemistry (coating), and possibly charge, govern translocation across epithelial and endothelial cell layers. In particular, the studies summarized by Mehta et al. (2004) and those performed by Heckel et al. (2004) using intravenous administration of albumin-coated gold NPs in rodents demonstrated receptor-mediated transcytosis (albumin-binding proteins) via caveolae. These 50–100 nm vesicles, first described by Simionescu et al. (1975), form from indentations of the plasmalemma and are coated with the caveolin-1 protein. Albumin, as the most abundant protein in plasma and interstitium, appears to facilitate NP endocytosis, as does lecithin, a phospholipid: even 240 nm polystyrene particles translocated across the alveolo-capillary barrier when coated with lecithin, whereas uncoated particles did not (Kato et al. 2003). The presence of both albumin and phospholipids in alveolar epithelial lining fluid may, therefore, be important constituents for facilitated epithelial cell uptake of NPs after deposition in the alveolar space.

However, as shown by results summarized in Table 4, surface coating of NPs with albumin clearly causes even the smallest particles to be internalized via caveolae. The presence of caveolae on cells differs: they are abundant in lung capillaries and alveolar type l cells but not in brain capillaries (Gumbleton 2001). In the lung, during inspiratory expansion and expiratory contraction of the alveolar walls, caveolae with openings around 40 nm disappear and reappear, forming

TABLE 4. Particle Size and Surface Chemistry-related Alveolar-Capillary Translocation.

Particle size (nm)	Type	Translocation	Localization/effect	Reference
5–20	Gold, albumin coated	Yes	Via caveolae	Mehta et al. (2004)
8	Gold, albumin coated	Yes	Via "vesicles"	König et al. (1993)
8	Gold, albumin coated	Yes	Via caveolae	Heckel et al. (2002)
18	Iridium	Yes[A]	Extrapulmonary organs	Kreyling et al. (2002)
30	Gold	Yes	Platelet	Berry et al. (1977)
35	Carbon	Yes	Liver	Oberdörster et al. (2002)
60	Polystyrene, positive charge	Yes[B]	Thrombus, early	Nemmar et al. (2002) Silva et al. (2005)
60	Polystyrene, negative charge	?	No thrombus	Nemmar et al. (2002)
80	Iridium	Yes[A]	Extrapulmonary organs	Kreyling et al. (2002)
240	Polystyrene, lecithin	Yes	Monocyte	Kato et al. (2003)
240	Polystyrene, uncoated	No		Kato et al. (2003)
400	Polystyrene	No	No thrombus	Nemmar et al. (2004)
	Surface coating (chemistry) charge, size govern translocation			

[A]minimal; [B]indirect evidence

vesicles that are thought to function as transport pathways across the cells for macromolecules (Patton 1996). Knowledge from virology about cell entry of biologic NSPs (viruses) via clathrin-coated pits and caveolae mechanisms should also be considered (Smith and Helenius 2004) and can shed light on the mechanism by which engineered NPs may enter cells and interact with sub-cellular structures.

Evidence in humans for the translocation of inhaled NPs into the blood circulation is ambiguous, with one study showing rapid appearance in the blood and significant accumulation of label in the liver of humans inhaling ^{99}Tc-labeled 20 nm carbon particles (Nemmar et al. 2002), whereas other studies using the same labeled particles reported no such accumulation (Brown et al. 2002; Mills et al. 2006). Taking into consideration all of the evidence from animal and human studies for alveolar translocation of NPs, it is likely that this pathway also exists in humans; however, the extent of extrapulmonary translocation is highly dependent on particle surface characteristics/chemistry, in addition to particle size. Translocation to the blood circulation could provide a mechanism for a

direct particle effect on the cardiovascular system as an explanation for epidemiologic findings of cardiovascular effects associated with inhaled ambient UFPs (Pekkanen et al. 2002; Wichmann et al. 2000) and for results of clinical studies showing vascular responses to inhaled elemental carbon UFPs (Pietropaoli et al. 2004). In addition to direct alveolar translocation of NSPs, cardiovascular effects may also be the corollary of a sequence of events starting with particle-induced alveolar inflammation initiating a systemic acute phase response with changes in blood coagulability and resulting in cardiovascular effects (Seaton et al. 1995) (Fig. 7).

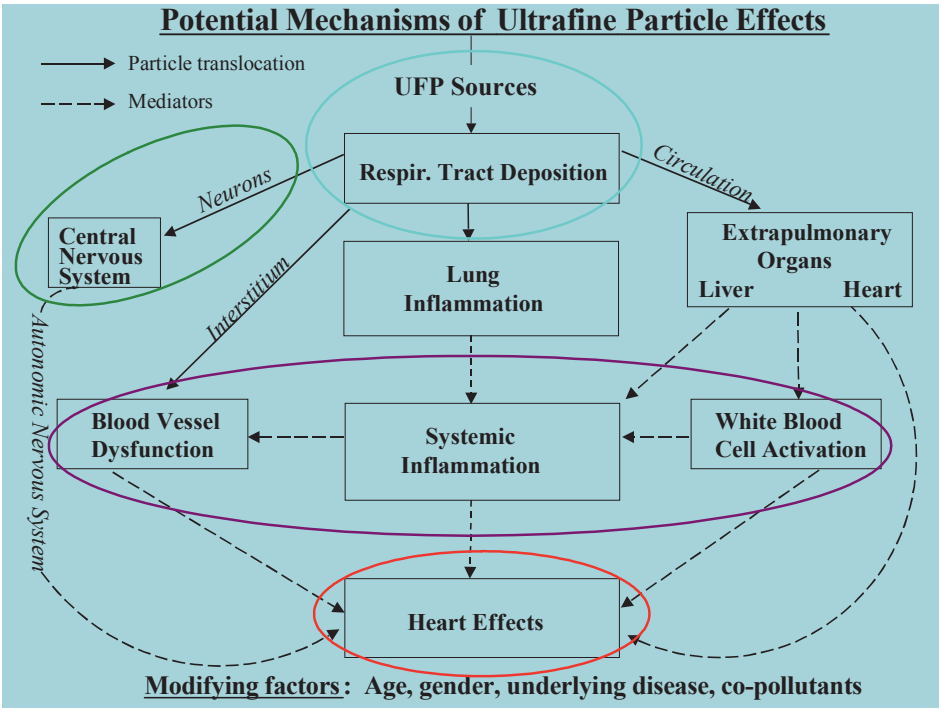


Figure 7. Potential mechanisms of ultrafine particle effects.

Once NSPs have translocated to the blood circulation, they can be distributed throughout the body. The liver is the major distribution site via uptake by Kupffer cells, followed by the spleen as another organ of the reticuloendothelial system, although coating with polyethylene glycol (PEG) almost completely prevents hepatic and splenic localization so that other organs can be targeted (Akerman et al. 2002). Distribution to heart, kidney, and immune-modulating organs (spleen, bone marrow) has been reported. For example, several types of NPs, ranging from 10 to 240 nm, localized to a significant degree in bone marrow after intravenous injection into mice (Table 5).

TABLE 5. Translocation of Nano-Sized Particles in the Blood Circulation to Bone Marrow In Mice.

Particle size (nm)	Type	Finding	Reference
~10	PEG-quantum dots	Fast appearance of QDs in liver, spleen, lymph nodes, and bone marrow (mouse)	Ballou et al. (2004)
<220	Metallo-fullerene	Highest accumulation in bone marrow after liver; continued increase in bone marrow but decreases in liver (mouse)	Cagle et al. (1999)
90–250	HAS-coated polylactic acid nanoparticles	Significant accumulation in bone marrow, second to liver (rat)	Bazile et al. (1992)
240	Polystyrene (non-biodegradable) polylisohexylcyonacrylate (biodegradable)	Rapid passage through endothelium in bone marrow, uptake by phagocytizing cells in tissue (mouse)	Gibaud et al. (1996, 1998, 1994)

Such target specificity may be extremely valuable for drug delivery; for example, drug delivery to the CNS via blood-borne NPs requires NP surface modifications in order to facilitate translocation across the tight blood–brain barrier via specific receptors (e.g., apolipoprotein coating for LDL-receptor–mediated endocytosis in brain capillaries) (Kreuter 2001, 2004; Kreuter et al. 2002). Such highly desirable properties of NPs must be carefully weighed against potential adverse cellular responses of targeted NP drug delivery, and a rigorous toxicologic assessment is mandatory.

2.1.4. Neuronal Uptake and Translocation

A translocation pathway via neuronal structures for solid particles in the respiratory tract involving neuronal axons is apparently specific for NPs. Respective studies are summarized in Table 6.

TABLE 6. Studies of Neuronal Translocation of Nano-sized Particles from Respiratory Tract.

1941	*Bodian and Howe*: *Olfactory* axonal transport of Polio-virus (30 nm) after intranasal instillation in chimpanzee. Transport velocity: 2.4 mm/h
1970	*de Lorenzo*: *Olfactory* axonal transport of 50 nm colloidal gold after intranasal instillation in squirrel monkey. Transport velocity: 2.5 mm/h
1998	*Hunter and Undem*: Rhodamine-labeled 40 nm microspheres translocation via sensory nerves of *TB region* to ganglion nodosum in hamster after intratracheal instillation.
1999	*Hunter and Dey*: Retrograde tracing of trigeminal neurons from nasal epithelium with Rhodamine-labelled ~40 nm microspheres after intratracheal instillation.
2004	*Oberdörster et al*.: 13C particles (CMD ~36 nm) translocation to *olfactory* bulb after inhalation exposure in rats.
2006	*Elder et al*.: Mn-oxide particles (CMD ~30 nm) inhalation exposure in rats; increased Mn and inflammatory response in olfactory bulb.

This pathway was described more than 60 years ago, yet it has received little or no attention from toxicologists. This pathway comprises sensory nerve endings of the olfactory and the trigeminus nerves and an intricate network of sensory nerve endings in the tracheobronchial region. These early studies concerned a large series of studies with 30-nm polio virus intranasally instilled into chimpanzees and rhesus monkeys (Bodian and Howe 1941a, b; Howe and Bodian 1940). Their studies revealed that the olfactory nerve and olfactory bulbs are, indeed, portals of entry to the CNS for intranasally instilled nanosized polio virus particles, which could subsequently be recovered from the olfactory bulbs. The close proximity of nasal olfactory mucosa and olfactory bulb requires only a short distance to be covered by neuronal transport (Fig. 8). Bodian and Howe (1941b) determined the transport velocity of the virus in the axoplasm of axons to be 2.4 mm/h, which is very well in agreement with neuronal transport velocities measured later by Adams and Bray (1983) for solid particles (up to 500 nm) directly microinjected into giant axons of crabs, and by de Lorenzo (1970) for silver-coated colloidal gold (50 nm) in squirrel monkeys.

Olfactory Nerve Translocation Pathway

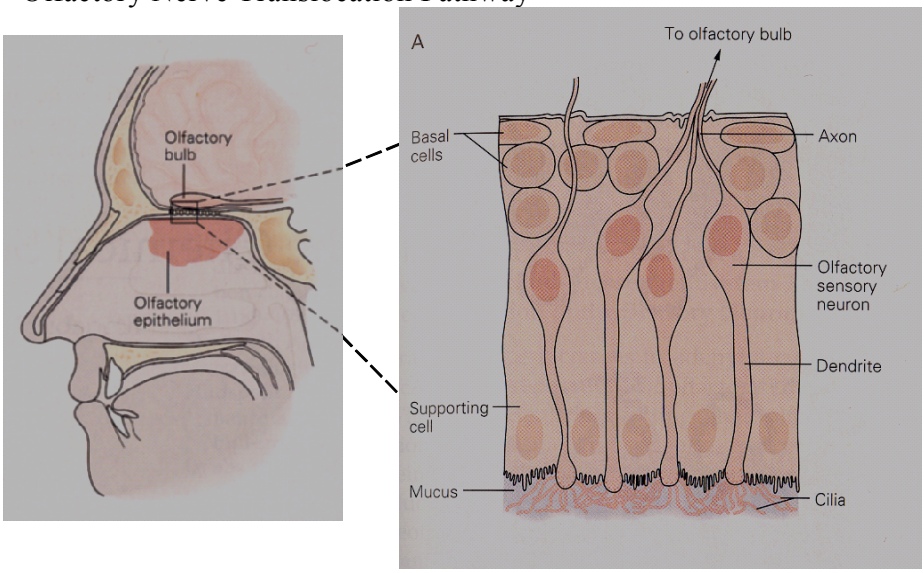


Figure 8. Close proximity of olfactory mucosa to olfactory bulb of the CNS. Inhaled NP, especially below 10 nm, deposit efficiently on the olfactory mucosa by diffusion, similar to airborne "smell" molecules which deposit in this area of olfactory dendritic cilia. Subsequent uptake and translocation of solid NP along axons of the olfactory nerve has been demonstrated in nonhuman primates and rodents. Surface chemistry of the particles may influence their neuronal translocation. (From Kandel et al. 2000.)

The de Lorenzo (1970) study demonstrated in squirrel monkeys that intranasally instilled silver-coated colloidal gold particles (50 nm) translocated anterogradely in the axons of the olfactory nerves to the olfactory bulbs. The 50 nm gold particles even crossed synapses in the olfactory glomerulus to reach mitral cell dendrites within 1 h after intranasal instillation. An interesting finding in this study – and important for potential adverse effects – was that the NPs in the olfactory bulb were no longer freely distributed in the cytoplasm but were preferentially located in mitochondria.

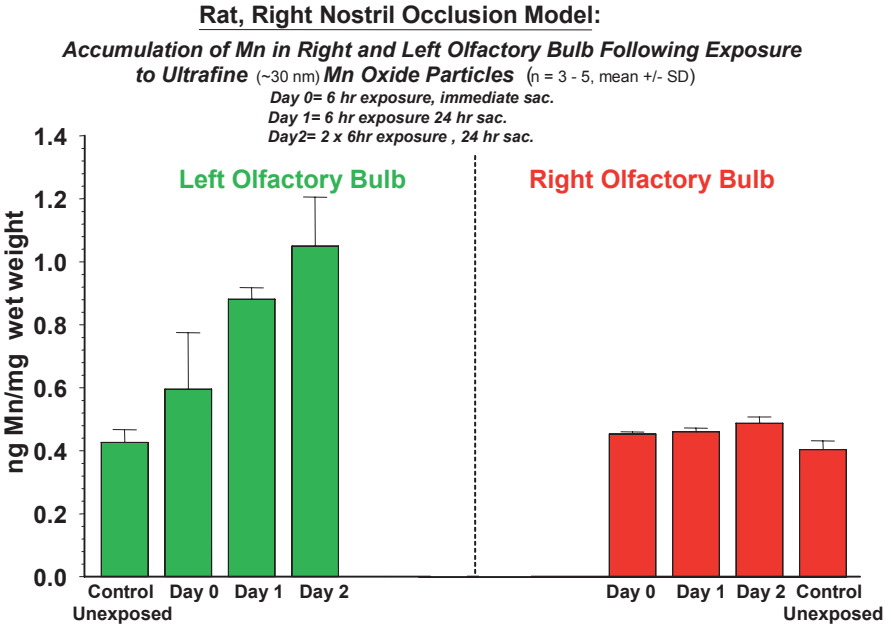


Figure 9. Occlusion of the right nostril of rats during 6 h inhalation of nanosized Mn-oxide particles (~30 nm CMD, ~450 μg/m^3) resulted in accumulation of Mn only in the left olfactory bulb only at 24 h after dosing. (From Elder et al. 2006.)

Newer studies indicated that this translocation pathway is also operational for inhaled NPs. Inhalation of elemental ^{13}C UFPs (CMD = 35 nm) resulted in a significant increase of ^{13}C in the olfactory bulb on day 1, which increased further throughout day 7 postexposure (Oberdörster et al. 2004). Results of another inhalation study with solid nanosized (CMD = 30 nm) manganese oxide particles in rats showed after a 12 day exposure to ~450 μg/m^3 (6 h/day, 5 days/week) a more than 3.5-fold significant increase of Mn in the olfactory bulb, compared with only a doubling of Mn in the lung. When one nostril was occluded during a 6-h exposure, Mn accumulation in the olfactory bulb was

restricted to the side of the open nostril only (Fig. 9) (Elder et al. 2006). This result contrasts with 15 day inhalation of larger-sized MnO_2 particles in rats (1.3 and 18 μm mass median aerodynamic diameter) where no significant increases in olfactory Mn was found (Fechter et al. 2002). This was to be expected given that the individual axons of the fila olfactoria (forming the olfactory nerve) are only 100–200 nm in diameter (de Lorenzo 1957; Plattig 1989). The most recent study by Elder et al. (2006) with inhaled Mn-oxide also showed significant increases of TNF-α message and protein in the olfactory bulb (Fig. 10).

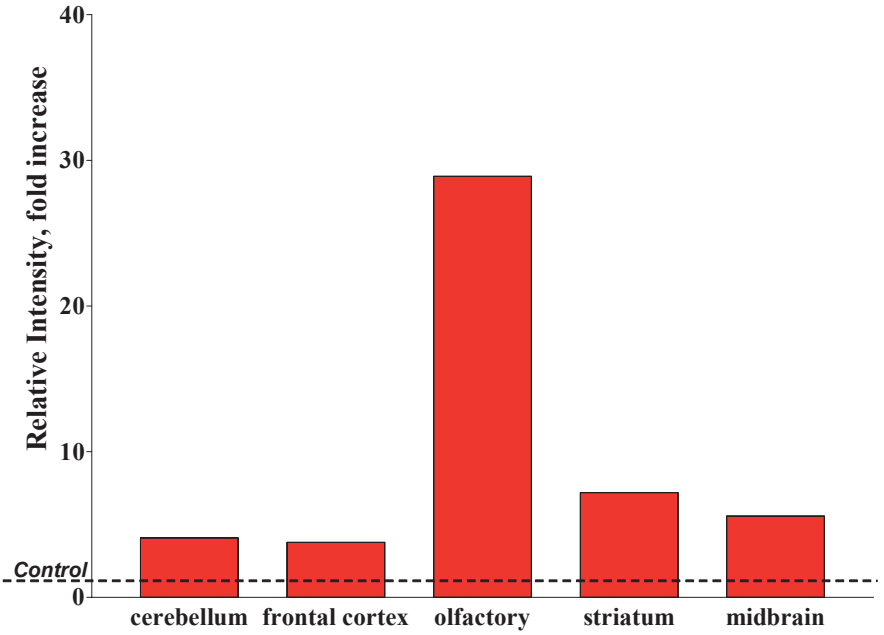


Figure 10. Brain region TNF-α protein expression changes after 12 days of exposure to ultrafine Mn-oxide in rats. (From Elder et al. 2006.)

Translocation into deeper brain structures may possibly occur as well, as shown in rats for soluble Mn (Gianutsos et al. 1997), was not observed in a recent inhalation study with soluble Mn in monkeys (Dorman et al. 2006). Further evidence for movement of NPs along axons and dendrites in humans is provided by knowledge accumulated by virologists who have long understood the movement of human meningitis virus through olfactory and trigeminal neurons and, the trigeminal neuron to trigger outbreaks of herpes cold sores in humans (Kennedy and Chaudhuri 2002; Terasaki et al. 1997).

There are additional neuronal translocation pathways for solid NPs via the trigeminus nerve and tracheobronchial sensory nerves (Table 6). A study by Hunter and Dey (1998) in rats demonstrated the translocation of intranasally instilled rhodamine-labeled microspheres (20–200 nm) to the trigeminal ganglion inside the cranium via uptake into the ophthalmic and maxillary branches of the trigeminus nerve that supplies sensory nerve endings throughout the nasal mucosa. In another study, Hunter and Undem (1999) instilled the same microparticles intratracheally into guinea pigs; they found neuronal translocation of these solid microparticles to the ganglion nodosum in the neck area that is networked into the vagal system. This finding may be relevant for ambient UFPs because it can be hypothesized that cardiovascular effects associated with ambient particles in epidemiologic studies (Utell et al. 2002) are in part due to direct effects of translocated UFPs on the autonomic nervous system via sensory nerves in the respiratory tract.

In the context of potential CNS effects of air pollution, including ambient UFPs, two recent studies with exposures of mice to concentrated ambient fine particles and UFPs should be mentioned. Campbell et al. (2005) and Veronesi et al. (2005) found significant increases of tumor necrosis factor-α or decreases in dopaminergic neurons, supporting the hypothesis of ambient PM causing neurodegenerative disease. A study by Calderon-Garcidueñas et al. (2002) may also point to an interesting link between air pollution and CNS effects: these authors described significant inflammatory or neurodegenerative changes in the olfactory mucosa, olfactory bulb, and cortical and subcortical brain structures in dogs from a heavily polluted area in Mexico City, whereas these changes were not seen in dogs from a less-polluted rural control city. However, whether direct effects of airborne UFPs are the cause of these effects remains to be determined.

Although the existence of neuronal translocation of NPs has been well established, size alone is only one particle parameter governing this process. Surface characteristics of NPs (chemistry, charge, shape, aggregation) are essential determinants as well, and it should not be assumed that all NPs, when inhaled, will be distributed by the mechanism described here. It should be kept in mind, however, that the unique biokinetic behavior of NPs – endocytosis, transcytosis, neuronal and circulatory translocation and distribution – which makes them desirable for medical therapeutic or diagnostic applications – may be associated with potential toxicity. For example, NP-facilitated drug delivery to the CNS raises the question of the fate of NPs after their translocation to specific cell types or to subcellular structures in the brain. For example, does mitochondrial localization induce oxidative stress? How persistent is the coating or the core of the NPs? A respective safety evaluation is key.

3. Exposure via Skin

A potentially important uptake route is through dermal exposure (Table 7).

TABLE 7. Skin as a Portal-of-Entry for Nanoparticles.

Translocation pathways to living tissue
• *across cells of stratum corneum*
• *between cells of stratum corneum*
• *via hairshaft follicle*
• *via sweat glands*
• *through inflamed /injured skin*
Distribution from epidermal/dermal region
• *lymphatic vessels*
• *blood vessels*
–venous
–arterial
–sensory neurons
–touch
–pain
–pressure
–warmth

The epidermis, consisting of the outer horny layer (stratum corneum), the prickle cell layer (stratum spinosum), and basal cell layer (stratum basale), forms a very tight protective layer for the underlying dermis (Fig. 11). The dermis has a rich supply of blood and tissue macrophages, lymph vessels, dendritic cells (Langerhans, also in stratum spinosum of epidermis), and five different types of sensory nerve endings. Broken skin represents a readily available portal of entry even for larger (0.5–7 μm) particles, as evidenced by reports about accumulation of large amounts of soil particles in inguinal lymph nodes of people who often run or walk barefoot; this can be associated with elephantiatic lymphedema (podoconiosis) (Corachan et al. 1988; Blundell et al. 1989). Tinkle et al. (2003) hypothesized that unbroken skin when flexed–as in wrist movements–would make the epidermis permeable for NPs. They demonstrated in a proof-of-concept experiment that, indeed, flexing the skin, but not flat skin, resulted in penetration of even 1 μm fluorescent beads to the dermis. The follow-up question about access of particles in the dermis to the circulation is answered by the aforementioned reports of podoconiosis, that is, uptake into the

lymphatic system and regional lymph nodes. Subsequent translocation of NPs beyond lymph nodes to the blood circulation is likely to occur as well, as shown in studies with small asbestos fibers (Oberdörster et al. 1988).

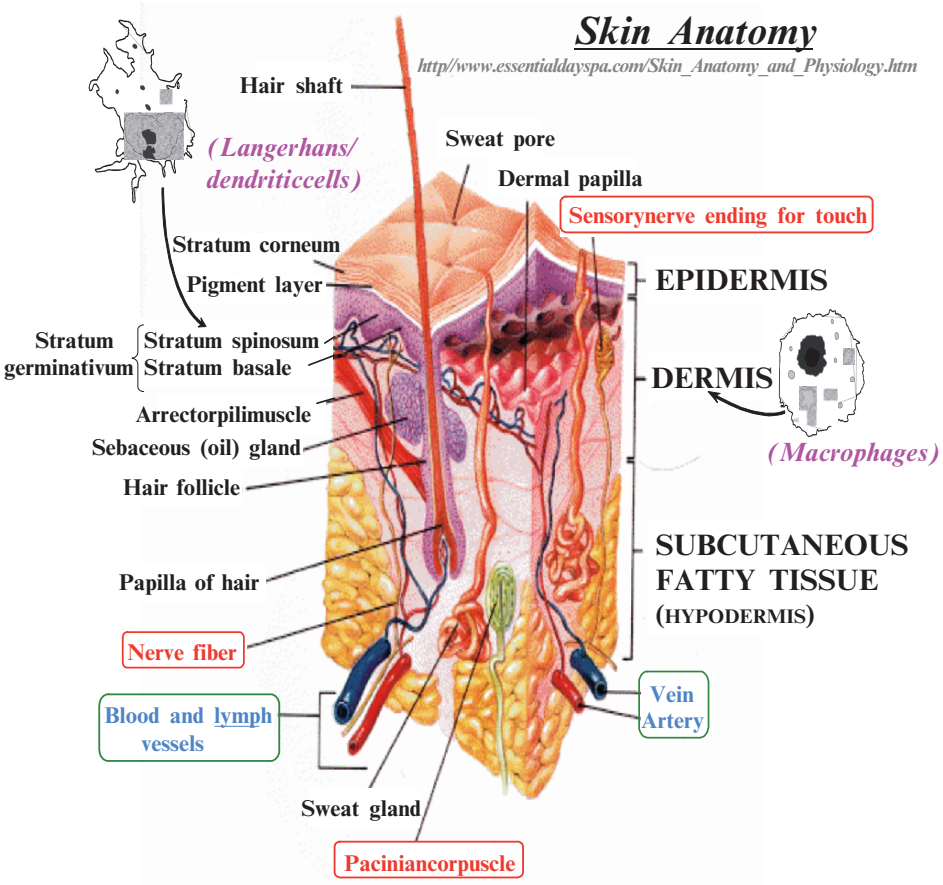


Figure 11. The epidermis represents a tight barrier against NSP penetration. Quantitatively, dermal translocation will, therefore, be minimal or nonexistent under normal conditions but increases in areas of skin flexing (Tinkle et al. 2003) and broken skin. Once in the dermis, lymphatic uptake is a major translocation route, likely facilitated by uptake in dendritic cells (epidermis) and macrophages; other potential pathways may include the dense networks of blood circulation and sensory nerves in the dermis. (Figure adapted from: http/www.essential dayspa.com/Skin_Anatomy_and_ Physiology.htm).

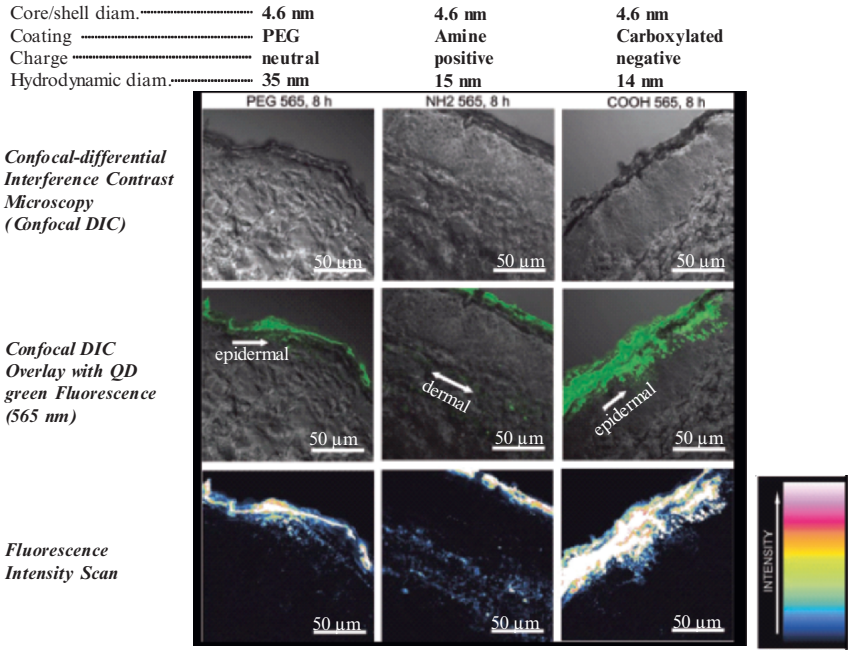
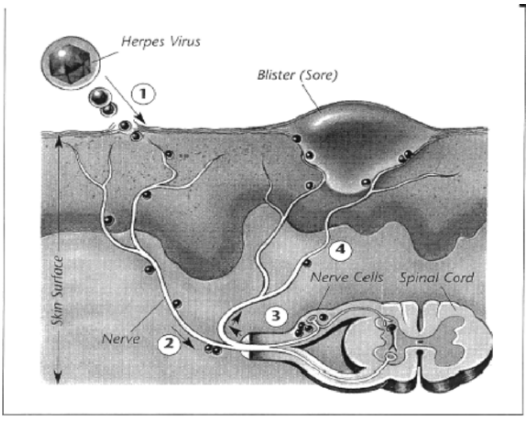


Figure 12. Quantum dots (*Invitrogen*) penetration of pig skin after 8 h treatment. (From Ryman-Rasmussen et al. 2006.)

A recent study by Ryman-Rasmussen et al. (2006) demonstrated translocation of quantum dots in a pig skin ex vivo model (Fig. 12): Quantum dots of neutral, positive and negative charge were found to penetrate to dermis and epidermis, however, the amount being translocated within 8 h could not be quantitated and is likely to be very low. Using confocal laser scanning microscopy, Verma et al. (2003) also showed that nano liposomes, depending on their size, did penetrate human abdominal skin.

Recent studies by Kim et al. (2004) in mice and pigs with intradermally injected near infrared quantum dots confirmed that NPs, once in the dermis, will localize to regional lymph nodes, which makes these particles very useful for *in vivo* imaging. Likely transport mechanisms to the lymph nodes are skin macrophages and dendritic (Langerhans) cells (Ohl et al. 2004; Sato et al. 1998); this raises a question about potential modulation of immune responses, after interaction of these NP-containing macrophages and dendritic cells with T lymphocytes. For example, Chen et al. (1998) were able to raise antibodies in mice specific for C60 after intraperitoneal injections of C60 conjugated to thyroglobulin and serum albumin. Clearly, research is needed to determine whether and under what conditions NPs can be recognized by the immune

system, following any route of uptake into the organism. Another question relates to the potential of sensory skin nerves to take up and translocate NPs. Given that this mechanism has been demonstrated for the nasal and tracheobronchial regions of the respiratory tract, how likely is this to occur in the dermis layer of the skin with its dense supply of different types of considering data on neuronal uptake and translocation of NPs after intramuscular injection. For example, nanosized ferritin and iron-dextran, after injection into the tongue of mice, labeled the neurons of the hypoglossal nuclei, and injection of both of these NPs into facial muscles of mice also resulted in synaptic uptake; cationized ferritin was also detected in cell bodies of facial neurons, indicating that electrical charge is of importance for incorporation into axons and axonal transport (Arvidson 1994; Malmgren et al. 1978; Olsson and Kristensson 1981). Other studies using intramuscular injection of ferritin (~112 nm), irondextran (11 or 21 nm), and gold protein (20–25 nm) NPs also showed rapid penetration through the basal lamina into the synaptic clef of the neuromuscular junction, but this was restricted to only the smaller NPs, implying that there may be a size-dependent penetration of the basal lamina with a threshold somewhere between 10 and 20 nm (Oldfors and Fardeau 1983).



The herpes virus passes through your skin (*1*). It travels through your body (*2*) and settles at nerve cells near your spine (*3*). When something triggers a new bout of herpes, the virus leaves its resting place and travels along the nerve, back to the surface of the skin (*4*).

Figure 13. Dermal translocation of nanosized materials may follow the same pathways taken by viruses. (From: "Planning your Pregnancy and Birth" 3rd edn. American College of OB/GYN.)

Neuronal transport of NPs along sensory skin nerves is well established for herpes virus (Fig. 13). After passing through the skin – especially broken skin –

the viruses are transported retrogradely along dendrites of sensory neurons to the dorsal root ganglion, where they remain dormant until a stress situation triggers anterograde translocation along the dendrites back to the skin (Kennedy and Chaudhuri 2002; Terasaki et al. 1997). Future studies need to determine whether and to what degree such translocation along sensory skin neurons also occurs with NPs penetrating the epidermis.

4. Subcellular Distribution

NPs can enter cells via different endocytotic mechanisms, as outlined in previous sections, involving specific sites on the cell's surface such as caveolae or clathrin-coated pits. Newer data also demonstrated non-endocytotic diffusional cell uptake for nano-sized particles (Geiser et al. 2005). Once in the cytoplasm, there are several possibilities of interacting with subcellular structures, the cytoskeleton, the mitochondria, the endoplasmic reticulum, the golgi apparatus, and the nucleus itself. Localization of nano-sized materials in mitochondria after entry into cells has been shown by several investigators (Table 8; Fig. 14).

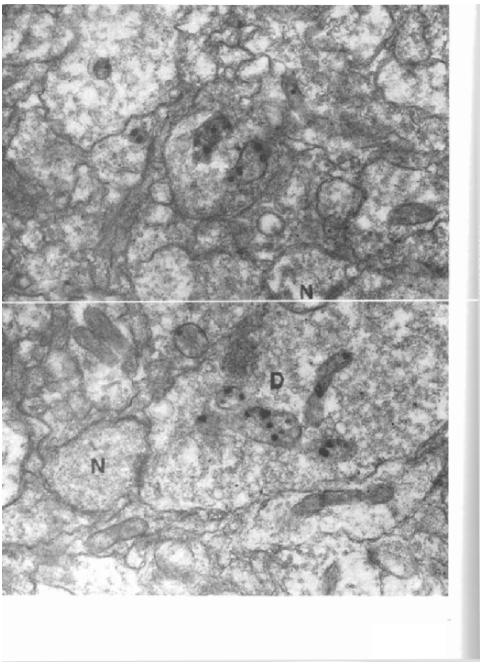


Figure 14. Squirrel monkey, intranasal colloidal gold particles (50 nm): Translocation to mitochondria of dendrites in mitral cells (D) of olfactory bulb after crossing the olfactory nerve (N)/mitral cell synapse. (From de Lorenzo, 1970.)

TABLE 8. Mitochondrial Localization after Dosing with Nano-sized Particles.

Material and Cell Type	*Reference*
Gold nanoparticles, squirrel monkey mitral cells of olfactory bulb	de Lorenzo (1970)
Colloidal gold, Rhesus monkey, sustentacular cells of olfactory mucosa	Gopinath et al. (1978)
Fullerene derivative, *in vitro*, fibroblast cell line	Foley et al. (2002)
Ambient UFP, *in vitro*, macrophage cell line	Li et al. (2003)
Micellar nanocontainers (*Block copolymer micelles*), *in vitro*, pheochromocytoma cells	Rodoslav et al. (2003)

4.1. WILL OXIDATIVE STRESS BE INDUCED?

Depending on particle chemistry, they may induce oxidative stress responses even resulting in apoptotic response, as shown by Li et al. (2003) in vitro using a macrophage cell line exposed to ambient UFP.

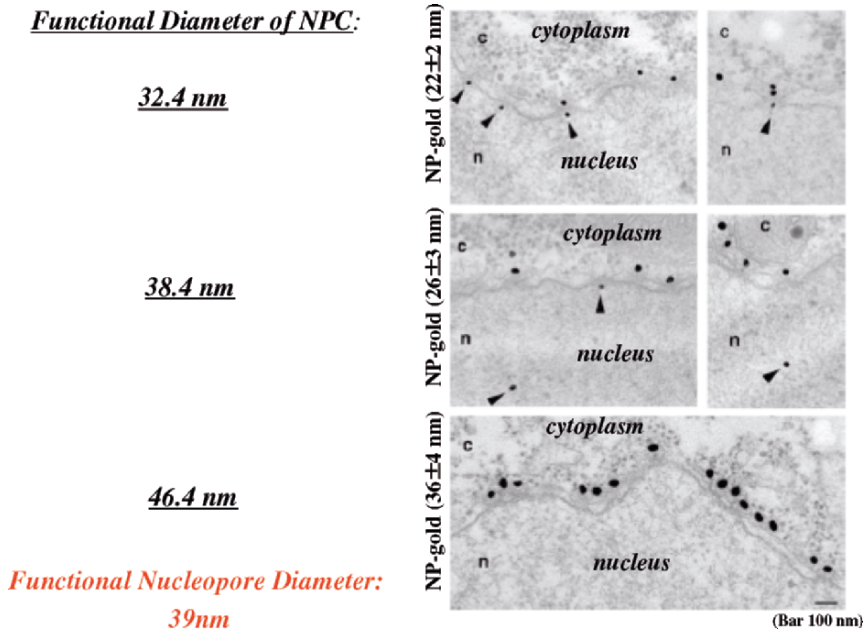
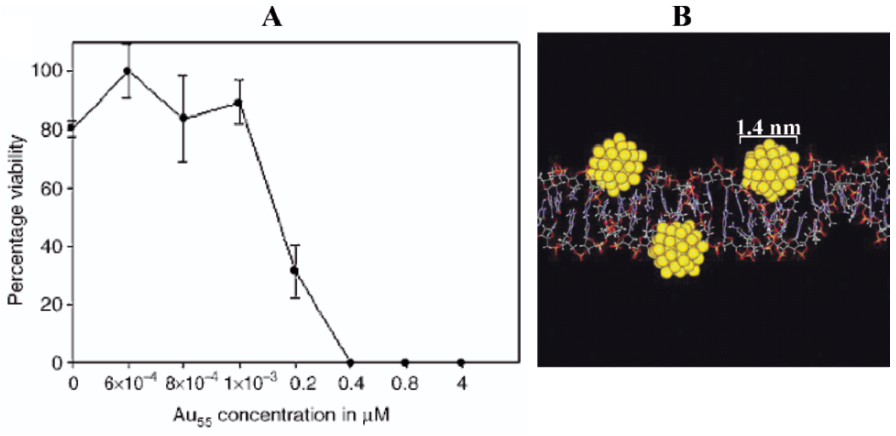


Figure 15. Nuclear import of nucleoplasmin-coated gold particles into oocyte nuclei via NPC. (From Panté and Kann, 2002.)

NP entry into the nucleus via the nucleopore complex (NPC) is another possibility. Using different sizes of nanogold particles, Panté and Kann (2002) determined the functional diameter of the NPC to be 39 nm (Fig. 15). Subsequently, Tsoli et al. (2005) reported that melanoma cells incubated with 1.4 nm gold particles (consisting of only 55 gold atoms) showed concentration dependent increased cytotoxicity, associated with nuclear disposition of these NPs. In fact, 50% of the gold particles in the nucleus was irreversibly attached to the major grooves of B-DNA (Fig. 16). The authors attribute the observed high cytotoxicity to the linking of the 1.4 nm particles to DNA. Confirmatory *in vivo* studies still need to be carried out; it would be of interest to determine as to whether the finding of gold–DNA interaction is specific for gold as the most electronegative metal linking with the negatively charged vicinity in the DNA groove (Tsoli et al. 2005)



A: Cytotoxicity of Au_{55} particles in BLM melanoma cells, 72h incubation
Cellular gold distribution:
57.5% cytoplasm
42.5% cell nucleus (50% DNA)

B: Au_{55} particles irreversibly attached to major grooves of B-DNA

Figure 16. Cellular uptake and toxicity of Au_{55} nanoparticles (1.4 nm). (From Tsoli et al. 2005.)

5. Summary and Outlook

The biokinetics of NPs are different from larger particles. When inhaled, they are efficiently deposited in all regions of the respiratory tract; they evade

specific defense mechanisms; and they can translocate out of the respiratory tract via different pathways and mechanisms involving endocytosis and transcytosis, including uptake and transport via neuronal structures and distribution via the blood circulation. When in contact with skin, there is evidence of penetration to the dermis followed by translocation via lymph to regional lymph nodes. A possible uptake into sensory nerves needs to be investigated. When ingested, systemic uptake via lymph into the organism can occur, but most ingested NP are excreted via feces. When in blood circulation, they can distribute throughout the organism, and they are taken up into liver, spleen, bone marrow, heart, and other organs. In general, translocation rates are

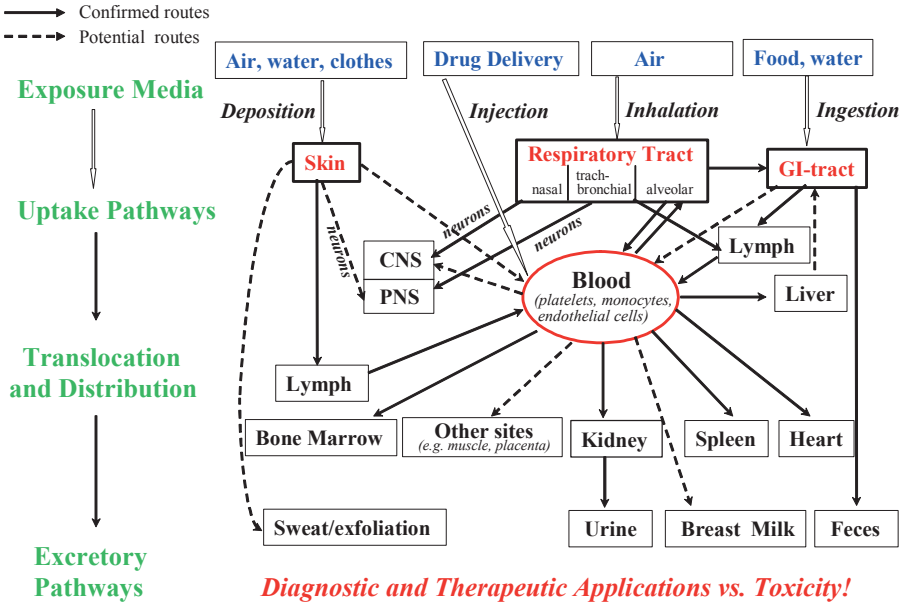


Figure 17. Biokinetics of NSP. While many uptake and translocation routes have been demonstrated, others still are hypothetical and need to be investigated. Largely unknown are translocation rates as well as accumulation and retention in critical target sites and their underlying mechanisms.These as well as potential adverse effects will be largely dependent on physicochemical characteristics of the surface and core of NSP. Both qualitative and quantitative changes in NSP biokinetics in a diseased or compromised organism need also to be considered.

largely unknown; they are probably very low but are likely to change in a compromised/diseased state (Fig. 17).

The biologic activity and biokinetics are dependent on many parameters: size, shape, chemistry, crystallinity, surface properties (area, porosity, charge, surface modifications, weathering of coating), agglomeration state, biopersistence, and dose. These parameters are likely to modify responses and cell interactions, such as a greater inflammatory potential than larger particles per given mass, translocation across epithelia from portal of entry to other organs, translocation along axons and dendrites of neurons, induction of oxidative stress, pro-oxidant, and antioxidant activity of NPs in environmentally relevant species, binding to proteins and receptors, and localization in mitochondria.

The principles of cellular and organismal interactions discussed in this article should be applicable for both ambient UFPs and NPs, even if the latter are coated with a biocompatible material. Knowledge about the biopersistence of this coating is as essential as is knowledge about the bioavailability of the core material that could have intrinsic toxic properties, for example, semiconductor metal compounds in sub-10 nm quantum dots consisting of cadmium and lead compounds. The very small size of these materials makes them available to the same translocation processes described for polydisperse UFPs, possibly even in a more efficient way because of their uniform size. When studying biologic/toxicologic effects, new processes of interactions with subcellular structures (e.g., microtubuli, mitochondria) will likely be discovered. The diversity of engineered nanomaterials – whether in medicine or in industrial applications – and of the potential effects represents major challenges and research needs for nanotoxicology, including also the need for assessing human exposure during manufacture and use. Identifying a hazard of a specific nanomaterials through appropriate toxicity testing, including dose–response assessment, by having some knowledge about the human exposure to this nanomaterials, will allow to characterize an associated risk, and if necessary to establish risk management measures (Fig. 18). For the risk assessment process, it is important to also consider the more susceptible parts of the population, the very young, the elderly and those with a compromised organ system, a lesson learned from epidemiological studies of the effects of fine particulate air pollution (Pope and Dockery, 2006). The goal to exploit positive aspects of engineered nanomaterials and avoid potential toxic effects can best be achieved through a multidisciplinary team effort involving researchers in toxicology, materials science, medicine, molecular biology, bioinformatics, and their subspecialties.

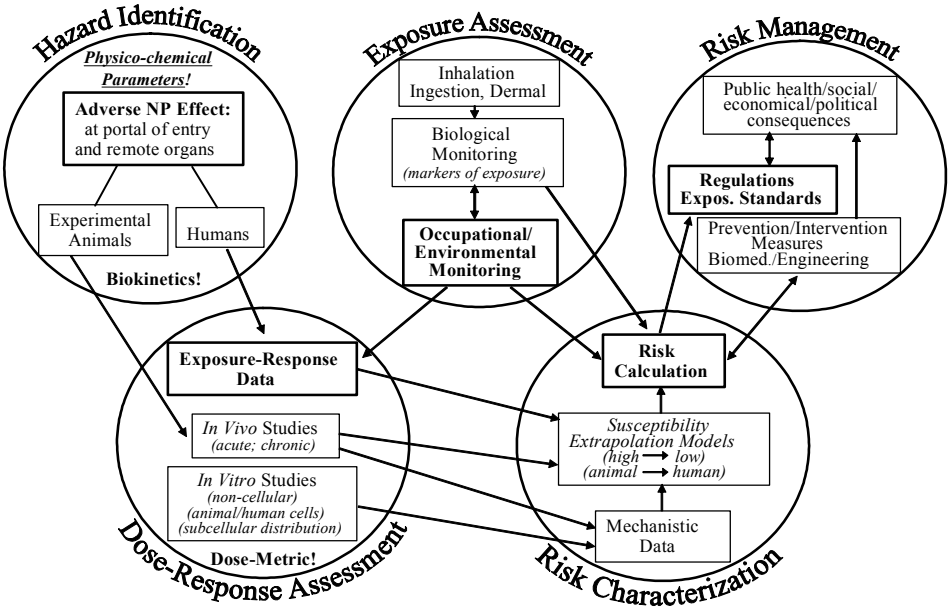


Figure 18. Risk assessment (NRC 1983) and risk management paradigm for engineered nanoparticles (NP). The four steps of risk assessment require answers to the questions: Do NP have adverse effects? What are the dose–response relationships? What are occupational/ environmental levels in different media? What is the calculated risk? Once a risk is determined, risk management decision can be established, including exposure standards and regulations and efforts for effective risk communication. (Modified from Oberdörster et al. 2004.)

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NANOPARTICLE EXPOSURE AND SYSTEMIC/CARDIOVASCULAR EFFECTS – EXPERIMENTAL DATA[1]

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Abstract: The most attractive properties of nanomaterials for medical and technological applications, including their small size, large surface area, and high reactivity, are also the main factors for their potential toxicity. Thus, some nanosized materials may induce not only a damage at the deposition site but also distant responses as a result of their transclocation and/or reactivity through the body. The exposure to low doses of these materials may modify the progression of existing pathophysiological conditions including cardiovascular diseases (CVD). In this respect, epidemiological and experimental studies have suggested an association between respiratory exposure to ambient ultrafine particles and the progression of cardiovascular disease. Our research efforts are currently directed to evaluate the cardiovascular effects, including vascular inflammation, blood cell coagulation status, atherosclerosis, as well as the related molecular mechanisms associated with respiratory exposure to different types of nanosized materials using animal models. Recently, we demonstrate that lung instillation of single wall carbon nanotubes (SWCNTs) is associated with a dose-dependent increase in oxidative vascular damage manifested by heme oxygenase-(HO-1) gene activation and mitochondrial alterations. Since these types of oxidative modifications are considered to play a role in atherogenesis, we further evaluated the effects of SWCNT respiratory exposure on atherosclerosis progression in ApoE–/– transgenic mice, a widely used model of human atherosclerosis. The accumulation of toxicological data on engineered nanomaterials will allow for development of adequate risk assessment and regulations.

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[1] Disclaimer: The findings and conclusions in this article are those of the authors and do not necessarily represent the view of the National Institute for Occupational Safety and Health.

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1. Introduction

Nanotechnologies will revolutionize our life including medicine, but they also pose important toxicological questions that are related to the unique nature of materials and processes at the nanometer scale. Human contact with nanomaterials can be related to targeted exposure through therapeutics and cosmetiscs or untargeted exposure through occupational and environmental contamination. The most attractive features of nanomaterials including their small size, large surface area, and reactivity might also be the main factors for their toxicity. In this regard, they may induce not only damage at the penetration site but also can lead to unexpected distant responses, involving the immune system, cardiovascular system, liver, kidney, and brain, as a result of their transclocation and reactivity through the body. Evidence exist that very small particles can partition into the blood or the central nerve system (rev. in Oberdörster et al. 2005). For example, ultrafine size air pollutants are thought to be involved not only in pulmonary toxicity but also cardiovascular dieases and mortality (rev. in Brook 2004).

Engineered nanosized particles are new materials of emerging technological importance in different industries (Colvin 2003; Hood 2004). The US National Science Foundation estimated that millions of workers would be needed to support nanotechnology industries worldwide within 15 years. One direction of the nanomaterial industries is developing new carbon nanomaterials. Carbon atoms can be arranged into diverse geometries, forming a number of stable nanostructures. For example, a graphene sheet can be folded, usually in the presence of metal catalyst, to form a long single wall carbon nanotube (SWNT) with a diameter of ~1 μm. Bare carbon atoms can also be organized into spherical structures as fullerenes (buckyballs). The most stable and readily available fullerene is C_{60} having an average diameter of 0.72 nm. In addition to these single layer structures, large nanotubes and fullerenes can also be synthesized forming multiwall nanotubes (MWNT) or onion-like clusters, respectively (Park et al. 2003). Fullerenes, because of their strong electronegativity, can be combined with metals and other molecules to form for example metallofullerens.

The current state of toxicologic science and knowlege provides opportunities to study the toxic effects of nanomaterials in parallel with their development and discovery of their potential use. In order to facilitate the hazard evaluation of new nanomaterials, complex and systemic studies, including pharmacokinetic, cellular, molecular, physiological tests, and mathematical modeling should be

conducted as a part of multidisciplinary integrative research. The accumulation of toxicologic data on engineered nanomaterials will allow for development of adequate risk assessment and regulations.

At the National Institute of Occupational Safety and Health, we developed an integrative research program for evaluation of nanomaterial toxicity related to possible occupational exposure (http://www.cdc.gov/niosh/topics/nanotech). This collaborative multidisciplinary program provides unique possibilities for evaluation pulmonary and systemic toxicity of engineered nanomaterials. The methodological approaches, tested under this program, will help for the development of predictive tests for estimation of the toxicity of new nanomaterials based on their physicochemical characteristics, potential to induce oxidative stress, inflammation, specific pulmonary and systemic toxicity.

2. Respiratory Particulate Matter (PM) Exposure and Cardiovascular Toxicity

In addition to the well-established cardiovascular risk factors, such as high cholesterol levels, diabetes mellitus, and hypertension, many nontraditional risk factors, including concomitant infections, systemic autoimmune diseases, and chemical exposure, have been suggested to influence atherosclerotic process and precipitate disease complications (Libby 2000b; Ross 1999; Simeonova and Luster 2004). In this respect, epidemiological and experimental studies have recently found a positive association between air pollution and adverse cardiovascular outcomes (Brook et al. 2004; Kunzli et al. 2005; Peters et al. 2004; Pope et al. 2004). Although the putative biological mechanisms and factors linking air pollution to heart diseases remain not well understood, it is well accepted that PM respiratory exposure, specifically the smaller size PMs ("thoracic particles" PM_{10} <10 μm in aerodynamic diameter, "fine particles" $PM_{2.5}$ <2.5 mm, and "ultrafine particles" UFPs <0.1 μm), plays a significant role in the risk for cardiovascular disease and mortality. Short-term exposure to elevated PMs has been associated with increased acute cardiovascular mortality, particularly with at-risk subset of population, while pro-longed exposure has been considered a causative factor for atherosclerosis and reduced life expectancies (rev. in Brook et al. 2004). For example, recent epidemiological studies reported an association between $PM_{2.5}$ and overall cardiovascular mortality including death from ischemic heart disease (IHD), a consequence of atherosclerosis (Pope et al. 2004) as well as increased carotid intima-media thickness, a direct measure of atherosclerosis (Kunzli et al. 2005). Consistently, hyperlipidemic rabbits exposed to PM_{10} or ApoE–/– mice exposed to $PM_{2.5}$ develop advanced coronary and/or aortic atherosclerosis (Chen and Nadziejko 2005; Sun et al. 2005). The small size particles have been found to travel into the systemic circulation after pulmonary

experimental exposure (Nemmar et al. 2002; Oberdörster, et al. 2002). Evidence from human and toxicological exposure studies suggest that oxidative stress and inflammation is most likely involved in particle-mediated cardiovascular effects (Barclay et al. 2005; Brook et al. 2004; Sun et al. 2005).

Transition metals such as iron are considered to play a significant role in particle toxicity probably through "Fenton reactions" (Barchowsky and O'Hara 2003; Yuan and Brunk 1998). These reactions are involved in generation of exacerbated oxidative stress and related inflammatory responses. Transition metals may affect the cardiovascular system indirectly (through pulmonary inflammation or pulmonary neural reflexes) or directly (through penetration of particles or soluble metals into blood circulation). The plausibility for direct vascular effects of PM-associated metals is supported by the epidemiologically demonstrated association between increased blood cadmium levels as a result of cigarette smoking and accelerated peripheral atherosclerosis (Navas-Acien et al. 2004). Recently, it has been reported that long-term inhalation exposure to particles of combustion – derived fugitive emission induces myocardial injury in rats susceptible to spontaneous cardiomyopathy, Wistar-kyoto (WKY) rats, and water-leachable zinc has been suggested as involved in this cardiotoxicity (Kodavanti et al. 2003). Additionally, metals such as arsenic and lead have been related to cardiovascular effects (Nash et al. 2003; Simeonova and Luster 2004).

3. Nanoparticles – Hypothesis and Research Approaches

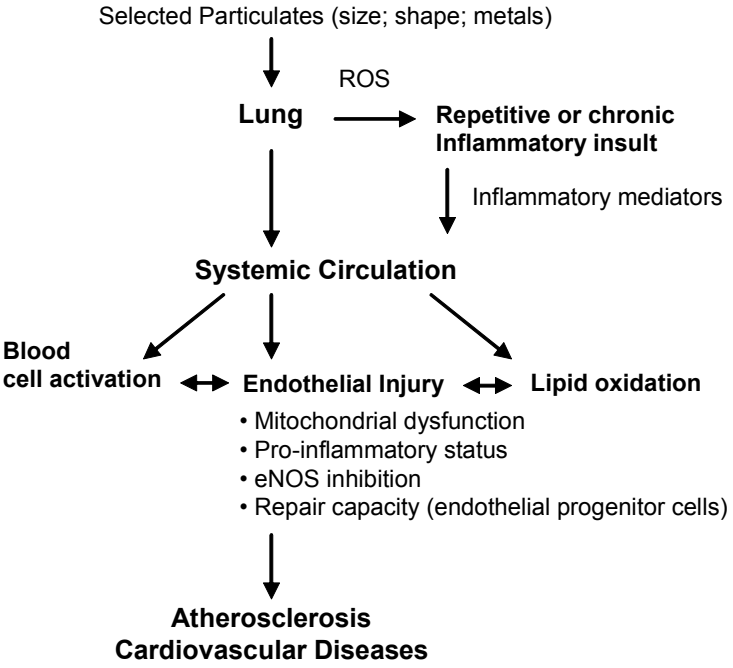
Long-term occupational respiratory exposure to nanoparticles, in addition to its pulmonary toxicity, may induce systemic effects of oxidative stress and inflammation which can modify the cardiovascular diseases (CVD) related to atherosclerosis. The unique physical characteristics (shape, size, surface area) and the metal constituents of these particles are major predictors for vascular toxicity. Smaller particles have greater deposition in the deep lung, a high surface area-to-mass ratio and prolonged clearance, potentially leading to lung inflammatory responses. The transition metals of the particles may accelerate the reactive oxygen species (ROS)-driven pulmonary toxicity. As a result, mediators might be released into the circulation leading to impaired endothelial as well as blood cell homeostasis (coagulation activation). Furthermore, nanoparticles may penetrate into the circulatory system and induce direct vascular toxicity. All stages of atherosclerosis can be modified by oxidative stress and inflammation. The primary underlying pathophysiological mechanisms of particle-mediated atherogenic effects might include the following: induction of oxidative-sensitive transcription factors and related inflammatory mediators (cytokines, chemokines, specifically monocyte chemoattractant protein

(MCP-1)-CCR2 axis, acute phase proteins); oxidative/inflammatory modifications in the endothelium (an altered ratio of oxidants/antioxidants, mitochondrial damage, apoptosis, eNOS inhibition; expression of adhesion molecules, specifically vascular cell adhesion molecule (VCAM-1); oxidative modifications of low-density lipoprotein (LDL); blood cell activation and coagulation abnormalities (increased blood levels of fibrinogen; tissue factor expression); and exhaustion of endothelial regenerative capacity. An early major event in atherogenesis is local recruitment of monocytes into the subendothelial space followed by phagocytosis of oxidized LDL, which is facilitated by the MCP-1 interaction with its receptor CCR2 and the endothelial expression of VCAM-1. The pathophysiological relevance of these mechanisms will vary dependent on the dose, duration of the exposure and physicochemical characteristics of the nanoparticles.

The observational human studies on the role of chemical exposure including particles and metals, in progression of atherosclerosis and related CVD may be influenced by numerous factors (e.g., characteristics of the exposure, personal, and life style confounders and population size). Understanding of the underlying biological mechanisms of the interaction between chemical exposure and atherogenesis can not be achieved without studies in relevant animal models. Recently, we characterized the application of an experimental mouse model for studying the role of metals in atherosclerosis (Simeonova et al. 2003). In these studies we were able to support the epidemiological findings linking arsenic exposure and atherosclerosis.

We designed studies to evaluate oxidative damage, inflammation, and coagulation status of plasma and heart/aorta in mice exposed through pharyngeal aspiration/inhalation to nanoparticles. Furthermore, we evaluate the effects of nanoparticle exposure on the genesis/progression of atherosclerosis using ApoE–/– mice or LDLR–/–, susceptible to atherosclerosis. If our findings link nanoparticle exposure to atherosclerosis, studies, using *in vivo* (mouse models) and *in vitro* (endothelial, smooth muscle, endothelial-progenitor cells), will be conducted to elucidate the putative role of several factors and mechanisms, e.g., vascular mitochondrial dysfunction, in these pathophysiological processes. In general, the conceptual objective of our studies is that nanoparticle pulmonary exposure may induce systemic effects which are relevant to atherogenesis or atherosclerosis progression. The systemic effects are related to ROS/inflammation – dependent cascades of signaling pathways which trigger endothelial dysfunction and impaired vascular repair mechanisms (as presented on the Diagram 1).

Diagram 1. Proposed processes by which certain materials influence CVD



4. SWCNT – Experimental Data

From the carbon nanomaterials, SWCNT recently elicited a great deal of interest due to their unique electronic and mechanical properties. SWCNT can be metallic or semiconducting thus offering amazing possibilities to create a broad spectrum of nanoelectronic devices as well as composite materials with extraordinary features (Sinnott and Andrews 2001; Subramoney 1998). Global revenues from carbon nanotubes (CNT) in 2006 are estimated at – $230 million with a growth rate of – 170%, which provides potential for workplace and even eventual general exposure (Donaldson et al. 2006). Concerns have been raised over occupational CNT exposure because they have several properties associated with potential adverse effects.

The extreme small size of SWCNT renders their chemical and physical properties fundamentally different from other particles with similar composition (Hood 2004). Several studies evaluated the potential pulmonary and cellular toxicity of SWCNTs. Lam et al. (2004) demonstrated that a single intratracheal

instillation in mice with three different types of SWCNT resulted in dose-dependent granulomas and some evidence of interstitial inflammation. Quartz and carbon NP were used as controls, and the author concluded that, on an equal mass basis, SWCNT in the lungs were far more toxic than carbon black and quartz. Histological images of the lungs in these studies showed that nanotubes induce significant granuloma formation and fibrous tissue accumulation compared to the control exposure. Based on the histological evaluation the inflammation was not a consistent finding. Warheit et al. (2004) evaluated the acute toxicity of intratracheally instilled SWCNT in rats. In this study, the authors showed that SWCNT exposure induced a transient inflammation and a nondose accumulation of multifocal granulomas. Shvedova et al. (2005) studied mice exposed to SWCNT of 99.7% weight elemental carbon and 0.23% weight iron. The primary nanotubes were 1–4 nm in diameter, but, as delivered by pharyngeal aspiration, two distinct particle morphologies were observed: aggregates and dispersed material. These two lung accumulations were associated with two types of responses – granulomas around the aggregates and diffuse fibrosis around the more dispersed SWCNT. Control mice were exposed to ultrafine carbon black or quatz and these exposures did not cause the response observed with SWCNT. All three studies demonstrated that SWCNT induce pulmonary toxicity which is different than this induced by graphite.

In the light of the pulmonary toxicological studies, we evaluated the cardiovascular responses in SWCNT-exposed mice (Li et al. 2006). To screen for systemic oxidative effects of SWCNT exposure, *Ho1-luc* reporter mice or C57BL/6 mice (at least 4 mice per treatment) were exposed to SWCNT in doses raging between 10 and 40 μg/mouse by single intrapharyngeal instillation and were sacrificed at time points including 1, 7, 28, and 56 days after exposure. These experimental settings were selected to correspond to the pulmonary toxicity studies by Shvedova et al. (2005). To evaluate SWCNT effects on atherosclerosis progression, a chronic process, ApoE–/– mice (10 mice per treatment) were exposed by pharyngeal aspiration to a medium dose SWCNT (20 μg/mouse) via multiple exposures (once every other week for 8 weeks). In all studies the control animals were exposed by pharyngeal aspiration to vehicle – sterile phosphate-buffered saline (PBS).

Our studies demonstrated that SWCNTs, under the described conditions, have the potential to influence CVD. A single intrapharyngeal instillation of SWCNTs induced heme oxygenase-1 (HO-1) activation, a marker of oxidative insults, in lung, aorta, and heart tissue in HO-1 reporter transgenic mice. Furthermore, we found that C57BL/6 mice, exposed to SWCNT (10 and 40 μg/mouse), developed aortic mtDNA damage at 7, 28, and 60 days after exposure. The SWCNT exposure also induced mitochondrial glutathione

depletion, and increased mitochondrial protein carbonyl formation in aortic tissue. Mitochondrial components have been reported to be highly susceptible to oxidative stress, mediated by metabolic defects and environmental insults (Madamanchi et al. 2005) and mitochondrial dysfunction is emerging as an important pathophysiological factor in a number of CVD including atherosclerosis (Binkova et al. 2001; Ballinger et al. 2002, 2005). Oxidative alterations of mitochondria result in compromised metabolic processes, such as oxidative phosphorylation, which can trigger endothelial dysfunction, a leading mechanism in atherosclerosis progression (Choksi et al. 2004). Altered endothelial activities lead to a series of events including vasoconstriction, increased adhesiveness resulting in inflammatory cell infiltration and platelet-thrombus formation (Cai and Harrison 2000; Libby 2000a, b). Combination of multiple cardiovascular risk factors which work through similar processes, such as mitochondrial dysfunction, may lead to synergistic acceleration of atherosclerosis progression and precipitation of its complications. Although the role of mitochondrial distress in atherosclerosis related to respiratory particle exposure has not been well explored, it is clearly demonstrated that cigarette smoke and hypercholesterolemia result in mtDNA damage and greater plaque formation (Knight-Lozano et al. 2002). Consistently, atherosclerosis was accelerated in SWCNT exposed ApoE–/– mice primed with a high-fat diet as measured by plaque morphometric analysis (Fig. 1). Although SWCNT exposed ApoE–/– mice did not have altered lipid profiles, they had exacerbated plaque development in the aorta and brachiocephalic arteries. The histopathology, including granulomas around the agglomerated SWCNT, fibrotic tissue in the granulomas and along the small SWCNT depositions (more dispersed material), in the lung of these mice was similar to the previously described pulmonary alterations in C57BL/6 mice after a single exposure to SWCNT.

Pulmonary exposure to SWCNT may induce cardiovascular effects either directly or indirectly through mitochondrial oxidative perturbations which can result in altered vessel homeostasis. SWCNT, although with a very low content of iron – induced lung pathophysiological responses, associated with deposition of particle agglomerates and histopathological alterations in the lung. Hypothetically, it is possible that individual SWCNTs can translocate from the lung into the systemic circulation causing direct cardiovascular endothelial dysfunction. It has been reported that nanoparticles treated with albumin and/or surfactant proteins cross the alveolo-capillary barrier to gain access to the systemic circulation (Kato et al. 2003; Oberdörster et al. 2005). The proximity between epithelial type I and endothelial cell alveolar membrane structures might play a role in the particle translocation mechanisms. Since the SWCNT

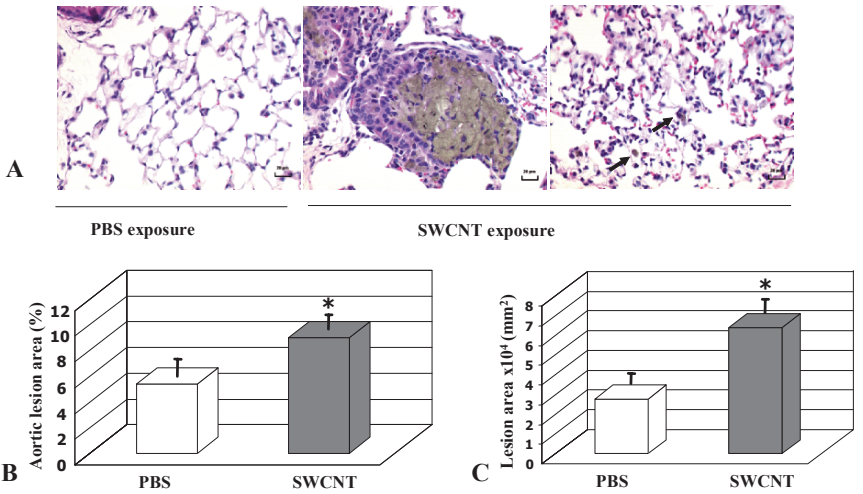


Figure 1. Lung histopathology and atheroma formation in ApoE–/– mice repeatedly exposed to SWCNTs (20 μg/ mouse every other week in two months) and fed a fat chow diet for four weeks followed by a regular chow for four weeks (adapted from Li et al 2006). A Representative images of lung (A) and morphometric analysis of the plaque size in the aortas (B) and the brachiocephalic arteries (C).

are not well recognized and cleared by lung macrophages (Kagan et al. 2006; Shvedova et al. 2005), nanotubes, dispersed or disintegrated from the agglomerates, may persist in the alveolar space which will facilitate their access into the systemic circulation. In contrast to SWCNTs, similar dose of UfCB agglomerated particles which are readily phagocytized by alveolar macrophages, did not produce lung fibrosis or granulomas (Shvedova et al. 2005), nor induce cardiovascular mtDNA damage. It is also possible that indirect processes are responsible for the cardiovascular effects induced by SWCNT exposure. This could occur if either mediators, released from the lung into the systemic circulation, or hypoxemia, associated with altered pulmonary function seen after SWCNT exposure (Shvedova et al. 2005), lead to oxidative modifications and low-level systemic inflammation. Several inflamematory mediators known to play a role in atherosclerosis were measured in the ApoE–/– mice evaluated for the atherosclerotic lesions. Although SWCNT exposure was associated with atherosclerosis acceleration, significant differences in the plasma levels of IL-6, IL-10, MCP-1, TNF-α, and IFN-γ were not observed. Furthermore,

SWCNT exposure was not related to increased inflammation in the vascular wall prior formation of the plaques. If SWCNTs with higher content of iron are deposited in the lung, chronic inflammation in the lung as well as systemic circulation might be induced. This will be tested in future studies. A third hypothesis for SWCNT exposure mediated cardiovascular effects is through platelet activation in the lung circulation. The pulmonary circulation is considered a site for platelet formation (Martin et al. 1983; O'Sullivan and Michelson 2006) and recently, it has been demonstrated that SWCNT can directly activate platelet aggregation *in vitro* (Radomski et al. 2005). Furthermore, transforming growth factor β 1(TGF β1), which is involved in platelet activation (Hoying et al. 1999), was found to be significantly increased in the lung of SWCNT-treated mice (Shvedova et al. 2005) and the time course of its increase paralleled the occurrence of cardiovascular mitochondrial dysfunction. Although, a link between activated platelets and cardiovascular mitochondrial distress has not been clearly established, it is well understood that that both platelet activation and mitochondrial damage lead to endothelial dysfunction and atherosclerosis (Ballinger 2005; Ross 1999).

5. Conclusions

Overall, these initial studies demonstrate that respiratory exposure to high concentrations, mostly agglomerated, SWCNT provokes not only pulmonary toxicity but vascular effects related to mitochondrial oxidative modifications and accelerated atheroma formation. Taken together, the findings are of sufficient significance to warrant further studies which should evaluate the systemic effects of SWCNT under inhalation exposure paradigms more likely to occur in the workplace or environment, such as low-level chronic inhalation exposure. Studies in progress, involving labeled SWCNT as well as detailed analysis of the role of lung platelet activation, will provide more insight into the mechanisms of the cardiovascular mitochondrial dysfunction in SWCNT-treated animals.

These studies also demonstrate that evaluation for systemic effects in parallel with pulmonary toxicity studies provides more complete toxicological information which will help in predicting the risk and development of safety regulations for the nanomaterial production and use.

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PULMONARY EFFECTS OF SINGLE-WALLED CARBON NANOTUBES: INFLAMMATORY RESPONSE, OXIDATIVE STRESS/SIGNALING, AND RECOGNITION BY MACROPHAGES[a]

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Abstract: Nanosized materials and nano-scaled processes are believed to be beneficial for many industries as well as for medicine. However, irrational or unjustified use of nanomaterials may also pose risks to human health and the environment, particularly if their potential toxicological impacts are unknown. This review discusses several important issues relevant to pulmonary toxicity of nanoparticles, especially single-walled carbon nanotubes, such as their direct cytotoxic effects, their ability to cause an inflammatory response, and induction of oxidative stress. Further, recognition and engulfment of nanotubes by macrophages as they relate to phagocytosis and biodistribution of nanotubes in tissues and circulation are discussed. Possible involvement of lung fibroblasts in pulmonary responses to nanotubes is also considered.

Keywords: occupational exposure, free radicals, lung fibrosis, neutrophils, transition metals

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[a]Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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1. Introduction

Revolutionary ideas and understanding by physicists of potentially unique and unusual behaviors of single atom assemblies with properties significantly different from larger macroscale molecular arrangements resulted in the development and building of nano-materials and nanodevices. Over the last decade, the break-throughs in nanotechnology have already revolutionized our lives in different fields through the manufacturing of new types of products from computer chips and energy-saving batteries, to composite construction materials, highly effective catalysts, and chemical sensors (Roco 2004). In the biomedical field, the advantages and impact of nanotechnology are particularly evident. They include significant advances in many fields of medicine from growing artificial organs and tissues for replacement therapies, to development of miniaturized and highly specific biosensors and diagnostic devices, and formation of new effective tools for drug delivery. In the field of public health, the examples of benefits offered by nanotechnologies are also impressive. One good example of improved nanobased techniques is filtration to provide purified high-quality water. Thus, benefits of nanotechnologies in improvements of quality of life are immense but so are the potential risks and dangers. This is mostly because these unique features of nanomaterials may be associated with their unpredictable effects on health and environment. Therefore, public acceptance and consensus of the society is not unequivocal and concerns have been raised that nanotechnologies should be subject to stringent regulations. It is clear, however, that the regulatory process should be based on solid research, which includes both basic studies of interactions of nano-materials with cells and biofluids as well as more applied toxicological assessments of nanomaterials.

Five organs – lung, skin, gastrointestinal tract, nasal olfactory structures, and eyes – are the prime targets for direct interactions with nanomaterials (The Royal Society and The Royal Academy of Engineering Report 2004; US EPA 2006). However, appearance of nanoparticles in these tissues can initiate their binding and transfer into general circulation and translocation into distant organs, including the cardiovascular system and brain (Oberdorster et al. 2005; Nel et al. 2006). The latter process includes specific interactions of nanoparticles with cells of immunesystem, particularly macrophages. Therefore, in this brief review, we will concentrate on three major aspects of nanotoxicology as it relates to pulmonary: (1) inflammatory response, (2) oxidative stress, and (3) recognition and engulfment by macrophages.

2. Pulmonary Inflammatory Responses to SWCNTs *In Vivo*

Three major responses are induced by exposure of animals to SWCNTs delivered through pharyngeal aspiration: (1) direct damage of cells, particularly of type II epithelial cells, (2) encapsulation of agglomerated forms of SWCNTs and formation of granulomas, and (3) nongranulomatous inflammatory reaction to dispersed SWCNTs and resultant interstitial fibrosis. Our previous work has detailed these specific features of inflammatory response to aspired SWCNTs and established that SWCNT caused unusual pulmonary effects in C57BL/6 mice that combined a robust acute inflammation with early onset of progressive fibrosis and granulomas formation. Appearance and accumulation of typical biomarkers of cell damage such as increase in the total protein, lactate dehydrogenax (LDH), and gamma-glutamyl transferase activities in broncho-alveolar lavage were accompanied by augmented levels of 4-hydroxynonenal (a characteristic secondary product of lipid peroxidation with profibrotic properties (Shvedova et al. 2005) as well as depletion of glutathione and other low-molecular weight thiols (the major water-soluble antioxidants) in lungs. At the cellular level, a characteristic yet accelerated inflammatory response was evidenced by an early arrival and accumulation of neutrophils, followed by influxes of lymphocytes and macrophages (Shvedova et al. 2005). Accordingly, there was rapid induction of proinflammatory cytokines, including TNF-α and IL-1β, followed by the production of pro fibrogenic transforming growth factor (TGF)-β1. A "pre-mature" onset of progressive fibrosis in mice exhibited two distinct morphologies: (1) SWCNT-induced granulomas mainly associated with hypertrophied epithelioid cells surrounding SWCNT agglomerates and (2) diffuse interstitial fibrosis and alveolar wall thickening likely associated with deposition of dispersed SWCNT. Comparing with well-documented responses to standard particles – equal doses of ultrafine carbon black particles or fine crystalline silica (SiO_2) – demonstrated that they were remarkably less effective than SWCNTs in inducing granulomas or alveolar wall thickening and caused a significantly weaker pulmonary inflammation and damage. Not surprisingly, the exuberant inflammatory response induced by SWCNTs resulted in functional respiratory deficiencies and decreased bacterial clearance (*Listeria monocytogenes*). Combined with other reports in the literature (Lam et al. 2004; Warheit et al. 2004; Muller et al. 2005), our results suggest that occupational exposures to respirable SWCNT particles at the current permissible exposure limit (PEL) (for graphite particles) may represent a significant risk of developing some lung lesions.

3. Interactions of Pulmonary Inflammation with Oxidative Stress

Most commonly used methodologies to manufacture SWCNTs include employment of transition metal catalysts, such as Fe and Ni. Inflammation involves production of reactive oxygen species (ROS), mostly superoxide radicals and hydrogen peroxide, and reactive nitrogen species (nitric oxide and resulting peroxynitrite) by immune cells – PMNs and macrophages (Kagan et al. 2006). Combination of transition metals with oxygen radicals is known to synergistically enhance each other to cause disproportionally high inflammatory response and oxidative damage. Therefore, it is not surprising that SWCNTs-induced inflammatory response is characterized by accumulation of typical biomarkers of oxidative stress. Among those depletion of total antioxidant reserves (decreased levels of glutathione and other low-molecular weight thiols as well as protein SH-groups), consumption of the major lipid-soluble antioxidant of membranes (vitamin E), and accumulation of a typical biomarker of lipid peroxidation (4-hydroxy-nonenal) were all documented in mice exposed to SWCNTs by pharyngeal aspiration (see above). If the mutual enhancement of transition metal-catalyzed oxidative stress and inflammatory response are indeed major contributors, then SWCNTs containing metals may induce greater lung damage than purified SWCNT where the metal catalysts have been removed. It is logical to expect that animals with compromised antioxidant defenses would be more sensitive and elicit an enhanced inflammatory response. To test this experimentally, we employed C57BL/6 mice with dietary vitamin E-deficiency. This was achieved by maintenance of the animals on vitamin E-insufficient diet as described by (Kisin et al. 2006). In these animals, vitamin E-deficiency also caused decreased levels of other antioxidants (vitamin C and GSH) and elevated levels of lipid peroxidation products. Most notably, these animals displayed very high levels of both acute and chronic inflammatory responses. This was evidenced by higher amounts of inflammatory cells (PMNs, macrophages), higher levels of released cytokines (TNF-α, IL-1, IL-6, TGF-β, IL-10), as well as more pronounced deposition of collagen in the lung (data not shown).

4. Interactions of SWCNTs with Macrophages

Macrophages are the primary responders to different particles that initiate and propagate inflammatory reactions and oxidative stress (MacNee 2005; Tao et al. 2003). Recognition, tethering, engulfment, and digestion of nanoparticles by macrophages may be important for regulation of inflammatory response as well as for the fate of nanopartciles, their distribution and potential biodegradation. Interaction of particles with macrophages commonly results in the activation of their NADPH oxidase system leading to the production and release of (ROS),

mainly superoxide radicals (Park 2003). The latter dismutates (spontaneously or via SOD-catalyzed pathways) to yield hydrogen peroxide. Elimination of superoxide is important to prevent its very effective interaction with nitric oxide which results in the formation of peroxynitrite (ONOO-), a potent oxidant causing massive nitration of protein tyrosine resides. Accumulation of nitrotyrosines has been associated with oxidative/nitrosative stress and tissue damage (Bayir et al. 2005; Ricciardolo et al. 2006). Alternatively, the product of superoxide radical dismutation, H2O2, can be decomposed, in the presence of transition metals, to yield another very potent oxidant, hydroxyl radical (OH•) via a well known Haber–Weiss mechanism. Because OH• radicals can indiscriminately attack essentially any biomolecule and cause its oxidative modification, excessive generation of hydroxyl radicals has been also linked to oxidative damage (Klebanoff 2005; Berg et al. 2004). In this pathway, the presence of catalytically active transition metals is critical to the fate of superoxide/H2O2 produced by inflammatory cells (macrophages). The presence of transition metals (Fe, Ni) as obligatory decorating metals in the manufacturing of SWCNTs may have a very substantial impact on the induction of macrophage-driven reactions. Therefore, in our previous work, we focused on two types of SWCNT: (1) iron-rich (nonpurified) SWCNT (26 wt% of iron) and (2) iron-stripped (purified) SWCNT (0.23 wt% of iron) to study their interactions with RAW 264.7 macrophages. SWCNT with high iron content displayed high redox activity in a cell-free model system and generated higher levels of EPR-detectable ascorbate radicals resulting from ascorbate oxidation. In the presence of zymosan-stimulated RAW 264.7 macrophages, iron-rich SWCNT were more effective in generating hydroxyl radicals spin-trapped with 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), than purified SWCNT. Similarly, EPR spin-trapping experiments in the presence of zymosan-stimulated RAW 264.7 macrophages showed that non-purified SWCNT more effectively converted superoxide radicals generated by xanthine oxidase/xanthine into hydroxyl radicals as compared to purified SWCNT. After stimulation of RAW 264.7 macrophages (with zymosan- or PMA), iron-rich SWCNT caused significant loss of low-molecular weight thiols and accumulation of lipid hydroperoxides. Thus, transition metal contaminations in SWCNT substantially affect redox-dependent responses of macrophages (Kagan et al. 2006).

5. Recognition and Engulfment of Nanotubes by Macrophages

Redox effects of SWCNTs and their transition metal content with macrophages can be realized during the immediate contact of particles with cells or at a distance through ROS and other reactive intermediates generated by transition

metals. Because the lifetime of free radical intermediates is short it is likely that remote interactions are less effective than direct particle/cell contacts. It is therefore important to assess the extent to which SWCNTs are recognized and engulfed by macrophages. Previous work has established that functionalized SWCNTs, particularly those carrying negative charge on their surface, are readily ingested by macrophages (Kagan et al. 2002; Dumortier et al. 2006). In contrast, non-functionalized SWCNTs are poorly recognized by macrophages and do not effectively induce typical macrophage activation responses, such as superoxide production by NADPH oxidase or NO• production by iNOS (Kagan et al. 2006). Significant literature indicate that recognition of apoptotic cells by macrophages is largely and universally dependent on the appearance on the cell surface of an acidic phospholipid, phosphatidylserine (PS), which is normally confined to the cytosolic leaflet of plasma membrane (Kagan, et al. 2003). In other words, the externalization of PS during apoptosis generates an "eat-me" signal for macrophages. Further, non-apoptotic cells with externalized PS are well recognized by macrophages resulting in the suppression of their ROS and RNS production (Serinkan et al. 2005; Tyurina et al. 2006). Based on these findings, we hypothesized that coating of SWCNTs with PS could interface them with macrophages and stimulate the recognition, tethering, and engulfment. This may be important for regulation of the inflammatory response to SWCNT. Moreover, this approach can be utilized for targeted delivery of specialized cargos – regulators, inhibitors – into macrophages aimed at control of their functions. Indeed, PS-coated SWCNTs are readily taken up by macrophages both *in vitro* and *in vivo*.

6. Possible Involvement of Lung Fibroblasts in Pulmonary Responses to SWCNTs

As macrophages are the primary cells involved in responding to a variety of particles, the effects of SWCNT on macrophage cell lines have been actively investigated. However, recent studies indicate that particle exposure can induce release of cytokines from lung fibroblasts, in addition to macrophages (Rao et al. 2004, 2005). Therefore, we performed a comparative study in which we examined the expression of several cytokines implicated in the inflammatory response, in alveolar macrophages, lung fibroblasts and type II alveolar epithelial cells (type II cells) following exposure to SWCNT *in vitro*.

We found (Table 1) that exposure of primary rat alveolar macrophages to SWCNT resulted in marked upregulation of mRNA expression of five genes (IL-1β, iNOS, MCP-1, MIP-1α, and MIP-2), moderate upregulation of three other genes (osteopontin, TGFβ-1, and TNF-α), and essentially no change in two genes (IL-6 and IL-10). The mRNA levels at the two points studied reveal that with the exception of MIP-1α and TGF-β1, induction tends to return to the

basal level at 18 h post-exposure, suggesting that the upregulation might be transient. To evaluate if upregulation of message was accompanied by a corresponding increase in the protein levels, we measured the protein concentrations of three cytokines (IL-1, MCP-1, and TNF-α) in the culture supernatants of AM exposed to SWCNT. Protein levels of IL-1 and MCP-1 were increased only 18 h post exposure, whereas there was significant increase in TNF-α at both 4 and 18 h time points (Table 2).

TABLE 1. Changes in mRNA Expression In Vitro after SWCNT Exposure.

	AM	Fibroblasts	Type II
GM-CSF	ND	++++	–
IL-1β	++	+++++	–
Il-6	–	++++	++
IL-10	–	+++	–
iNOS	+++	+++	–
MCP-1	+++	–	–
MIP-1a	++	ND	–
MIP-2	++++	++++	–
Osteopontin	+	ND	ND
TGF-β1	+	–	–
TNF-a	+	+	–

Quantitation of mRNAs by Real-Time RT/PCR. The cytokine mRNA levels in the cells were measured at 4 h (AM) and 18 h (Fibroblasts and Type II cells) post SWCNT exposures using a SYBR Green PCR kit with the ABI 5700 Sequence Detector (PE Applied Biosystems, Foster City, California). Total RNA was isolated using RNAqueous™ -4PCR kits (Ambion, Austin, Texas) from AM (≈2 million cells). One to two micrograms of the DNAse I-treated RNA was reverse transcribed, using Superscript II (Life Technologies, Gaithersburg, Marylard). The cDNA generated was diluted 1:100 and 15 μl was used to conduct the PCR reaction according to the SYBR Green PCR kit instructions. The comparative C_T (threshold cycle) method was used to calculate the relative concentrations (User Bulletin #2, ABI PRISMR 7700 Sequence Detector, PE Applied Biosystems, Foster City, California).

Primary rat lung fibroblasts when exposed to SWCNT upregulated for 18 h the expression of seven genes (GM-CSF, IL-1β, IL-6, IL-10, iNOS, MIP-2, and TNF-α). There was no change in MCP-1 and TGF-β1 (Table 1). Consistent with upregulation of mRNA levels, the expression of the protein levels in the fibroblast culture supernatants (Table 3) was upregulated for five of the proteins measured (GM-CSF, IL-1β, IL-6, IL-10 and TNF-α).

Exposure of primary rat type II epithelial cells to SWCNT caused moderate upregulation of only IL-6 mRNA levels (Table 1). As there was no increase in IL-6 mRNA levels in AM exposed to SWCNT, it is proposed that the main sources of IL-6 after exposure to SWCNT in the lung are fibroblasts and type II alveolar epithelial cells.

TABLE 2. Levels of Inflammatory Mediators in Alveolar Macrophanges after Exposure to SWCNT.

SWCNT (μg/ml) / Cytokines (pg/ml)	Exposure, h	0	30	60	90
MCP-1	4	339±64	549±47	663±63*	460±170
	18	685±91	1405±61*	1701±106*	1611±148*
TNF-a	4	7±4	169±58*	314±65*	252±43*
	18	7±3	101±27*	184±29*	236±40*
IL-1a	18	37±22	559±115*	879±141*	1176±189*

*$p < 0.05$, versus control.

Measurement of Cytokines. Levels of cytokines were assayed in the cell medium at 4–18 (AM) and 18 h (Fibroblasts) following SWCNT exposures to primary rat macrophages, fibroblasts, and epithelial type II cells. The concentrations of TNF-α, IL-1α, MCP-1, and GM-CSF (sensitivity of assay is 5–7.3 pg/ml) were determined using Bender MedSystems, Rat Cytokines 6plex kit (Bender MedSystems, Burlingame, California). The concentrations of IL-6 and IL-10 (sensitivity of assay is 5–7.3 pg/ml) were determined by BD Cytometric Bead Array, Rat Inflammation Flex Set (BD Biosciences, San Diego, California).

TABLE 3. Levels of Inflammatory Mediators in Fibroblasts after Exposure to SWCNT.

SWCNT μg/ml / Cytokines pg/ml	0	30	60	90
MCP-1	7±4	51±6*	70±9	103±10
IL-1a	16±8	559±116*	879±142*	1176±189*
GM-CSF	95±41	1314±368*	1793±419*	3303±916*
Il-6, $x10^3$	26.6±4.1	50.6±2.4*	47.0±4.7*	50.5±1.8*
IL-10	1±0	26.7±10.9*	31.1±13.0*	46.1±8.7*

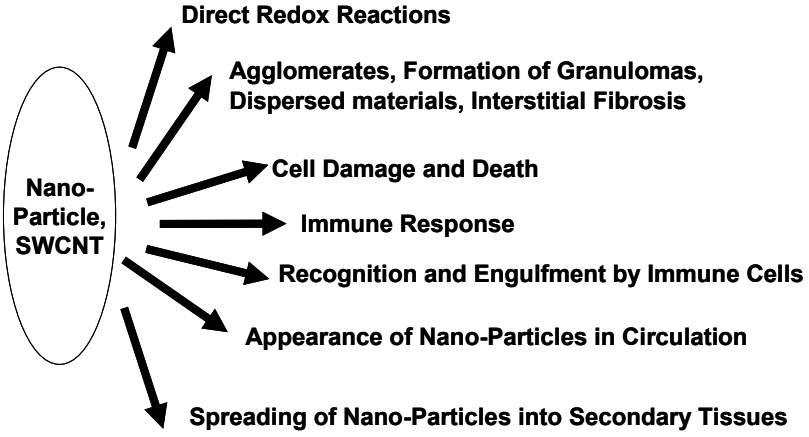
*$p < 0.05$, versus control.

The fact that the majority of the mRNA levels of the genes upregulated in AM after exposure to SWCNT tubes tend to return to basal levels after 18 h is consistent with the *in vivo* results reported by Shvedova et al. (2005) that IL-1β and TNF-α protein levels in BAL fluid were up on day 1 and returned to basal levels on day 3. Interestingly, TGF-β1 levels were shown to be high at day 7 in the same study and it was one of the two messages still high at 18 h in the current study (the other one was MIP-1α).

Our results show that the main source of inflammatory mediators in the lung after exposure to SWCNT, are alveolar macrophages and fibroblasts. In the past, inflammatory mediators produced by alveolar macrophages have been the main focus in particle-induced lung disease. The current study, along with our previous studies (Rao et al. 2004, 2005), indicate that fibroblasts are an important source of inflammatory mediators after particle exposure. The responsiveness of fibroblasts to SWCNT *in vitro* correlated with the rapid and progressive interstitial fibrosis observed after pharyngeal aspiration of SWCNT in a mouse model.

7. Concluding Remarks

While toxic effects of SWCNTs have not been extensively studied, exisiting data indicate that they may be harmful to living organisms (US EPA, 2006). As shown in Schema 1, the SWCNTs toxic effects may be realized through the formation of granulomas (mostly from large agglomerates structures of SWCNTs) and interstitial fibrosis (mostly due to more dispersed SWCNT structures). Direct redox reactions of SWCNTs and their transition metal contaminations may dysregulate anti-prooxdiant balance in cells and biofluids. Of special interest are interactions of SWCNTs with immune cells, particularly macrophages. Apparently, nonfunctionalized SWCNTs lack essential signals on their surface, hence are not effectively recognized by macrophages. However,



Schema 1. SWCNT Toxic Effects

functionalization of SWCNT and incorporation of negatively charged groups on their surface facilitates their recognition by phacytozing cells. This difference may be important for understanding pathways for tissue distribution of SWCNT in the body and their appearance in systemic circulation. It is also important that responses realized through the release of cytokines can be induced by SWCNTs not only through their interactions with the cells of immune system but also via their effects on fibroblasts. Obviously, further rigorous and extensive *in vitro* and *in vivo* nanotoxicological studies are necessary to determine their mechanisms of toxicity, biodegradation pathways, pulmonary distribution and translocation, before risk assessment can be conducted and appropriate prevention strategies can be developed and implemented to assume the safe production and use of carbon nanotubes.

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INHALATION OF NANOMATERIALS: SHORT OVERVIEW OF THE LOCAL AND SYSTEMIC EFFECTS

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Abstract: In this chapter inhalation of nanomaterials and its main effects on the lung are discussed. The behavior of inhaled particles differs significantly from that of inhaled gaseous or volatile compounds. The deposition of solid material in the respiratory tract is dependent on material-specific physicochemical characteristics and host factors. Three distinct mechanism of deposition are described: impaction, sedimentation, and diffusion. Particles between 10–50 nm are deposited mainly in the alveoli, smaller and larger ones are more efficiently deposited in the higher regions. Fibers with a small diameter can also penetrate deep into the lungs, even a small portion of long fibers (>>20 μm) can enter the alveolar space.

Clearance: The removal of solid material from the lungs is carried out by two distinct mechanisms. The mucociliary escalator, a very efficient mechanism, dominates the clearance from the airways and the nose; in the alveolar region the clearance predominantly takes place by macrophage phagocytosis. Nano-sized particles are more likely to hamper the clearance resulting in a higher burden, possibly amplifying the observed effect. The persistence of material in the lung also depends on the material-specific biodurability.

What makes nanoparticles dangerous? The biological effects of nanomaterials do not just depend on the intrinsic toxicity of the material. Parameters such as size, surface area, the charges carried by the particle in contact with the cell membranes and the chemical reactivity also play a role. The primary effects observed after inhalation of nanomaterials are the induction of oxidative stress and inflammation.

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Extrapulmonary effects of inhaled nanoparticle: Inhaled ultrafine (nanoparticles) particles probably can be translocated from the lumen of the lung into the systemic circulation and have a direct effect on, e.g., blood clotting. It has also been observed that nanoparticles can, via the olfactory bulb, enter the brain, and is possibly linked to neurological disorders.

Keywords: nanoparticles, toxicology, exposure, pulmonary effects, systemic effects

1. Introduction

It is believed that the lung, of all major exposure sites of the body is the most important one for involuntary exposure to man-made nanoparticlulates. The respiratory tract consists of two different parts; nose and airways (transporting the air in and out the lungs) and alveoli (gas exchange areas). The nose and the airways have a relatively robust barrier, an active epithelium protected with a viscous layer of mucus. In the gas exchange area, the barrier between the alveolar wall and the capillaries is very thin. The air in the lumen of the alveoli is only 0.5 μm (500 nm) away from the blood flow. The large surface area of the alveoli, 140 m^2 in adults, and the intense air–blood contact in this region makes the alveoli less well-protected against environmental damage when compared with airways.

Two critical aspects, from a health effect point of view, are important: first, how can nanomaterials have a local effect in the lungs and second, can nano-materials move from this portal into the body and affect other organs?. In this chapter the focus will be on the deposition and clearing of nanomaterials in the lung.

2. Inhalation, Deposition, and Pulmonary Clearance of Insoluble Solids

2.1. INHALATION AND DEPOSITION

The behavior of inhaled particles differs significantly from that of inhaled gaseous or volatile compounds. The deposition of volatile compounds in the lungs is mainly dependent on the water solubility of the compound – the more the compound is water soluble the less deep it will penetrate in the lung. The deposition of particulate matter is mainly dependent on its aerodynamic diameter.

Aerodynamic diameter
The behavior (settling velocity) of spherical particle can be described by Stokes law:

$$V_s = \frac{2r^2 g(\rho_p - \rho_f)}{9\,\eta}$$

where: V_s is the particles' settling velocity
g is the acceleration due to gravity
ρ_p is the density of the particles
ρ_f is the density of the fluid
r is the Stokes radius of the particle
η is the fluid viscosity.

When particle matter is not perfectly spherical, then the aerodynamic behavior is expressed as an idealied spherical particle, with a density of 1 g/cm^3 and having the same inertial properties as the studied particle. Therefore particles with a same aerodynamic diameter (a.d.) can have different dimensions.

The deposition of solid material in the respiratory tract is dependent on; (1) material-specific factors (physical-chemical characteristics) such as particle size and shape, relative weight, charge, etc.; and (2) on host factors such as the anatomy of the respiratory tract including diameter of the airways, branching angle of airways, but also tidal volume, air speed (physical exercise), and health status (bronchoconstriction, hypersecretion of mucus) (Frampton et al. 2004; Oberdorster 1994; Borm 2002; Sanfeld 2003; Daigle et al. 2003).

Five distinct mechanisms concerning the deposition of solid material can be described:

1. Particles suspended in the air flowing through the airways can impact the wall when the airflow abruptly changes direction; here the inertia forces play an important role.
2. Particles can sediment from the air when the air speed is lower than the settling velocity of the particle. These two mechanisms are dependent both on air velocity and size and on relative density of the particle.

3. Particles in the air will collide with air molecules and this will induce random movement – Brownian motion. Due to this motion the particle will move from the lumen to the epithelium. This mechanism (diffusion) is observed when the airflow velocity is low and the particles are light and small. The air speed in the lungs decreases with increased branching of the airways and is almost zero in the alveolar region. Therefore impaction is most often observed in the higher airways, sedimentation becomes more important in the more distant airways and diffusion is most important in the alveoli.
4. Interception happens in the respiratory tract when a nonspherical particle contacts the airway wall. For elongated particles (e.g., fibers), interception is an important respiratory tract deposition mechanism. The chance of particle interception increases as the airway diameter becomes smaller.
5. Electrically charged particles can interact with the epithelium due to charge differences. If the particle charge plays a role, the deposition will differ from the expected "normal" regional deposition based on characteristics such as size, shape, and density.

Taking into consideration the former mechanisms, pulmonary deposition of particle matter has been simplified as follows: particulates with an a.d. of more than 5 μm will deposit in the upper airways (nose and trachea), smaller particles will also deposit deeper in the airways, and those with an a.d. of less than 2.5 μm can travel down to the alveoli. Recently it has been shown that deposition efficiency of nanoparticles at the three pulmonary regions is not linear with the size. Particles with a size between 10 and 50 nm are deposited mainly in the alveoli, smaller and larger ones are more efficiently deposited in the higher regions (Oberdorster et al. 1994, 2005).

Fibers are defined, in pulmonary toxicology, as solid materials with a length-to-diameter ratio of at least 3:1. Their a.d. can be used to judge their penetration into the lungs, although other factors also play a role in their deposition, fibers with a small diameter, and a relative small length, can penetrate deep into the lungs, while very long fibers (>>20 μm) more easily get stuck (interception) already in the higher airways (although some long fibers can enter the alveolar space) (Lippmann 1990; Moore 2001; Oberdorster 2000, 2001, 2002; Warheit et al. 2001).

2.1.1. Clearance

The removal of solid material from the lungs is carried out by two distinct mechanisms: the mucociliary escalator dominates the clearance from the nose and airways; in the alveolar region the clearance takes place predominantly by macrophage phagocytosis.

The mucociliary escalator, driven by the cilia of the epithelium, is an efficient transport system pushing the mucus, which covers the airways, together with the trapped solid materials toward the mouth.

The phagocytosis of particles and fibers results in activation of macrophages and induces the release of chemokines, cytokines, reactive oxygen species, and other mediators. Chronic stimulation of macrophages can result in sustained inflammation and progress to lung damage – such as fibrosis and emphysema (Donaldson and Tran; Seaton et al. 1995). The phagocytosis efficiency is affected by the chemical composition, by the dose, and the intrinsic toxicity of the material or, typically for solid fibrous materials, by the physical–chemical characteristics of the material; moreover, fibers too long to be phagocytized (fibers longer than the diameter of the alveolar macrophage) will not (or very slowly) be cleared (Brown 2001; Oberdorster 2002; Schins 2002; Berman 1995; Kreyling 2004).

The dose dependency of the clearance has been studied in some detail. If the inhaled concentrations are low, such that the deposition rate of the inhaled particles is less than the clearance rate, then the retention half time in the alveoli is about 70 days. If the deposition rate of the inhaled particles exceeds this clearance rate, the retention half time can increase significantly. Overload is a condition in which the phagocytotic capacity of the alveolar macrophages is impaired, thus their efficiency to take up the inhaled particulates decreases resulting in a higher burden (Oberdorster 1995; Muhle 2003; Mossman 2000). Overload is a phenomena mainly observed in rats and is dependent on the volume of the particles phagocytized by the macrophages and is reached when particles in the lung reach a level which corresponds with a volume of about 1 μl/g of lung (Tran et al. 2000). Clearance from the lung depends not only on the total mass of particles inhaled but also on the particle size and, by implication, on particle surface, as shown already (Oberdorster 1994); a sub-chronic 3 months inhalation exposure of rats to ultrafine (~20 nm) and fine (~200 nm) titanium dioxide (TiO_2) particles showed that the ultrafine particles cleared significantly slower and translocate more to interstitial sites and to regional lymph nodes when compared to the fine TiO_2 particles (Warheit et al. 2006). It has to be remarked that cytotoxic particles also impair clearance, often at a much lower lung burden, compared to particle overload. Also significant species differences exist in response to overload: mice and hamsters are less prone to developing chronic inflammation and pulmonary fibrosis; and lung tumors have been observed only in rat studies (Elder et al. 2005; Oberdorster 1995).

In summary, nanosized spherical solid materials will easily enter the alveoli, although a large amount will deposit in the upper and lower airways. The particles can be cleared from the lungs, as long as the clearance mechanisms are

not affected, due to particulate toxicity or overload (mainly observed in rat). It is also important to note that particle number or specific particle surface area are probably better indicators for maximum tolerated exposure level than total mass, suggesting that the biological effects are linked to the surface reactivity (Oberdorster 2005; Donaldson, and Stone 2003).

3. Bio-persistence of Inhaled Solid Material

The main determinants of biopersistence are clearance (species-specific, physiological) and material-specific biodurability (physical–chemical processes), and is mainly important for fibrous materials.

The alveolar clearance of fibers depends on the ability of alveolar macrophages to phagocytoze them. Macrophages containing fibers longer than their own diameter (in humans longer than 20 μm) may not be mobile and are unable to clear the fibers from the lung (Berman et al. 1995).

The biodurability of a fiber depends on its dissolution and the possibility to break the fiber mechanically into smaller, shorter fragments (Hesterberg et al. 1998; Warheit et al. 2001). When breakage occurs longitudinally, resulting in more fibers of the same length but smaller diameter, e.g., amosite asbestos or brown asbestos, the biodurability is larger (Berman et al. 1995; Wylie et al. 1997). Other types of fibers (e.g., amorphous) break perpendicular to their long axis, resulting in fibers that can be engulfed by the macrophages (Searl 1997).

It is self-evident that the slower clearance of the fibers from the tissue (high biopersistence), the higher the tissue burden over time; and the longer the residence of fibers in a tissue, the higher the probability of an adverse response (Lippmann 1990). Although length plays a crucial role (Stanton hypothesis), it does not strictly indicate that all fibers longer than the lower threshold are equally active or that shorter fibers are not. Although fibers less than 5 μm in length did not appear to contribute to lung cancer risk in rats while fibers of more than 40 μm in length imposed the highest risk (Stanton and Wrench 1972; Berman et al. 1995; Schins 2002).

Inhaled fibers, which are persistent in the alveoli, can interact with the pulmonary epithelial cells or even penetrate the alveolar wall and enter the lung tissue. These fibers are often described as being in the “interstitial” where they may lie between or within the cells making up the alveolar walls. Biopersistent solid materials, certainly those containing mutagenic potency and which remain for years in the lungs, increase the risk of developing cancer (Oberdorster 2000, 2002; Muhle and Pott 2000).

There are no indications that the biodurability of fibers with a diameter <100 nm will differ from those with a larger diameter. But considering that not much is known on the long-term health effects of purposely made fibrous

nanomaterials, the biodurability of these materials should be evaluated carefully. Biodurability tests must be performed before releasing any products containing nonfixed (imbedded) materials.

An important group of nanofibers, from a technological point of view, are carbon nanotubes. Already in the first toxicological reports, signs of toxicity were reported, which demonstrated the pulmonary effects of single-walled carbon nanotubes *in vivo* after intratracheal instillation, in both rats and mice (Service 2003; Warheit et al. 2003; Lam et al. 2003). Lam et al. (2006) reviewed the existing literature on production, exposure, and toxicity of carbon nanotubes (CNT). From this report it can be concluded that CNT possess a certain health risk, however the exposure will play a crucial role because these materials tend to aggregate into large clumps which are relatively difficult to be inhaled, as reported in the study of the National Institute for Occupational Safety and Health (NIOSH); showing that none or only a small fraction of the nanotubes present in the air can be inhaled, (Maynard et al. 2004; Donaldson et al. 2006).

It has been mentioned above that spherical TiO_2 ultrafine particles, which are cleared significantly more slowly from the alveoli, showed more translocation to interstitial sites and to regional lymph nodes compared to the fine particles at equal dose. Thus, besides the greater biological effects of ultrafine particle, the difference in toxicokinetics in the lung results in a higher burden (Moolgavkar et al. 2001).

Moreover, inhaled nanomaterials inhibit more effectively the phagocytotic activity of pulmonary macrophages, an effect that can be linked to specific surface area of the material. In addition some nanomaterials induce a chemotactic response resulting in higher concentration of inflammatory cells (PMN) in the lungs and more active migrating macrophages (Renwick et al. 2001, 2004; Barlow et al. 2005).

4. Systemic Translocation of Inhaled Particles

The impact of inhaled particles on other organs has been reported in several epidemiological studies. Most research has concentrated on the possible consequences of particle-related malfunction of the cardiovascular system, such as arrhythmia, coagulation, etc. (Yeates and Mauderly 2001). However, the autonomic nervous system, as well as the olfactory nerves may be a target for inhaled particulates (Gold et al. 2000; Liao et al. 1999; Oberdorster et al. 2005).

In humans, translocation of inhaled ultrafine carbon particles, labeled with technetium (^{99m}Tc), into the blood circulation was studied by (Nemmar et al. 2002; Kawakami et al. 1990). In addition to human studies, *in vitro* and in experimental animal studies, extrapulmonary translocation of ultrafine particles

after intratracheal instillation or inhalation has been reported (Geys et al. 2006; Nemmar et al. 2001; Kreyling et al. 2002; Oberdorster et al. 2002; Takenaka et al. 2001).

However, the translocation mechanism is still unclear. Nemmar et al. demonstrated that technetium (^{99m}Tc) labeled carbon particles, which are very similar to the ultrafine fraction of actual pollutant particles, diffused rapidly – within 5 m – into the systemic circulation (Nemmar et al. 2001, 2004). The authors concluded, therefore, that it was unlikely that phagocytosis by macrophages, and/or endocytosis by epithelial and endothelial cells, are solely responsible for particle translocation to the blood, but that paracellular mechanism probably also play a role. More recently, it was shown, morphologically, that inhaled polystyrene particles are transported into the pulmonary capillary space, presumably by transcytosis (Kato et al. 2003). The amount of ultrafine particles that translocate into blood and extrapulmonary organs differed among these studies. Passage of solid materials from the pulmonary epithelium to the circulation is not restricted to nanoparticles, and depends on the surface characteristics of the material (Kato et al. 2003).

In inhalation experiments with rats, using 13C-labeled particles, Oberdorster et al. (2001) found that nanosized particles (25 nm) were present in several organs 24 h after exposure. The most extraordinary finding was the discovery of particles in the central nervous system (CNS). The authors examined this phenomenon further and found that particles, after being taken up by the nerve cells, can be transported via nerves (in this experiment via the olfactory nerves) at a speed of 2.5 mm h (Oberdorster 2001, 2002). It has also been shown that, following intranasal delivery, polystyrene microparticles (1.1 μm) can translocate to tissues of the systemic compartment (Eyles et al. 2001).

The issue of particle translocation still needs to be clarified, since several negative studies have been reported (Mills et al. 2006). Thus, the role of factors governing particle translocation such as the route of exposure, dose, size, surface chemistry, and time course should be investigated and it should also be investigated how and to what extent lung inflammation modulates the extrapulmonary translocation of particles.

5. Why are More Studies Needed Before People are Potentially Exposed to Nanoparticles.

In “nanotoxicology,” as in any branch of toxicology, two distinct characteristics play a role: on one hand, the material-specific and intrinsic toxicity and, on the other hand, more general but specific nanoparticle-induced responses (Donaldson and stone 2003; Hoet et al. 2004a, b).

Material-specific responses can often be understood and/or explained by material-specific toxic responses, e.g., dissolution of the material, surface area, and surface coating (Borm et al. 2006; Schmidt, and Vogelsberger 2006; Hohr et al. 2002; Beck-Speier et al. 2001; Yin et al. 2005). More general nanoparticle-dependent responses, certainly in lung and liver, are often categorized as free-radical production and inflammatory responses (Oberdorster et al. 2005; Kreyling et al. 2004).

5.1. EFFECT OF SIZE AND SURFACE AREA

As reported above, *in vivo* and *in vitro* it has been shown that nanosized particles inhibit phagocytosis compared to fine particles and can change the chemotactic behavior of macrophages significantly (Renwick et al. 2001, 2004). In the lung, tumor incidence of chronically inhaled TiO_2 of nanosized particles (20 nm diameter) at low exposure (10 mg/m³) was significant higher than high exposure (250 mg/m³) of 300 nm particles (Lee et al. 1986). It was shown that tumor incidence correlated better with specific surface area than with particle mass (see above) (Oberdorster et al. 1994; Driscoll et al. 1997). Also carbon black particulates, which can be considered as relative inert particles, of similar size and composition, with significant difference in specific surface area (300 versus 37 m²/g) gave significant different biological effects (inflammation, genotoxicity, and histology).

5.1.1. Effect of Surface Charges

Beside the particle size and surface area the surface characteristics play a dominant role in the biological behavior of material in the body. Polycationic macromolecules show a strong interaction with cell membranes *in vitro*. A good example can be found in the Acramin F textile paint system, a mixture of different linker molecules of which three proved to be very toxic when inhaled. These three polycationic paint components exhibited considerable cytotoxicity to pulmonary cells such as primary cultures of rat and human type II pneumocytes, and alveolar macrophages. It was shown that the multiple positive charges play an important role in the toxic mechanism (Hoet et al. 2001, 1999).

The half-life and body distribution of quantum dots, a promising nanomaterial in medicine because of multiple applications such as tumor targeting, drug carrier, visualizing (imaging) of biological molecules was studied (Dubertret et al. 2002; Michalet et al. 2005). CdSe quantum dots with different surface characteristics, coated with short-chain or long-chain polythlene glycol (PEG) were compared (Ballou, et al. 2004). The short-chain PEG-750 coated quantum dots were found in the lymph nodes and the spleen 24 h after dosage. The long-chain (PEG-5000) coated quantum dots were less apparent in the lymph nodes

but more abundant in the liver, spleen, and bone marrow. This coating allowed a slow clearance from the body, and as a result the dots persisted in the body 133 days after dosing.

Regardless the uptake route, the body distribution of particles, is most dependent on the surface characteristics and the size of the particles. It is an important issue when considering drug design to deliver medication to the right target.

5.1.2. Oxidative Stress and Inflammation

Particle-induced pulmonary inflammation can result in protective and adverse cellular responses in a dose-dependent manner. It has been hypothesized that oxidative stress plays a central role in the toxicity of nanomaterials (Donaldson et al. 2003; Wilson et al. 2002). Li et al. (2003) proposed a "hierarchical oxidative stress model" in response to diesel exhaust particle exposure. This model suggests that depending on the dose, different levels of a response can be reached. Particle matter, at low dose, induces cyto-protective responses (tier 1), e.g., through the activation of antioxidant response elements, inducing the expression of several antioxidant and phase II drug metabolizing enzymes (e.g., heme oxygenase 1 and glutathione-*S*-transferase). At higher doses, this level of protection will fail and the oxidative stress (tier 2) will lead to mitogen-activated protein kinase/nuclear factor kB activation and pro-inflammatory effects. At even higher doses, a further escalation (tier 3), will trigger disturbances of the mitochondrial function resulting in cellular apoptosis or necrosis. This indicated that a weakened antioxidant defense can increase susceptibility toward PM-induced airway inflammation, increase susceptibility to infection, and maybe to asthma. Alternatively, it explains the existence of susceptible human subsets. Xiao et al. (2003) showed that the hierarchical oxidative stress model can be applied in a macrophage cell line. Recently it has been reported that relative inert nanomaterials, such as carbon black can induce oxidative stress *in vitro* (Koike and Kobayashi 2006; Brown et al. 2001).

6. Summary and Discussion

Reviewing the knowledge collected concerning health effects of nanomaterials, we have to conclude that is still premature to draw any final conclusions, simply because too little has been investigated. However, due to the existing knowledge collected from environmental studies (e.g., carbon black and TiO_2) it is possible to have a general view on the possible hazards and risks.

A first important remark to make is that current knowledge is based mainly on epidemiological and experimental work concerning environmental particle pollution, most often referred to as coarse (10 – 2.5 μm), fine (2.5 – 0.1 μm)

and ultrafine particulates (UFP) (<0.1 μm). We can certainly learn from this research but it has to be taken into account that exposure to purpose-made nanomaterials differs from exposure to environmental particles in several ways: UFP, often arising from combustion, has a complex composition, with no uniform size. Some compounds are soluble in biological systems while others often have a very uniform (crystalline) structure and size, and are often not soluble. Beside these differences, it has to be taken into account that some effects of particulate matter will be more generic; not discriminating between the nature (chemical composition) of the materials: e.g., activation or inhibition of phagocytosis, cellular uptake or other cellular interactions. Also, as the particle diameter decreases the surface area increases significantly, which results in increased surface activity. Often the health effects can be better correlated with the total surface area of the material rather than with its total mass.

Beside the expected and predictable risks, we must keep in mind that nanomaterials can induce a biologic response in a way we are not familiar with from the previous studies of known compounds. For example, it has recently been observed that green light emitting quantum dots are more toxic *in vitro* compared to red light emitting dots, simply because of the difference in DNA damage emitted by the light (Hoet, et al. 2004 a, b; Lovric et al. 2005).

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INTERACTIONS OF ORGANIC COMPOUNDS WITH MINERAL PARTICLES, AND THE DETECTION OF CELL COMPONENTS IN BACTERIA BY SPECTROSCOPIC METHODS – CONNECTIONS TO NANOSCIENCE

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Abstract: Nanoscience includes numerous disciplines which deal with living and nonliving structures with dimensions below 100 nm. Even before the term "nanoscience" was explicitly introduced, research on the interactions between mineral, organic, and bio-particles, some of them naturally occurring in nano-dimensions, was ongoing. In this review, we summarize the results on experimental transformation of different organic substances on the surfaces of small mineral particles and the importance of these events for soil and aquatic environments. Attention was devoted also to the interactions of phenoloxidases, i.e., enzymes produced by different microorganisms, with clay particles, which can be of importance in remediation of chemically polluted sites. The molecular structure of microbial cells that might be used for tailoring of nano-sized bio-systems should be elucidated. In our work, Fourier-Transform Infrared (FTIR) spectroscopy was used to detect such bacterial structures. In this paper, some trends in the investigation of bio-, micro- and nanosystems are also indicated.

Keywords: bio-, microsystems, nanosystems, organic compounds, mineral particles, microbial activity, spectroscopic methods

1. Introduction

At the beginning of the 21st century, nanotechnology appears a novel, promising approach in men's endeavor to enhance quality of life in many areas. This versatile technology is based on enormous amounts of information that was

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obtained in the last decades by using advanced analytical methods in physics, chemistry, and biology. From a stage of general knowledge of understanding in depth different processes, the nanoscience currently is directed to specific tailored technologies and procedures. Nanomedicine, and nanoengineering, both harbor a broad scale of possible applications, represent wide fields of a prospective use of the nanoscience knowledge.

In environmental microbiology, the term "nanobacteria" has been proposed for spherical cells of about 100 nm in diameter. Supposedly, nanobacteria, mainly forming biofilms, exist in many natural environments. Reports of them being living organisms are controversial; what is called nanobacteria might be an artifact of chemical or geochemical reactions of some nonliving materials (Madigan et al. 2003). Nevertheless, recently, i.e., in January 2006, the American Society for Microbiology (ASM) organized a special conference on "Bio-, Micro- and Nanosystems", in San Francisco, California. In this conference, the nanoscience and nanotechnology was discussed from the microbiological points of view.

We will discuss here data on alterations observed in some simple organic compounds that are in contact with small inorganic particles. Among the methods for studying these interactions as well as for analyses of the molecular structure in microbial biomass, the UV, Vis, and/or IR spectroscopy appeared to be advantageous tools.

2. Transformation of Organic Compounds on Small-Sized Mineral Particles

Using the power of nanotechnology it will be possible to manipulate matter, to place molecules of interest where and when we want, and to obtain desired configuration and function. To succeed in this, the information provided from model experiments, carried out with well-defined substances under well-defined conditions, and by using well-approved analytical techniques, will be beneficial. The interactions of simple organic compounds with mineral particles are of a particular interest for the theory, and possibly also for the practical uses of various nanotechnologies. Even more, their understanding may contribute to the elucidation of the origin of first inorganic–organic molecules and small grains in our Solar System (Kwok 2004).

Long before nanotechnology became an independent subject to discuss, we have performed laboratory experiments in order to better understand transformation and the possible functionality of different organic substances that come in close contact with clay minerals. It is well known that clay minerals represent the most powerful natural mineral adsorbents which affect both biological and nonbiotic processes in soils and sediments (Filip 1973,

1975; Dixon and Weed, 1977). Due to high surface area and cation exchange capacity, e.g., some 766 m^2/g, and 110 meq/100 g, respectively, for smectites (Borchardt 1977; Gast 1977), the interaction of clay minerals with different organic compounds may bring about a variety of results of environmental importance (Harter 1977; Huang and Bollag 1998). Since, a high percentage of smectites such as montmorillonite, occurs in soils in a less than 80 nm particle size, and the respective interlamellar space of the particles may vary between 5 and 20 Å, i.e., 0.5–2nm (see the citations above), physicochemical interactions between organic substances and clay minerals undoubtedly belong into the frame of nanoscience.

The main attempt of our experiments was to contribute to the elucidation of a nonbiotic formation of dark colored humic-like substances. Humic substances themselves are basically spherical particles with average molecular masses of 20,000–50,000 Da, and a particle size between 4 and 150 nm (Steelink, 2002; Sutton and Sposito 2005). They are known to interact with a wide spectrum of inorganic and organic elements and compounds through a diverse array of physical-chemical reactions that can decrease but sometimes also enhance bioavailability and toxicity of chemicals to biota (Haider et al. 1975; Schnitzer 1986; Hayes and Himes 1986; Fava and Piccolo 2002).

In our laboratory experiments with cultures of microscopic fungi capable to form different phenols, we found an enhancement in the formation of humic-like polymers if montmorillonite was added to the growth medium (Filip et al. 1972 a, b). Thus, we tried to establish whether montmorillonite affects nonbiotic polymerization of some simply structured phenols into humic-like substances. In our experiments, oxygen uptake by 5-methylpyrogallol either alone or in a mixture with a few other phenolic substances, and furthermore the browning reaction of pyrogallol, was tested in the presence of muscovite and montmorillonite saturated or not with different cations.

The data in Table 1 show that the oxygen uptake of phenol mixtures was not enhanced by the presence of montmorillonite. In some cases it was even lower than in the controls. As the sorption of the phenols used in our experiments could be omitted at the given pH values according to previous tests, a support of heterogenic catalysis through the clay surfaces should rather be neglected in this case. Only with the addition of Fe^{2+} ions, the oxygen uptake increased a little at pH 8. This effect was not detected at pH 7, probably because the iron cations became firmly fixed at the clay mineral surfaces. Thus, our experimental results indicate that montmorillonite-particles apparently do not exert catalytic effects on the oxidative polymerization of the phenolic substances which was observed in cultures of some soil fungi, and which became evidently visible by a browning of cultural media. Rather, fungal exoenzymes, e.g., phenoloxidases, played an important role in those effects.

TABLE 1. Oxygen uptake from reaction mixtures of some phenolic substances with and without montmorillonite (0.5 % w/v) added. (From Filip et al. 1977.)

Reaction mixture montmorillonite	μmol O_2, pH 7		μmol O_2, pH 8	
	Control	Montmorillonite	Control	Montmorillonite
5 μmol 5-MPG[a]	5.6	5.5	6.1	6.2
+ 20 μmol PCA	6.0	5.8	6.4	6.2
+ 20 μmol CA	6.5	6.5	9.3	9.2
+ 20 μmol ORC	6.0	5.8	6.3	6.5
5 μmol 5-MPG + 10 ppm Fe^{2+}	8.6	7.8	11.2	12.4
+ 20 μmol PCA	8.6	6.6	12.6	13.5
+ 20 μmol CA	10.4	8.1	15.8	16.2
+ 20 μmol ORC	9.7	8.6	13.9	14.0

Abbreviations: [a]5-MPG = 5-methylpyrogallol, PCA = protocatechuic acid; CA = caffeic acid; ORC = orcinol; Fe^{2+} = $FeSO_4$

Recently, the use of nanosized Fe_0 particles or bimetallic combinations of Fe_0 and catalytic metals such as Pb gained attention. Such nanoparticles might be delivered to deep soil and groundwater zones to force the removal of different contaminants (Glazier et al. 2003). Also in this respect, the nanosized Fe_0 seems capable of exhibiting greater catalytic activity than microsized particles, due to the larger surface area (Nurmi et al. 2005).

Since the browning phenomena are typical in humification processes in all terrestrial and aquatic environments, further experiments were carried out in our laboratory with pyrogallol, a trihydric phenol, which can be easily oxidized by molecular oxygen to dark in color polymeric products. An oxidative transformation of pyrogallol was measured in the UV range of light, in which a freshly prepared colorless pyrogallol solution distinctly absorbs at 266 nm. In our tests, 100 mg of pyrogallol was dissolved in 40 ml of 0.1 N sodium acetate solution buffered at pH 6.0. With the exception of controls, 40 mg of finely ground quartz or clay minerals (fraction <2μm) were added. The clay minerals used (kaolinite, muscovite, montmorillonite) were saturated with H^+, K^+, Ba^{2+}, Ca^{2+}, Al^{3+}, or Fe^{3+} ions before use. The reaction mixtures were shaken at 100 rpm at a light intensity of 600 W/m^2. After 4, 24 and 48 h, parallels were centrifuged at 12,000 *g* for 20 min. The spectra of pyrogallol solutions free from clay minerals were collected in a wave range of 250–600 nm. Extinction values at 300 nm were used for the evaluation of color intensity. The data in Table 2 show an increase in the optical density in a pyrogallol solution from 4 to 48 h. If compared with the reaction of a single pyrogallol, the extinction was only slightly influenced by the addition of kaolinite or montmorillonite, and

therefore the respective values are not shown in the table. However, the addition of muscovite, a mica-type clay, evidently affected the extinction values, and enhanced the formation of a brown polymer substance in the pyrogallol solution. Surprisingly, the saturation of muscovite by either mono-, di-, or trivalent cations did not affect the browning reaction.

Similar results can be observed in Figs. 1 and 2. Figure 1 shows spectral changes of a pyrogallol solution as a result of reaction with only molecular oxygen. A secondary oxidation by electron transfer could additionally be taken into consideration when clay minerals are present in the reaction mixtures. As already mentioned, the absorption spectrum was little changed by the presence of kaolinite or montmorillonite in the mixture. Muscovite, however, influenced the UV spectra quite extensively. In Fig. 2, the spectral curve of the 48 h solution shows at 266 nm only a small absorption shoulder instead of an original strong band. Furthermore, below the 300 nm the spectral curve shows no longer any clear-cut bands. These phenomena resemble UV spectra of soil humic substances (Filip et al. 1976). Conclusively, our results show that not all, but some clay minerals can act as catalysts in a non-biotic oxidative polymerization of single phenols which typically occurs in a humification process. Naidja et al. (1998) reported such a capacity for birnessite, a naturally occurring, poorly crystalline oxide of tetravalent Mn and one of the most common forms of mineralized Mn in soils and sediments. In general, the humification of simple organics may include different individual processes, and as a result, the macromolecural structures of humic substances in water and soil environments may vary strongly in dependence of the origin, solution chemistry, and the associated mineralogy, but mainly they appeared as nano-sized particles (Myneni et al.1999).

TABLE 2. Influence of muscovite on a browning reaction of pyrogallol in a sodium acetate solution at pH 6. (From Filip et al. 1977.)

Reaction mixture	Extinction values at 300 nm		
	After 4 h	After 24 h	After 48 h
Pyrogallol (PYR)	0.063	0.500	0.592
PYR + Muscovite-H^{1+}	0.076	0.750	1.260
PYR + Muscovite-K^{1+}	0.086	0.755	1.130
PYR + Muscovite Ca^{2+}	0.070	0.740	1.250
PYR + Muscovite-Al^{3+}	0.070	0.480	1.220
PYR + Muscovite-Fe^{3+}	0.063	0.580	1.040

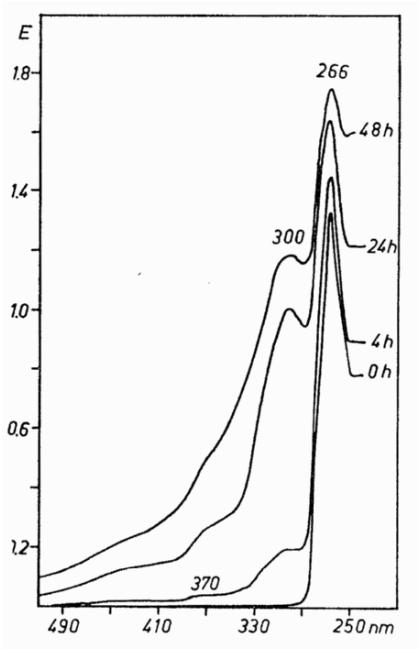

Figure 1. UV spectra of a pyrogallol solution (From Filip et al. 1977.)

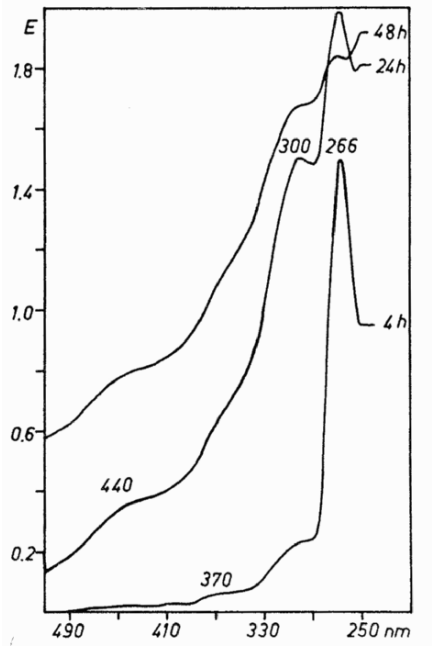

Figure 2. UV spectra of a pyrogallol solution with 0.1% (w/v) of muscovite added. (From Filip et al. 1977.)

3. Interactions of Phenoloxidases with Organic Substrates and Particulate Mineral Adsorbents

If considering phenoloxidases as agents capable of transforming different organic chemicals (see above), their respective reactions should be experimentally examined for possible importance, e.g., in decontamination of chemical pollution in terrestrial and aquatic environments. In our laboratory, the oxidation of 49 species of phenols and aromatic amines by laccase, i.e., polyphenoloxidase from the fungus *Polyporus versicolor*, and a tyrosinase from *Agaricus bisporus* was tested (Claus and Filip 1990a; Filip and Claus 1995). Thirty-eight compounds (78%) were oxidized by laccase and 7 (14%) by tyrosinase. Anisol was the only substance which was weakly oxidized by tyrosinase and not by laccase. Alkyl-substituted phenols were rapidly oxidized by laccase, but no reaction occurred with some bulky molecules such as 2,6-ditert-butyl-4-methylphenol. Alkyl-chloro-substituted phenols (seven compounds) were oxidized by laccase. Of 16 halogenated phenols, including F and Br containing substitutes, 11 were oxidized by laccase. The reaction velocity decreased with an increasing degree of halogen substitution. No oxygen consumption was observed with pentachlorophenol. No reaction occurred also with nitro-substituted phenols. Furthermore, no synergistic reactions were observed when laccase and tyrosinase were used simultaneously in substrate solutions. With para-substituted phenols, reaction velocity decreased in the sequence: amino > isopropyl > fluoro > chloro > nitro residues. Substituents in metapositions, e.g., 3-chlorophenol, and 3-aminophenol, prevalently exerted negative effects on reaction velocity. The oxidation of chlorinated compounds was often accompanied by a different degree of dehalogenation. By using tyrosinase, chloride became released from some substrates even if no oxygen consumption was detected.

Under natural conditions, i.e., in soils and sediments, the activity of phenoloxidases is affected, and mainly altered by the presence of different mineral and organic particles (Filip and Preusse 1985). The reason is to be seen in the adsorption of the respective biomolecules on clays and clay–humic complexes, the intensity of which depends, e.g., on prevailing pH values. In Fig. 3 this is shown for laccase from *P. versicolor*. In the presence of bentonite clay, enzyme activity was rapidly reduced at pH < 5, and disappeared completely at pH 3, thus, indicating that all the enzyme was adsorbed. In the presence of kaolinite which has much lower exchange capacity and surface area, the decrease of laccase activity was slower, and at pH 2 little activity still remained detectable. Similar results were obtained also for other enzymes tested by Claus and Filip (1988).

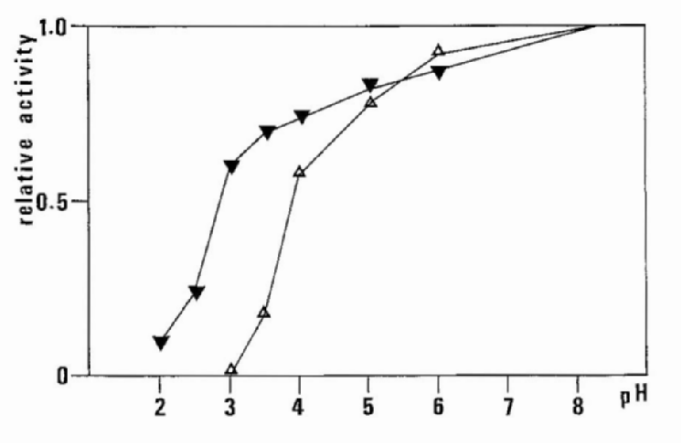

Figure 3. Adsorption of laccase from *P. versicolor* on bentonite (Δ) and kaolinite (▼) at different pH values. (From Claus and Filip 1988.)

In Table 3 the influence of different adsorbents of the activity of laccase from *P. versicolor* and tyrosinase from *A. bisporus* is summarized. At adequate pH values, both phenoloxidases were strongly adsorbed on bentonite and a bentonite–humus complex, resulting in almost total disappearance of free enzyme activity. Kaolinite and quartzsand were much less effective. In fact, due to the presence of bentonite or bentonite–humic acid complexes not only the enzymes activity but also the growth of phenoloxidase producing fungi, in the respective cultures was significantly inhibited (Claus and Filip 1990b).

TABLE 3. Residual activity (%) of laccase and tyrosinase in supernatants after enzyme adsorption on clays and a clay-humic acid complex. (Modified from Claus and Filip 1988.)

Enzyme	pH	Adsorbents			
		Bentonite	Kaolinite	Bentonite+Humic Acid	Quartzsand
Laccase	3.0	0.0	61.0	0.0	65.0
Tyrosinase	5.0	0.6	37.0	0.8	59.0

4. Effects of Particulate Mineral Adsorbents on Growth and Metabolic Activity of Microorganisms

As already mentioned in this paper, the existence of nanobacteria remains a matter of a controversial discussion. On the other hand, there is no doubt that nanosized solid particles, both mineral and organic ones, belongs to natural components of soils and sediments, which themselves are environments most densely inhabited by different microorganisms. A high percentage of smectite-

type clay minerals, such as montmorillonite, occurs in soils in a size fraction < 80 nm (Borchardt 1977). If considering all this, there is no wonder that in a microscopically examined soil suspension, microorganisms can be regularly observed as forming larger or smaller aggregates with clay particles. Mainly, the microbial cells appear adsorbed at the clay surface or they form aggregates with other cells, but also single microbial cells can be coated by a thin layer of nanosized mineral particles (Filip 1967). In our early review, we summarized the state of research in the respective field (Filip 1973). Similar phenomena, but including organic particles, were observed in our consecutive studies on biological stabilization of sewage sludge (Leschber et al. 1984). Furthermore, different micro- and/or nanosized particles proved capable in preventing a heat-stress in a mixed population of garbage microorganisms (Filip 1978a). Thus, it becomes a matter of interest to determine in some more detail whether microbial growth and activity can be influenced by the presence of clays or other small-sized solid particles. The respective results obtained by Haider et al. (1970) showed that a fraction <200 nm of montmorillonite when added (0.5%) to cultures of *Saccharomyces cerevisiae* or *Candida utilis*, did not influence cell growth under anaerobic conditions. If oxygen was present, however, the growth of the yeasts, both in stationary and shake cultures, was enhanced up to 90% for *S. cerevisiae* and up to 235% for *C. utilis.* The presence of montmorillonite also resulted in an increase of carbon dioxide production in relation to consumption of oxygen, causing an increase in the values of a respiration quotient. This indicates increasing glycolytic utilization of the primary substrate (glucose). Such an effect could be also manifested by increased formation of intermediate metabolic products which, on the other hand, could be effectively used for the synthesis of cell mass. This assumption was confirmed, e.g., in cultures of *Streptomyces violaceus-niger* as documented in Table 4. The values of an "economic coefficient" (amount of biomass × 100, divided by the amount of glucose consumed) demonstrate clearly a higher efficiency of biomass formation in cultures containing montmorillonite particles (Martin et al. 1976).

TABLE 4. Economic coefficient values of the cell synthesis by *S. violaceus-niger* as affected by addition of Ca^{2+}-montmorillonite. (From Martin et al. 1976.)

Incubation days	Control	Montmorillonite (0.25% w/v)
3	27.6	29.5
6	13.6	23.2
9	10.3	16.3
15	7.3	11.2
21	5.3	10.6

In section 2, an enhancement in fungal formation of humic substances was mentioned. In order to show more clearly the extent to which the energy source (glucose) can be converted into the fungal humic-type secondary metabolites, the ratios of humic substances synthesized to glucose, and to biomass formed were calculated for aerobic cultures of a soil microscopic fungus *Epicoccum nigrum*. As documented in Table 5, after 20 and 30 days of incubation, these ratios were greatly increased in the presence of different amounts of montmorillonite particles in a cultural medium.

During the first 10 days of the *E. nigrum* cultivation, the distance between the structural sheets in montmorillonite expanded from 12 to 18 Å, and between 20th and 30th days, the interlayer spacing contracted back to 12 Å again. Apparently low-molecular weight (and/or small in size) substances, such as single phenols excessively produced at the beginning of fungal growth were removed in this way, first. Later on, the same phenols were apparently exchanged for ammonium ions (much smaller in weight and size) that became released in the course of the autolysis of fungal cells. At enhanced pH values (pH 7–8), and due to spontaneous oxidative polymerization reactions, the released phenolic substances could serve an additional source of enzymatic polymerized dark in color humic-like substances in the cultures of *E. nigrum* fungus (Filip 1975).

TABLE 5. Ratios of humic substances (HS) to biomass formation, and to glucose consumed in stationary cultures of *Epicoccum nigrum*. (From Filip et al. 1972b.)

Montmorillonite (% w/v)	20 days g of HS ×100		30 days g of HS ×100	
	per 1g biomass	per 1 g glucose	per 1 g biomass	per 1 g glucose
Control	0	0	4.1	1.5
0.25	18.2	4.7	17.5	4.2
0.5	15.7	5.8	18.0	4.5
1.0	15.8	4.1	29.6	5.4

All these phenomena observed in our laboratory experiments demonstrate some differences but also an effectiveness of mineral solid particles, mainly sized in a nanometer scale, on biologic and nonbiotic processes of environmental importance.

5. Spectroscopic Detection of Molecular Structures in Different Microorganisms

Because the phenomenon of nanobacteria as mentioned in the previous parts of this paper remains unsettled, the detection of different molecular constituents in microbial cells evokes broad interest. In this way, a more detailed knowledge

on structural components of living cells can be obtained, and consecutively perhaps, one can better recognize whether or not all basic molecules of life could be harbored in individual nano sized cells. The Fourier-Transform Infrared (FTIR) spectroscopy belongs among methodical tools that can be used in aiming this goal. Comprehensive information on this powerful technique that can be used in many areas of science can be found in a book by Johnston (1991). Naumann et al. (1991) presented several examples on the use of FTIR spectroscopy in microbiology, and later, Naumann (2000) added further important details in a book chapter. The main advantage of FTIR is the relative simplicity in use, and the possibility to obtain spectral fingerprints of intact microbial cells and their entire components, including DNA/RNA, proteins, lipids, carbohydrates, and other structural units sized in nanoscale. Recently, Stopa and Morgan (2005) listed FTIR spectroscopy under methodical approaches to be used in the determination of different agents of a suspect biological attack. Ragoonanan et al. (2006) used FTIR in their analysis of droplets containing carbohydrate solution and cells of Geobacter sulfurreducens to determine damage in bacteria as a result of desiccation procedures.

In our early investigations, soil microbial biomass harvested from a cultural medium delivered a complex but well-differentiated infrared spectrum that clearly indicated various molecular groups in the cell mass (Filip 1978a, b). Recently (Filip et al. 2006), we summarized possible assignments and relative intensity of infrared bands in the biomass of some soil bacteria (Table 6).

In Figs. 4, 5, and 6 the FTIR spectra recorded from differently cultivated Azotobacter chroococcum, Bacillus subtilis and Pseudomonas aeruginosa are shown. *A. chroococcum*, a common free-living soil bacterium capable of fixing atmospheric nitrogen, developed a well-differentiated spectrum containing a strong triple-band at 1,726, 1,659 and 1,537 cm^{-1}. A very strong and sharp band at 1,726 cm^{-1} can be attributed to poly-β-hydroxybutyrate – a polyester compound that belongs to metabolites of this bacterium. The FTIR spectrum of starving *A. chroococcum* cells (Fig. 4B) was very similar to the standard spectrum. The cell mass harvested from a stationary growth phase absorbed strongly in a C–H stretching region (Fig. 4C).

The standard FTIR spectrum of *B. subtilis* (Fig. 5A) was dominated by absorption bands of amides I, II, and III at 1,660, 1,544, and 1,235 cm^{-1}. No C═O stretch could be detected at 1,720 cm^{-1} in difference to other bacteria. There were also no major differences detected between the standard spectrum of the bacterial biomass, and the ones obtained from either starved cells or from biomass harvested in a stationary phase of *B. subtilis* growth.

As shown in Fig. 6, the FTIR spectra of *P. aeruginosa* varied, in part, if the composition of nutrient medium was altered in which the bacterium was grown. The differences were most prominent in a cell mass harvested from nutrient broth

containing glutamate instead of ammonium-chloride as a source of nitrogen (Fig. 6D). The absorption at 3,300 cm^{-1} and at 2,960–2,850 cm^{-1} became stronger, and an absorption band at 1,743 cm^{-1} appeared. The 1,080 cm^{-1} band was transformed in a triplet with additional absorption at 1,155 and 1,030 cm^{-1}.

TABLE 6. Assignment and relative intensity of infrared absorption bands in some soil bacteria. (From Filip et al. 2006.)

Band (cm^{-1})	Possible assignment[a]	Relative intentity of IR bands in bacteria		
		Azotobacter chroococcum	*Bacillus subtilis*	*Pseudomonas aeruginosa*
3,400–3,000	H-bonded OH groups; NH_2 stretching (adenine, quanine, cytosine)	m, br	s, br	m, br
2,960–2,850	C–H stretching in aliphatic structures (fatty acids)	w, sh	m, sh	m, sh
1,720	C═O stretching (saturated esters)	vs, sh	nd	vw, br
1,660–1,535	NH_2 bending, C═O, C═N stretching (amide I and II)	vs, sh	vs, sh	vs, sh
1,467–1,455	C–H deformations of CH_2/CH_3 groups in aliphatic	v, sh	vw, sh	vw, sh
1,402	C–O in carboxylates	nd	vw, sh	nd
1,390–1,380	C–H bending, $–CH_3$ stretch (fatty acids)	w, sh	nd	vw, sh
1,290	P═O stretching (phosphoesters)	s, sh	nd	nd
1,240–1,230	C–N stretching (amide III)	m, sh	s, sh	s, sh
1,150–1,000	C–O, PO_2^- (glycopeptides, ribose)	m, sh	s, sh	vs, sh
980–800	P–O–C, P–O–P stretching (phospholipids, ribose-phosphate chain, pyrophosphate)	m, sh	vw, br	vw, br
550–515	C–O–C, P–O–C bonding (nucleic acids, aromatics)	v, sh	vw, br	w, br

[a]After Bellamy (1975) and Parker (1971).

Abbreviations: v = Very; vw = very weak; w = weak; m = medium; s = strong; vs = very strong; br = broad; sh = sharp; nd = not detected.

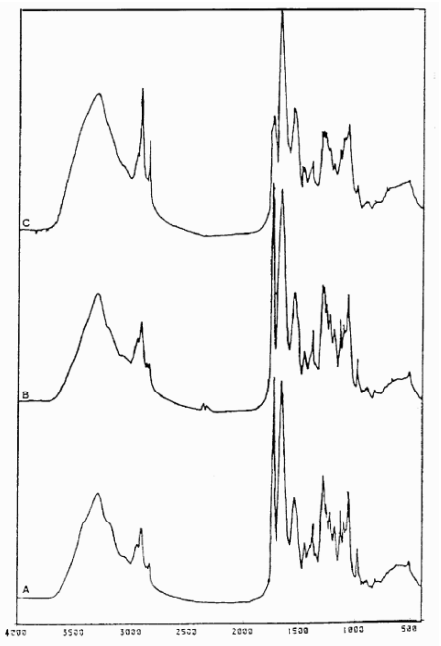

Figure 4. FTIR spectra of *Azotobacter chroococcum.* (A) Standard spectrum of bacteria grown 48 h in MNB; (B) Spectrum of a starving biomass; and (C) Spectrum of a biomass yielded in a late stationary phase of growth (i.e., after 3 days). (From Filip et al. 2006.)

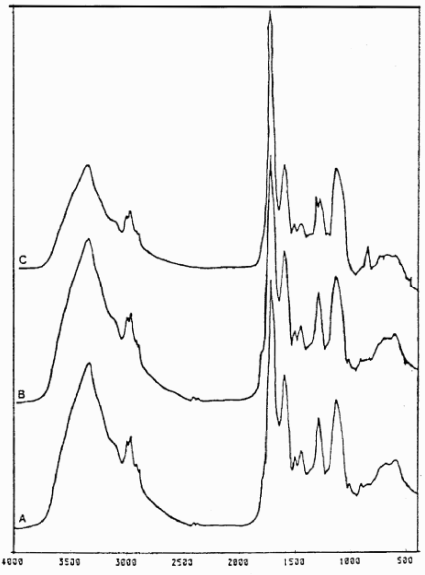

Figure 5. FTIR spectra of *Bacillus subtilis.* (A) Standard spectrum of bacteria grown 48 h in a minimum nutrient broth, (MNB); (B) Spectrum of a starving biomass; and (C) Spectrum of biomass yielded in a late stationary phase of growth. (From Filip et al. 2004.)

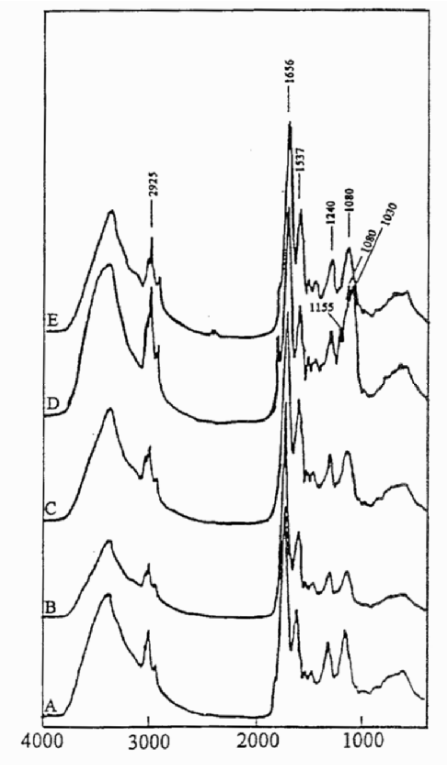

Figure 6. FTIR spectra of *Pseudomonas aeruginosa.* (A) Standard spectrum of bacteria yielded from a minimum nutrient broth (MNB); (B) Spectrum of biomass yielded from a full strength Lurie Bertani Broth; (C) Spectrum of a biomass yielded from MNB containing glutamate as the only C and N source; (D) Spectrum of a biomass yielded from MNB containing glutamate instead of ammonium chloride as N source; (E) Spectrum of a biomass from MNB containing fructose instead of glucose as C source. (From Filip and Herrmann 2001.)

AUV spectroscopic approach was also developed for estimation of soil microbial biomass (Turner et al. 2001). The authors recommend to measure the UV absorbance of 0.5 M K_2SO_4 extracts of chloroform fumigated soil samples at 280 nm. The procedure is based on presumption that molecular compounds released by fumigation, and containing carbon, nitrogen, and phosphorus, absorb in the near UV region. A strong correlation of the respective absorbance values with contents of microbial biomass C, as estimated by conventional methods, has been reported.

There is no doubt that detailed knowledge of individual molecular composites of bacterial biomass, inside and outside of cells, is a necessary precondition for the illumination of binding mechanisms between microbial cells and solid surfaces. This knowledge is of basic importance if specific bio-

organic-mineral particles should be designed and possibly utilized in novel nanotechnologies.

6. Current Trends in the Study of Bio-, Micro- and Nanosystems

In many fields of nanotechnology, different silica structures play a key role. Hildebrand (2006), e.g., reported molecular and microscopic investigations on a diatom, *Thalassiosira pseudonana*. The author attempts to illuminate biosilica formation by micro algae for possible use as a source of natural nanostructured materials. Gorby (2006) pointed out the capacity of some bacteria, ranging from oxygenic cyanobacteria to heterotrophic sulfate reducing bacteria, to produce electrically conductive appendages referred to as bacterial nanowires. Dissimilatory metal reducing bacteria such as *Shewanella oneidensis* and *G. sulfurreducens*, produce nanowires in direct response to electron acceptor limitation and facilitate electron transfer to solid phase iron oxides. Implications shall target energy distribution and communication in biofilms and other microbial communities, and possibly also applications for alternative energy (microbial fuel cells) and some nanoelectronic technologies. Of concern to biofilms, Adamo et al. (2006) pointed on the indirect importance of nematodes to vector bacteria and viruses, and to dismember biofilms. Different free-living soil nematodes such as *Rhabditis, Caernorhabditis elegans* and *Turbatrix aceti* feeding on bacteria, digested only about 70% of biomass consumed, and defecate 30% of viable cells, and in this way they can indirectly affect nanosystems based on bacterial biofilms. In our experiments with laboratory microcosms inhabited by the diplopod *Pachyiulus flavipes* and the woodlouse *Armadillidium vulgare*, plasmid transfer occurred from introduced bacterial strains into facultative-anaerobic gram-negative bacteria isolated from excrements of the invertebrates (Byzov et al. 1999). The results evidenced the possibility of a genetic, i.e., submicroscopic transfer of specific molecules in a natural biosystem.

Bionanofabrication represents a novel process that takes advantage of the specificity and catalytic efficiency of biological systems to create novel nano-scale structures. Niamsiri et al. (2006) concentrated their effort on polyhydroxyalkanoates (PHAs), a family of aliphatic polyesters produced by a variety of microorganisms as a reserve of carbon and energy. PHAs can be combined from more than 199 different monomers to give a variety of biodegradable materials with widely different physical properties. By varying reaction conditions, and using catalytic enzymes, the authors succeeded in creating *in vitro* novel polymeric structures via surface-initiated polymerization, some aspects of which we reported here in sections 2 and 3. The authors attempt to generate biocompatible PHAs-coated solid surfaces for tissue engineering, regulating cell attachment and growth.

Nanosized particles of nobel metals can be applied to a wide range of functions such as catalysis, optics and biosensing. Konishi et al. (2006) used bioreduction of some noble metals by microorganisms for the synthesis of respective nanoparticles. In a culture of *Shewanella algae* with H_2 as an electron donor, intracellular synthesis of gold nanoparticles was achieved at 25°C and pH 7. The 10–20 nm gold particles were located in the cell periplasm. Because many of the gold particles were detected in aqueous solution at pH 1, the authors believe that a gold-reducing enzyme becomes released from the periplasmic space at a low pH.

Methodical tools to elucidate basic interrelationships at a nanoscale level should be further developed. Subramanian (2006) recommends a three-dimensional electron microscopy with a resolution between 50 and 1000 nm for bridging some imaging gaps in nanobiology. A much higher resolution, i.e., ~2 nm, can be achieved by an atomic force microscopy (AFM). Plomp et al. (2006) applied AFM on endospores of *Bacillus* sp. and *Clostridium* sp. They found that AFM could address spatially explicit spore coat protein interactions, structural dynamics in response to environmental changes, and the life cycle of respective pathogens at near-molecular resolution. A stress-induced response of *Arthtobacter oxydans* to Cr (VI) exposure, resulting in the formation of a supramolecular crystalline hexagonal structure on the cell surface, could also be detected. Using a bacterium, *Clamedie trachomatis* the authors were able to identify surface exposed proteins versus proteins embedded in the outer cell membrane. These studies show *in vitro* AFM as a powerful new tool capable of revealing bacterial architecture, structural dynamics and variability at nanometer-to-micrometer scales. Nevertheless, as shown in Fig. 5, the FTIR technique used in our investigations may deliver results of a similar value (Filip et al. 2004).

The recent discovery that metal-reducing bacteria are also capable of reducing solid electrodes enabled the study of microbial fuel cells or sensors utilizing bacteria as catalysts. Based on attachment and current production characteristics of *Geobacter metallireducens*, Hunt and Bond (2006) selected designs for refinement and miniaturization of nitride-coated silicon wafers, with the goal of producing arrays of porous anodes for growth testing in multiple electrode-reducing cultures, and flat anodes for high-resolution electrochemical characterization of microbial respiration.

As concluded by Montemagno (2006), currently, the merging of biotechnology, informatics, and nanotechnology will result in an integrative technology which should make the synthesis of a new class of smart biomaterials possible. Such materials will enlarge the potential to emulate much of the functionality associated with living systems into different nonliving forms. In this way, novel technologies will open an opportunity for realizing true advances in the manner in which technology interacts with nature.

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TOXICITY OF POLYMERIC NANOPARTICLES WITH RESPECT TO THEIR APPLICATION AS DRUG CARRIERS

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Abstract: The aim of this study was to explore the acute toxicity of titanium and silica nanopolymers by monitoring clinical markers of toxicity macroscopic organ pathology, hematological and biochemical parameters in an animal model. Intraperitoneal (0.2 g/kg body weight) or oral (2 g/kg body weight) administration of silica and titanium dioxide xerogels did not result in significant changes in the biochemical parameters characterizing liver function. The intraperitoneal application of silica dioxide xerogels resulted in increased kidney weight and creatinine levels, indicating an impaired kidney function. Hemoglobin concentrations and blood cell counts were within the normal range independently of xerogel type and route of administration. Oral administration of silica dioxide xerogels did not induce gastric mucosal damage. On the other hand, titanium dioxide xerogels administered orally in the same dose induced ulcer formation in 60% of the treated animals. Overall, these results suggest that silica dioxide xerogel can be further investigated as a possible drug carrier matrix.

Keywords: polymeric nanoparticles, silica and titanium xerogels, *in vivo* animal studies

1. Introduction

Polymeric nanoparticles have been intensively studied for their wide use in diagnostics of viral infections, tumors (Labib et al. 1991; Schroeder et al. 2000), and therapeutic applications including gene therapy, transfer, and release of hemoglobin, insulin and other drugs in the blood (Yu and Chang 1996; Zhang et al. 2001). Currently, mainly organic polymeric nanoparticles have been

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intensively studied as drug carriers (Rihova et al. 2003; Soma et al. 2000; Gulyaev et al. 1999; Oh et al. 1999).

There are a few studies on silica polymeric nanoparticles in model systems focusing mainly on the physicochemical characteristics of the drug–matrix interactions and pharmacokinetics (Chen et al. 2004; Lai et al. 2003; Kortesuo 2001; Li et al. 2004). Silica xerogels are candidate materials for chemical sensors (Wei et al. 2000; Wolfbeis et al. 2000; Kumar et al. 2000; Chen et al. 1998; Reetz 1997) drug-delivery systems (Jain et al. 1998), and novel optical (Brusatin et al. 2000) and electrochromic (Rosseinsky and Mortimer 2001) applications. A recent study (Eriksson et al. 2001) evaluated the application of titanium dioxide polymeric nanomaterials as implants in orthopedics and demonstrated a good tolerance in humans. The literature on the application of titanium nanopolymers as drug carriers are insufficient. The toxicity of the free nanopolymers and the nanoparticles has not been well elucidated.

The aim of this study was to explore the acute toxicity of titanium and silica nanopolymers with respect to their application as drug carriers by monitoring clinical markers of toxicity, macroscopic organ pathology, hematological and biochemical parameters.

2. Materials and Methods

2.1. NANOMATERIALS

Titanium and silica nanopolymers, as xerogels, were synthesized by a sol-gel method and supercritical drying and characterized physicochemically and morphologically at the Electrochemical Research Laboratory of the University "Babes-Bolyai", Cluj-Napoca, Romania. The toxicity testing in experimental animals requires administration of water soluble materials. Preliminary studies on the solubility of titanium and silica dioxide xerogels demonstrated that the gels are not water-soluble over a 7-day period. Therefore, they were administered in the present study in the form of suspensions. Due to the high viscosity of the suspensions, it was not possible to use high concentrations and we were unable to determine LD100, LD50, LD20 or LD10. The highest concentrations used for the acute toxicity studies are shown in Table 1.

TABLE 1. Highest applicable concentrations of titanium and silica xerogels.

	Intraperitoneal administration	Oral administration
Highest applicable concentrations	50 g/l	500 g/l
Highest applicable doses	200 mg/kg	2,000 mg/kg

2.2. LABORATORY ANIMALS

Healthy male Wistar rats with an average weight 230 ± 17.3 and an average age of 80 days were used. The animals were housed at the animal facility of the Medical University of Varna under the following conditions: a 24 h free access to water and food, a 12 h light-dark cycle, and room temperature.

Animals were randomized in six groups (Table 2).

TABLE 2. Experimental animal randomization into groups.

Experimental groups	Intraperitoneal administration (number of animals)	Oral administration (number of animals)
Saline-treated controls	9	8
Silica dioxide xerogel-treated group	9	8
Titanium dioxide xerogel-treated group	8	8

Test substances were administered as a single dose after a 24 h period of fasting. Animals were observed for behavioral changes, neuromuscular reactions, and any adverse effects for a period of 8 h after the administration. Mortality was assessed at the end of the study.

The animals were anaesthetized with diethyl ether and blood samples were obtained. The animals were sacrificed by rapid decapitation and exsanguination. Internal organs were weighted and macroscopically examined for any abnormalities: liver, stomach, and kidney of orally treated animals and liver and kidney of intraperitoneally treated animals. The number and area of gastric mucosal ulcer defects were determined. In a case of petechiae, five of them were considered as a 1 mm^2 lesion. The experimental procedure was approved by the Medical University Ethics Committee (Varna) and performed with a strong consideration for ethics for animal experimentation.

2.3. HEMATOLOGICAL EXAMINATIONS

Blood was taken from the jugular vein and divided into portions. Heparin was added to the first portion and the blood was used for hematological assays. The microscopic preparations were stained after Romanovsky–Giemsa and differential blood count was performed. Red blood cell (RBC) counts and hemoglobin concentrations were determined using kits from Hospitex, Italy.

2.4. BIOCHEMICAL ASSAYS

The second blood portion was centrifuged at 1,500 rpm, at room temperature, and the serum collected was used for biochemical assays. Aspartate amino-transferase (AsAT) activity and creatinine levels were determined in serum using kits from LaChema, Czech Republic.

2.5. STATISTICAL ANALYSIS

Mean values and their standard deviations (SD) were calculated for each group. Mean values were compared using Student's *t*-test. A value of $p < 0.05$ was considered to be statistically significant.

3. Results

Body weight and weights of the liver and kidney of rats were not changed after oral administration of titanium and silica xerogels. The weights of kidneys were slightly increased in the group treated intraperitoneally with silica xerogel. Gastric mucosal ulcers were found in 60% of the animals treated orally with titanium dioxide xerogel. This result implies a possible adverse effect of TiO_2 on the gastric mucosa.

TABLE 3. Effect of silica and titanium xerogels on serum AsAT and creatinine levels.

Intraperitoneal administration			
Biochemical parameter	Controls (*n*=9)	SiO_2 (*n*=9)	TiO_2 (*n*=8)
Creatinine	50.93 ± 4.05	80.60 ± 12.90*	47.21 ± 1.34
AsAT	0.83 ± 0.07	0.90 ± 0.06	0.87 ± 0.04
Oral administration			
Biochemical parameter	Controls (*n*=9)	SiO_2 (*n*=9)	TiO_2 (*n*=8)
Creatinine	45.32 ± 1.71	45.73 ± 1.26	51.20 ± 3.75
AsAT	1.15 ± 0.05	1.13 ± 0.06	1.10 ± 0.05

*p< 0.05 compared to the control group.

AsAT and creatinine serum levels were used as indicators of liver and kidney function, respectively. A statistically significant increase of creatinine (by 60%) was found in the intraperitoneally silica dioxide xerogel treated group as compared to the controls (Table 3). The other experimental groups did not demonstrate kidney toxicity, based on the blood creatinine levels. No

significant variations in AsAT serum levels were observed in any of the experimental treatments, indicating a lack of liver toxicity.

We did not find significant changes in RBC count and hemoglobin levels for all experimental groups (Table 4).

TABLE 4. Effect of silica and titanium dioxide xerogels on hemoglobin concentration and RBC count.

Intraperitoneal administration			
Hematological parameter	Controls (*n*=9)	SiO_2 (*n*=9)	TiO_2 (*n*=8)
Hemoglobin	17.8 ± 0.37	18.4 ± 0.39	17.2 ± 1.02
RBC count	10.8 ± 0.25	11.1 ± 0.10	10.7 ± 0.58
Oral administration			
Hematological parameter	Controls (*n*=9)	SiO_2 (*n*=9)	TiO_2 (*n*=8)
Hemoglobin	18.2 ± 0.18	18.3 ± 0.22	18.3 ± 0.20
RBC count	11.5 ± 0.10	11.7 ± 0.14	11.5 ± 0.09

Differential blood count was performed to examine possible changes in the ratio and morphology of white and RBC lines induced by the administered xerogels. No significant differences between the xerogel treated and the control rats were found by intraperitoneal or oral routes of administration (Table 5).

TABLE 5. Differential blood count.

Intraperitoneal administration			
Cell types	Controls (*n*=9)	SiO_2 (*n*=9)	TiO_2 (*n*=8)
St	4.20 ± 2.40	7.20 ± 2.20	7.50 ± 4.90
Sg	38.00 ± 12.00	47.00 ± 13.00	36.00 ± 12.00
Eo	0.56 ± 0.53	0.56 ± 0.73	1.10 ± 1.60
Mo	1.10 ± 1.10	1.70 ± 1.20	1.80 ± 1.50
Ba	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ly	56.00 ± 14.00	42.00 ± 12.00	55.00 ± 15.00
Oral administration			
Cell types	Controls (*n*=8)	SiO_2 (*n*=8)	TiO_2 (*n*=8)
St	4.30 ± 3.30	3.60 ± 1.30	3.00 ± 2.30
Sg	28.00 ± 7.30	26.00 ± 12.00	33.00 ± 15.00
Eo	0.25 ± 0.71	0.38 ± 0.74	0.13 ± 0.35
Mo	0.75 ± 1.20	0.13 ± 0.35	1.40 ± 1.40
Ba	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ly	68.00 ± 8.20	70.00 ± 11.00	63.00 ± 18.00

The relative ratios of all cell types examined were within the reference range. Only one animal treated orally with silica dioxide xerogel showed erythrocyte damage.

4. Discussion

This is one of the first reports on *in vivo* toxicity assessment of inorganic silica and titanium dioxide xerogel nondegradable polymeric systems. A few reports studied the applicability as a potential drug carrier of silica xerogels, whereas data about titanium xerogels are scarce.

Due to the limited solubility and high viscosity of the aqueous solutions of titanium and silica dioxide xerogels, it was not possible to establish their LD100, LD50, LD20 or LD10. The xerogels administered in the form of suspensions at the highest applicable concentrations did not induce macroscopic organ pathology.

The intraperitoneal application of 0.2 g/kg body weight of silica dioxide xerogels in our experiments resulted in increased kidney weight and higher creatinine levels indicative of an impaired kidney function. It has been reported that silica is actively phagocytized by the macrophages or excreted in the urine through kidneys (Lai et al. 1998). Our experiments suggest that the intraperitoneal administration of silica dioxide xerogels has more adverse effects than oral (2 g/kg body weight) administration of the xerogel. In support of our findings Kortesuo (2001) did not detect any silica-related histological changes in liver, kidney, lymph nodes, and uterus in subcutaneous administration even though the amount applied was high (1.5 g/kg body weight) and comparable to the doses used in this study. Similarly we did not find significant variations in the biochemical parameters characterizing liver function after intraperitoneal and oral administration of silica and titanium dioxide xerogels. Hemoglobin concentration and blood cell counts were also within the normal range.

The plain silica xerogel implant (without drug substance) did not cause irritation of the surrounding tissue during 42-day treatment in mice (Kortesuo 2001). Sol-gel glass neither caused the inhibition of fibroblast growth nor induced a significant inflammatory response (Wilson et al. 1981, Palumbo et al. 1997). Oral administration of a high dose (2 g/kg body weight) of silica dioxide xerogels did not exhibit ulcerogenic effects in our study whereas titanium dioxide xerogels at the same dose induced ulcer formation in 60% of the treated animals. The lack of pro-inflammatory effects of silica dioxide xerogel on gastric mucosa suggests that it could be used as a drug carrier matrix in further *in vivo* studies.

Kortesuo (2001) has studied the drug release of toremifene from silica xerogel monoliths in mice and of dexmedetomidine from monoliths and microparticle suspension in dogs. The release of drugs was found to be faster than the degradation of the silica matrix, which varied from days to months with monoliths and from days to more than a year with microparticles. This, together with our findings about the low-silica dioxide xerogel toxicity suggests that these gels could be explored as potentially safe drug carriers.

In order to assess more precisely the toxicity of titanium and silica dioxide xerogels and their possible application as drug carriers, further experiments evaluating their chronic toxicity should be conducted.

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RISK ASSESSMENT APPROACHES AND RESEARCH NEEDS FOR NANOMATERIALS: AN EXAMINATION OF DATA AND INFORMATION FROM CURRENT STUDIES[1]

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Abstract: Risk assessment is an important component in the development of effective risk management strategies. Toxicology, epidemiology, and workplace exposure data are very limited for new engineered nanomaterials, although the number of toxicology studies in this area is growing rapidly. Data are available from existing human and animal studies of exposure to airborne ultrafine and fine particles and fibers, and these can be used as "benchmark" materials for comparison to new nanomaterials. In this paper, we examine several risk assessment and risk management options to evaluate and control exposures to reduce the risk of adverse health effects in workers producing or using nanomaterials. These options include quantitative risk assessment (QRA) using available dose-response data from chronic or subchronic inhalation studies in rodents; comparative potency analysis based on toxicological studies of ultrafine or fine particles; evaluation and adjustment of current exposure limits for similar materials; and hazard assessment and control approaches. As more data become available on new nanomaterials, more precise estimates using QRA can be developed. In the meantime, the existing scientific literature on particles and fibers can be used to develop preliminary hazard or risk estimates for nanomaterials and provide a basis for effective risk management approaches.

Keywords: nanoparticles, risk assessment, risk management

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[1]Disclaimer: The findings and conclusions in this article are those of the authors and do not necessarily represent the view of the National Institute for Occupational Safety and Health.

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1. Introduction

The burden of occupational disease is enormous in terms of both financial cost and loss of quality of life, and the reported rates are likely underestimates (Schulte et al. 2005). Occupational lung disease rates are highest among workers in dusty jobs, and historically these rates decrease when dust exposures are reduced (MMWR 2003). Airways disease remains a leading cause of death in the USA, and millions of workers are potentially exposed to agents that cause airways disease. Based on the epidemiological and toxicological studies of airborne particles and fibers, and our understanding of the mechanisms of particle and fiber toxicity, there is concern about the potential health risk of exposure to engineered nanoparticles. This increase in health and safety awareness also provides an opportunity to establish healthful work environments to prevent occupational disease among workers producing or using nanomaterials.[2]

Inhalation is the primary route of exposure for airborne particles or fibers in the workplace, although ingestion is a possible route of exposure, either by inadvertent consumption with food or drink, or as a result of mucociliary clearance of inhaled particles that may then be swallowed (ICRP, 1994). In addition, some studies suggest that exposure to nanoparticles may occur by other routes including dermal penetration (Tinkle et al. 2003; Ryman-Rasmussen et al. 2006) or neuronal transport to the brain (Elder et al. 2006). While nanoparticles entering the body by any of these routes have the potential to translocate to the blood circulation and nonpulmonary organs, based on studies in rats (Ferin et al. 1992; Kreyling et al. 2002, 2006; Oberdörster et al. 2002; Geiser et al. 2005), the respiratory tract remains a primary target organ for potential adverse effects of airborne particles including nanoparticles. Studies in humans have shown that nanoparticles are more likely to deposit in the respiratory tract than larger respirable particles, with the total deposition increasing to >90% for the smaller nanoparticles (<10 nm) (ICRP 1994; Jaques and Kim 2000).

[2]The terms ultrafine particle and nanoparticle are often used interchangeably, although nanoparticle is a newer term that generally refers to "engineered" particles. Ultrafines or nanoparticles have primary particle diameters less than 0.1 μm and are respirable (i.e., capable of depositing in the gas-exchange region of the lungs), as are fine particles (0.1–2.5 μm) and coarse particles (2.5–10 μm) in humans. Ultrafine or nanoparticles frequently occur as agglomerates or aggregates, although surface coatings and other treatments are sometimes used to improve dispersion of the nanoparticles. An agglomerate is a group of particles held together by relatively weak forces (e.g., electrostatic, surface tension), while an aggregate is a heterogeneous particle with components held together by relatively strong forces, and thus is not easily broken apart (ISO 2006). The term nanomaterial is used here in a general context to include nano-structured particles of any shape or configuration with primary particle(s) <0.1 μm.

The lungs respond in relatively few ways to the many different types of airborne particles and fibers. Chronic inflammation appears to be a common mechanism in particle- and fiber-associated lung diseases (Castranova 1998, 2000; Mossman and Churg 1998; Donaldson et al. 2005). Studies in rodents have shown that inhaled particles elicit a cascade of events involving cell mediators for oxidative stress, pulmonary inflammation, and fibrosis, as well as lung cancer in rats (Donaldson et al. 1998; Donaldson and Stone 2003; Knaapen et al. 2004; Bermudez et al. 2004; Elder et al. 2005; Oberdörster et al. 2005).

One of the key determinants in the toxicity of fine and ultrafine particles is particle surface area. The particle surface area dose of poorly soluble, low toxicity (PSLT) particles has been more closely associated with adverse lung responses in rats including inflammation and lung cancer than has the particle mass dose across a range of particle sizes (Oberdörster and Yu 1990; Oberdörster et al. 1992, 1994b; Driscoll 1996; ILSI 2000; Tran et al. 2000; Brown et al. 2001; Duffin et al. 2002; Zhang et al. 2003). That is, lower mass doses of ultrafine than fine particles were associated with adverse lung responses. Lung cancer was observed in rats only at a high mass dose (250 mg/m^3) of fine-size titanium dioxide (Lee et al. 1985), which is consistent with "overloading" of lung clearance (Morrow 1988). In contrast, elevated lung cancer was observed in rats exposed to much lower mass doses of ultrafine particles (~2–10 mg/m^3) (Heinrich et al. 1995; Nikula et al. 1995), which are within the range of current occupational exposure limits (OELs) for titanium dioxide and other poorly soluble particles (section 2.3). A recent study reported that particle size and surface area did not influence toxicity (Warheit et al. 2005); however, that study was not adequate to test those hypotheses because the lung responses to either fine or ultrafine TiO_2 were not significantly different from controls. Nanoparticles are more biologically active than larger particles of the same chemistry due to their greater surface area per mass (Oberdörster et al. 2005).

In addition to particle surface area, other physical and chemical properties can influence the toxicity of particles and fibers, including shape, size, solubility, charge, chemical composition (including surface coatings), and surface reactivity (Lison et al. 1997; Zhang et al. 1998; Dick et al. 2003; Donaldson et al. 2005; Maynard and Kuempel 2005; Donaldson et al. 2006). For example, quartz is considerably more toxic than PSLT even after adjusting for the particle surface area due to its highly reactive surface (Duffin et al. 2002). In addition, individual nanoparticles are small enough to be capable of entering cells and organelles, where they can cause oxidative damage and impaired cell function (Li et al. 2003; Geiser et al. 2005). Chemical composition and charge can also affect the ability of individual nanoparticles to

enter cells, interact with cellular proteins and other macromolecules, induce mechanisms of oxidative stress and cytoxicity, and translocate into the blood circulation and various tissues (Takenaka et al. 2001; Kreyling et al. 2002; Oberdörster et al. 2002; Semmler et al. 2004; Xia et al. 2006).

In humans, occupational exposure to fine and ultrafine particles has been associated with adverse lung responses similar to those observed in rodents (Castranova 2000), including pulmonary inflammation (Lapp and Castranova 1993; Vallyathan et al. 2000), pulmonary fibrosis, chronic obstructive pulmonary disease, lung function decrements, metal fume fever, respiratory symptoms, and cardiovascular effects (Mossman and Churg 1998; Antonini 2003; Donaldson et al. 2005; Maynard and Kuempel 2005). Elevated lung cancer has been reported in some of the studies of workers exposed to ultrafine particles (diesel exhaust and welding fume) (Steenland et al. 1998; Garshick et al. 2004; Antonini 2003) or fine particles (crystalline silica and coal dust) (Rice et al. 2001; Attfield and Costello 2001; Morfeld et al. 2002). In the general population, chronic exposure to particulate air pollution, a mixture which includes combustion-derived ultrafine particles (Donaldson et al. 2005), has been associated with increased morbidity and mortality from adverse respiratory and cardiovascular effects (Dockery et al. 1993; Ibald-Mulli et al. 2002; Pope et al. 2004), including elevated lung cancer (Pope et al. 2002).

Currently, the scientific data available to evaluate the potential hazard of nanomaterials or risk of exposure in workers include:

- Extensive toxicological and epidemiological studies of ultrafine or fine airborne particles and fibers
- Limited toxicological studies and no epidemiological studies of engineered nanomaterials
- Some data on ultrafine particle concentrations in the workplace and urban air
- Very limited data on nanoparticle concentrations in the workplace

In this paper, we describe the current data pertaining to an evaluation of the potential adverse health effects of occupational exposure to nanoparticles, provide several possible approaches for hazard and risk assessment using available data, and identify key data gaps and research needs.

2. Risk Assessment Approaches

A standard risk assessment structure in use today was developed by the National Research Council (1983). This risk assessment process is defined as "the characterization of the potential adverse health effects of human exposures to

environmental hazards"; and it includes four major steps: (1) hazard identification, (2) dose-response assessment, (3) exposure assessment, and (4) risk characterization (NRC 1983). Risk assessment within this framework can be quantitative and/or qualitative (Fig. 1).

Included in the risk assessment process are evaluations of the uncertainties in the methods and the factors influencing variability in the risk estimates in the population. Risk management is defined as "the process of evaluating alternative regulatory actions and selecting among them" (NRC 1983).

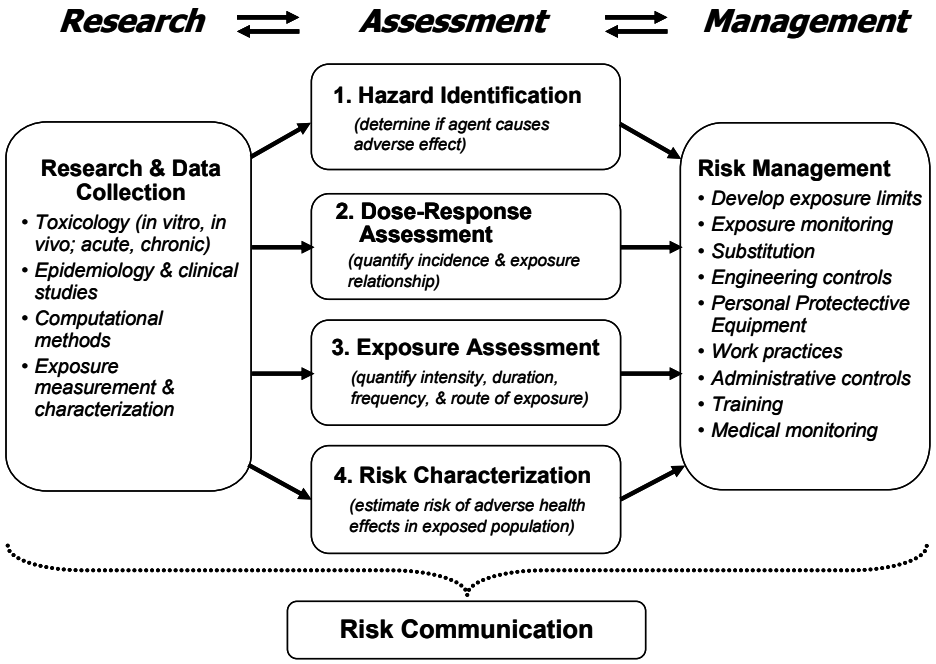

Figure 1. Risk Assessment framework – Scientific research and monitoring data provide the scientific basis to evaluate potentially hazardous substances, including assessment of the: (1) nature and level of hazard, (2) dose–response relationship, (3) exposures occurring in an occupational or ambient environment, and (4) risk of these exposures in a given population. These risk assessment findings are used to evaluate the risk management options, and these findings are disseminated to inform public health officials, workers, employers, the public, and regulatory agencies for public health decision-making. (Aadapted from NRC 1983).

The data needed for risk assessment come from scientific research and surveillance studies. The risk assessment findings are then used by risk managers to develop strategies to minimize or reduce the potential adverse health risks. The decision-making process in risk management may include ethical issues such as acceptability of risk and economic issues such as cost of control (NRC 1983; Schulte and Salamanca-Buentello 2006). Risk communication among public

health officials, workers, employers, and the public is essential throughout the risk assessment process.

2.1. QUANTITATIVE RISK ASSESSMENT

Quantitative risk assessment (QRA) is defined as "the estimation of the severity and likelihood of adverse responses associated with exposure to a hazardous agent" (Piegorsch and Bailer 2005). QRA methods are useful to estimate the exposure concentrations that are likely – or unlikely – to cause adverse health effects in workers. Dose-response data are required to perform QRA. If human dose-response data are available, a clear advantage of using those data for risk assessment is that no interspecies extrapolation is required. However, limitations and challenges in using human data may include: lack of quantitative exposure data; poorly characterized exposures and co-exposures; confounding factors; unmeasured sources of variability (e.g., genetic heterogeneity); and loss of study subjects to follow-up evaluation, which may obscure the relationship between exposure and disease, as in the "healthy worker survivor effect" (Gibb et al. 2002).

When human dose-response data are limited or not available, data from experimental studies in animals are often used to predict risk in humans. Scientific advantages of using animal (generally rodent) data in QRA may include: well-characterized and quantified exposures; controlled experimental conditions; reduced genetic variability (e.g., inbred strains); measurement of dose and disease markers; and investigation of biological mechanisms. A key limitation and source of uncertainty in using animal data is that extrapolation to humans is required. *In vitro* assays are also used in studies of biological mechanisms, and for initial toxicity screening (e.g., nanoparticles) (ILSI 2005). The utility of *in vitro* studies in hazard or risk assessment, including quantitative comparison of *in vitro* and *in vivo* responses (Faux et al. 2003), is an area of continuing research.

Steps in using existing rodent studies in QRA of airborne particles or fibers include:

1. Identify appropriate data, including the animal model, dose metric, and disease response of interest for human health risk assessment.
2. Analyze the dose–response relationship(s) in animals to estimate the dose(s) (administered or internal) associated with specified risk(s) of disease.
3. Calculate the human-equivalent dose(s), e.g., using allometric adjustment of administered dose or a human lung dosimetry model to estimate internal dose; normalize internal dose per unit lung mass or lung surface area in each species.

4. Determine human exposure conditions (airborne concentration, duration, frequency, breathing pattern) associated with the human-equivalent lung dose (e.g., either included in allometric calculations in step 3, or use a human lung dosimetry model to estimate exposures); characterize variability in human exposure, dose, and response relationships and uncertainties in models and methods, including interspecies extrapolation.

This approach has been used to illustrate QRA of working lifetime occupational exposure to airborne fine or ultrafine TiO_2, carbon black, or diesel exhaust particulate (Table 1) (Kuempel et al. 2006).

TABLE 1. Rat data-based estimates of workplace airborne particle concentrations (8 h TWA) over a 45-year working lifetime associated with 0.1% excess risk of lung cancer. (From Kuempel et al. 2006.)

Substance[a]	Human-equivalent exposure concentration (mg/m^3)			
	Allometric adjustment – concentration[*]	*BMDL as air mass*	*Lung dosimetry model – lung dose*[*]	*BMDL as mass*
	Lung mass extrapolation factor	Lung surface extrapolation factor	MPPD/ICRP models (CIIT & RIVM 2002)	Interstitial/ sequestration model[b]
TiO_2 (ultrafine)	–	–	0.14	0.073
CB (ultrafine)	0.19	0.11	0.24	0.12
DEP (ultrafine)	0.28	0.17	0.28	0.18
TiO_2 (fine)	8.7	5.1	1.3	0.68

[*] BMDL: lower 95% confidence limit of benchmark dose (Crump 1984) at 10% excess risk of lung cancer with linear extrapolation to 0.1% excess risk (based on the multistage model, third degree polynomial).

[a] DEP: diesel exhaust particulate; CB: carbon black.

[b] Kuempel et al. (2001a, b); Tran and Buchanan (2000).

In this analysis, the rat-based estimates of the working lifetime airborne concentrations (associated with 0.1% excess risk of lung cancer; 95% lower confidence limit) were similar for the ultrafine particles whether allometric scaling or lung dosimetry models were used, while the estimates differed for fine TiO_2–with higher working lifetime exposure estimates from allometric scaling. Although these analyses are limited, they suggest that external exposure may not always be a reasonable surrogate for the internal dose (e.g., due to nonlinear relationship between exposure and dose). Differences in the clearance kinetics of fine versus. ultrafine particles that may not be adequately described by current models could also influence the dose predictions (Kuempel and Tran 2002; Kuempel et al. 2006).

2.2. COMPARATIVE TOXICITY ANALYSES

2.2.1. Poorly Soluble Particles

A number of studies provide quantitative data to facilitate a comparison of various poorly soluble fine and ultrafine particles. Soutar et al. (1997) found that human and animal studies show consistent relative potencies of various

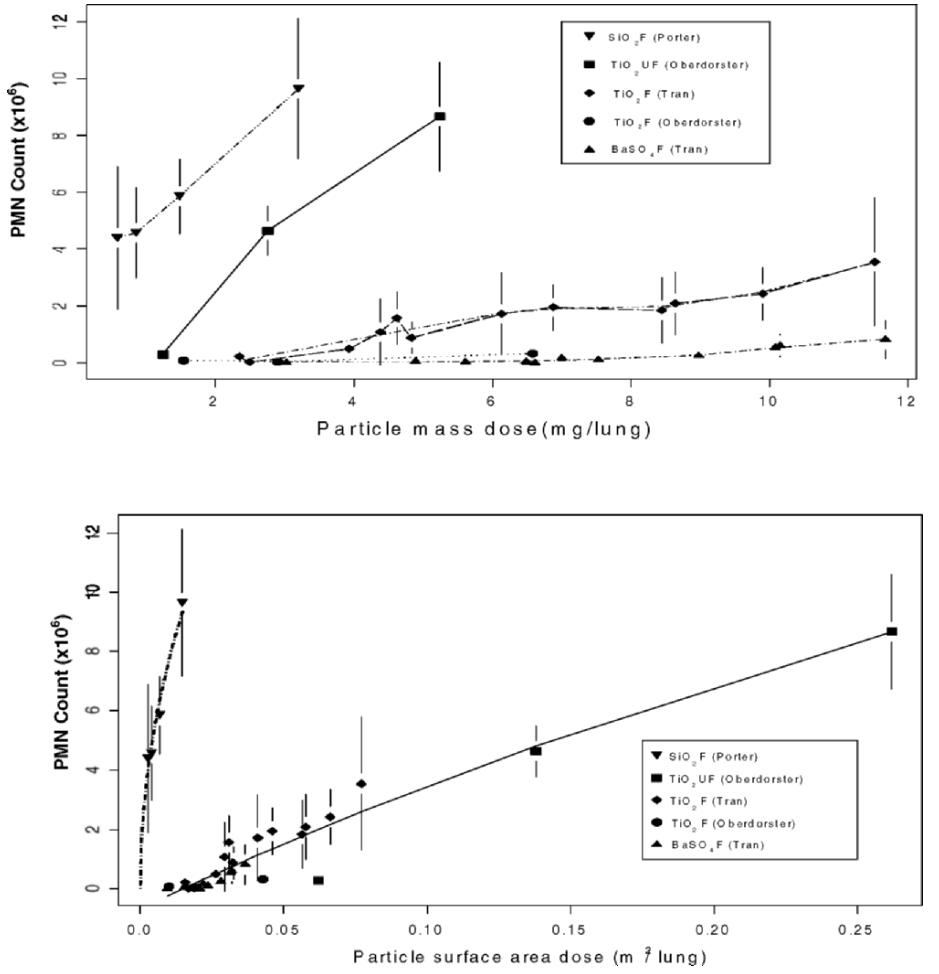

Figure 2. Relationship between pulmonary inflammation (polumorphonuclear leukocyte count) and either: (a) particle mass dose, or (b) particle surface area dose of high toxicity (crystalline silica) or low toxicity (titanium dioxide and barium sulfate) inhaled particles. (Data from Porter et al. 2001; Oberdörster et al. 1994a; Tran et al. 1999).

types of inhaled particles. Exposure in both animals and humans was expressed as airborne mass concentration. The human lung responses were pneumoconisosis and lung function deficits, and the rat lung responses were the number of cells in broncoalveolar lavage fluids and the clearance halftimes of particles from the lungs. Not all particle data were available in both species, but consistent findings were observed for those that were. In both humans and rats, crystalline silica was the most toxic, carbon black was intermediate, and polyvinylchloride (PVC) dust was relatively low toxicity. Coal dust (in humans) and fine rutile TiO_2 and printer/copier toner (in rats) were also relatively low toxicity. Ultrafine TiO_2 and diesel exhaust particulate were nearly as toxic as crystalline silica (based on reduced pulmonary clearance) in rats.

Some of the differences in relative toxicity of poorly soluble fine and ultrafine particles can be explained by the particle surface area. Figure 2a shows different dose–response relationships for pulmonary inflammation in rats exposed by subchronic inhalation to either fine crystalline silica, ultrafine TiO_2, fine TiO_2, or fine $BaSO_4$ – when dose is expressed as mass. However, when dose is expressed as particle surface area, ultrafine and fine TiO_2 and fine $BaSO_4$ show a consistent dose–response relationship, and crystalline silica remains more potent (Fig. 2b).

2.2.2. Carbon Nanotubes

Carbon nanotubes (NT) are commercially available nanoparticles with increasing worldwide production (Donaldson et al. 2005). The toxicity of CNT does not appear to be predictable from the "bulk" materials with the similar chemical composition (e.g., graphite). Single-walled carbon nanotubes (SWCNT) produced multifocal granulomas in the lungs of rats and mice exposed by intratracheal instillation or pharyngeal aspiration, without the persistent inflammation typical of lung responses to other poorly soluble particles and fibers (Lam et al. 2004; Warheit et al. 2004; Shvedova et al. 2005). SWCNT were shown to be more fibrogenic in mice than an equal mass of either ultrafine carbon black or fine quartz (Lam et al. 2004; Shvedova et al. 2005). Shvedova et al. (2005) estimated that workers would attain an equivalent lung burden to that causing adverse lung effects in mice if they were exposed to SWCNT over a period of 20 days at the current Occupation Safety and Health Administration (OSHA) permissible exposure limit (PEL) for graphite (5 mg/m^3). Lam et al. (2006) provided similar estimates. Metals used in the production of the SWCNT also affected the toxicity (e.g., nickel) (Lam et al. 2004), and could be an additional health hazard for workers. Limited data are available on workplace exposures to CNT, although one study found much lower mass concentrations of respirable SWCNT (~0.05 mg/m^3) (Maynard et al. 2004). A study of multi-walled carbon nanotubes (MWCNT) in mice by intratracheal instillation also reported pulmonary inflammation and fibrosis (Muller et al. 2005).

2.2.3. Polymer Fume

Among the most acutely toxic of the aerosols containing nanoparticles may be the high temperature-generated polymer fumes, including polytetrafluoroethylene (PTFE) (teflon). Freshly generated PTFE fume (temperature >425°C; nanoparticles ~18 nm diameter) caused severe lung inflammation, oxidative injury, hemorrhagic pulmonary edema, and death in rats exposed to only ~0.05 mg/m^3 for 15 min (Oberdörster et al. 1995). Hypotheses about the toxic mechanism of these fumes include: (1) PTFE particles may act as carriers for adsorbed reactive gases, and (2) radicals on surfaces of the freshly generated nanoparticles – either of which could cause lung tissue injury. When PTFE fume was "aged," the toxicity was considerably reduced – an effect attributed to an increase in particle size (due to agglomeration) and changes in the surface chemistry (resulting in reduced reactivity) (Johnston et al. 2000; Oberdörster et al. 2005).

Humans exposed to PTFE fume have also experienced adverse effects, including polymer fume fever and pulmonary edema; one worker died when an equipment malfunction caused overheating of the PTFE resin and release of the PTFE pyrolysis products in the workplace (Goldstein et al. 1987; Lee et al. 1997). It is clear that a fully enclosed, well-monitored system is required to protect workers from exposure to high-temperature, freshly generated fumes, where even relatively low mass concentrations can be extremely toxic and lethal.

2.2.4. Comparison of Risk Estimates in Humans and Rats

Using animal data in risk assessment should include an evaluation of the uncertainty of the animal model for predicting risks in humans. This may include a qualitative evaluation of the biological mechanisms influencing exposure, dose, and response relationships in each species. In addition, where data are available, it is useful to compare quantitative risk estimates based on data in each species to evaluate how well the animal model may predict disease risk in humans, by class of substance, route of exposure, or adverse effect.

Quantitative comparisons in rats and humans are available for lung cancer risk from inhalation exposure to some poorly soluble particles, including crystalline silica and diesel exhaust particulate. For example, Table 2 shows human-based and rat-based excess risk estimates for lung cancer in workers after a 45-year working lifetime at the current NIOSH recommended exposure limit (REL) for respirable crystalline silica (Kuempel et al. 2001c).

The rat-based risk estimates were derived using a rat lung dosimetry and dose-response model for inflammation (considered an early step in the pathway to

TABLE 2. Comparison of rat- and human-data based risk estimates for lung cancer in workers exposed to respirable crystalline silica. (Kuempel et al. 2001c.)

Species and study	External exposure (mg/m^3) in humans	Internal dose (mg/g lung) in rats or humans	Excess risk (%)	
			Maximum likelihood estimate	95% Upper confidence limit
Rat (Muhle et al. 1991)	NA	0.54[b]	3.9	8.9
Human (Rice et al. 2001)	0.05 [a]	0.54[b]	1.2	2.6
Human (Attfield and Costello 2001)	0.05 [a]	0.54[b]	1.3	2.1

[a]NIOSH recommended exposure limit (REL) for respirable crystalline silica and cristobalite.
[b]Internal dose predicted from human lung dosimetry model to be associated with 45-year working lifetime exposure at either REL or PEL.

particle-induced lung cancer); the estimates are within a factor of three or four, with the higher estimates from the rat data. This is as expected since inflammation occurs earlier (and at lower doses) than lung cancer – according to a mechanistic pathway involving particle-elicited oxidative stress, pulmonary inflammation, tissue injury and repair, and eventually cancer (Shi et al. 1998; Donaldson et al. 2006). When lung tumor response was compared in both species, the rat-based risk estimates were similar to those in humans, depending on extrapolation method (Kuempel et al. 2002). Another comparison of quantitative risk estimates based on data in rats or humans exposed to diesel exhaust particulate showed that the rat studies generally underpredicted the lung cancer risk estimate in humans (Stayner et al. 1998). While these are limited examples, they suggest that the rat is not overly sensitive (e.g., from particle overload of lung clearance) to the development of particle-induced lung cancer compared to humans; and that the rat may be a useful model for risk assessment of inhaled particles including nanoparticles.

2.3. ESTIMATING APPROXIMATE OCCUPATIONAL EXPOSURE LIMITS (OELS) FROM EXISTING OELS

2.3.1. Examination of Existing OELs

Based on the studies showing greater toxicity of nanoparticles compared to the same mass of larger particles of similar composition, it would be worthwhile to examine the range of current mass-based exposure limits with respect to worker health protection. Table 3 provides a compilation of some of the OELs in the USA which have relevance to nanomaterials.[3]

[3] Occupational Safety and Health Administration (OSHA) permissible exposure limits (PELs) and National Institute for Occupational Safety and Health (NIOSH) recommended exposure limits (RELs) are provided. PELs are statutory limits, while RELs are recommended limits.

TABLE 3. Examples of recommended and permissible exposure limits in the USA for some substances of relevance to use in nanotechnology. (From NIOSH 2005b.)

Substance	Chemical formula	NIOSH REL (TWA: mg/m^3)	OSHA PEL (TWA: mg/m^3 – unless stated otherwise)
Carbon-based particles			
Carbon black	C	3.5 0.1 (PAHs) Ca	3.5
Diesel exhaust particulate	Not listed	Ca	None
Graphite (natural)	C	2.5 (resp)	15 mppcf [d]
Graphite (synthetic)	C	none [e]	15 (total) [d] 5 (resp) [d]
Metal oxides and poorly soluble particles			
Particulates not otherwise regulated	Not listed	None[e]	15 (total) 5 (resp)
Titanium dioxide	TiO_2	1.5 (resp) – draft [a] 0.1 (ultrafine) – draft [a]	15 (total) [d]
Zinc oxide	ZnO	5 (dust, TWA) 15 (dust, ceiling) 5 (fume, TWA) 10 (fume, STEL)	15 (total) [d] 5 (resp) [d] 5 (fume) [d]
Metal dust and fumes			
Aluminum	Al	10 (total) 5 (resp)	15 (total) 5 (resp)
Beryllium	Be	Ca 0.0005 (not to exceed)	0.002 (TWA) 0.005 (ceiling) 0.025 (30 min maximum)
Cadmium (as Cd) [c]	CdO or Cd	Ca (dust or fume)	0.005 (dust or fume)
Cobalt [b]	Co	0.05	0.1[d]
Iron (salts, soluble, as Fe) [b]	Fe	1	none[d]
Manganese tetroxide (as Mn)	Mn_3O_4	None [e]	5 (ceiling) [d]
Manganese fume	Mn	1 (TWA) 3 (STEL)	5 (ceiling)
Molybdenum [b]	Mo	None [e]	15 [d]
Nickel [b]	Ni	Ca 0.015	1[d]
Selenium [c]	Se	0.2	0.2
Welding fume	Not listed	Ca	None[†]

[a]NIOSH (2005a).

[b]Used as catalysts to produce carbon nanotubes.

[c]Quantum dot components.

[d]OSHA PEL update attempt vacated by court in 1988.

[e] NIOSH provided comments to OSHA on August 1, 1988, regarding the "Proposed Rule on Air Contaminants" (29 CFR 1910, Docket No. H-020). In these comments, NIOSH questioned whether the PELs proposed were adequate to protect workers from recognized health hazards (NIOSH 2005b).

Abbreviations: TWA: time-weighted average (NIOSH: up to 10 h/day during 40 h workweek; OSHA: 8h/day over 40 h workweek); Ceiling: not to be exceeded during any part of the workday, instantaneous or 15 min TWA; STEL: 15 min period; Total: total particulate; Resp: respirable fraction of airborne particulate; mppcf: million particles per cubic foot of air (impinger sampler).

For example, the graphite PELs are frequently cited on material safety data sheets (MSDS) for CNT. In addition, many substances with OELs can be produced or generated with primary particles in the ultrafine or nanoparticles size range, including carbon black, titanium dioxide, beryllium, diesel exhaust particulate, and welding fume. Several of these substances are used as catalysts in the production of CNT (cadmium, cobalt, nickel, and iron), and some are components of quantum dots (cadmium and selenium).

A rough indication of the relative hazard of these materials may be inferred from comparison of these OELs. For example, among the OELs listed Table 3, the lower values tend to be for the metal dusts or fumes, suggesting these are more hazardous (e.g., beryllium, cadmium, cobalt, nickel, and selenium). However, OELs have been developed over a long period of time with a variety of rationales, and thus can be used as only an approximate guide rather than a true indication of relative toxicity. Most of the PELs in Table 3 were proposed for revision in the 1988 Air Contaminants Rule, including several which NIOSH had testified were not adequately protective of workers' health. When the 1988 Air Contaminants Rule was vacated by the court, the previous (less protective) PELs were restored. Moreover, OELs may be based on technical or economic feasibility factors in addition to health and safety considerations. Thus, the use of OELs to evaluate the safety of nanoparticles should be viewed with caution, both with regard to their adequacy for "bulk" materials and for nanoparticles specifically.

Draft RELs for fine and ultrafine titanium dioxide have been developed recently using QRA and dose-response data in rats (NIOSH 2005a). NIOSH interpreted the scientific literature as indicating that the rat lung tumor induction from TiO_2 inhalation was secondary to the pulmonary inflammation. Consistent with this mechanism, a statistical analysis showed evidence of a threshold dose for pulmonary inflammation in rats exposed to fine TiO_2 by subchronic inhalation. The dose–response relationships were related to the particle surface area dose, and the separate RELs for fine and ultrafine TiO_2 reflect the greater adverse lung responses observed for ultrafine TiO_2 on a mass basis. These RELs represent airborne concentrations that are estimated to be unlikely to result in adverse health effects in workers exposed up to a full working lifetime.

Developing OELs for nanoparticles is a critical occupational health need, especially for nanoparticles already in production and use. Until data are available for QRA of specific nanoparticles, more immediate and practical methods are needed to evaluate the potential hazard or risk of exposure to nanoparticles in the workplace and to develop risk management strategies.

2.3.2. Adjustment of Current OELs

One approach that has been discussed by industrial hygienists and occupational health scientists (although we have not seen it formally proposed) is to adjust and existing OEL by a factor at least as large as the ratio of the specific surface areas (m^2/g) for the "bulk" material (e.g., fine- or coarse-sized particles) and the nanoparticles of the same material. While this would be a very rough approximation, it may be an improvement over simply assuming that the current mass-based OELs developed for larger respirable particles are safe for workers exposed to the same mass concentration of nanoparticles. In addition, consideration should be given to the basis of the OEL for a given "bulk" material, including the type and quality of data and methods used, and the extent to which the OEL was based on health effects data versus technological or economic factors. This being said, the conceptual approach is supported by the scientific data of increased reactivity of nanoparticles due to their increased surface area per mass (a key property exploited in nanotechnology) and by the studies showing that this characteristic of nanoparticles also translates into greater reactivity in biological systems, as evidenced in a number of studies from pulmonary inflammation in rodents to lung tumors in rats (Oberdörster and Yu 1990; Driscoll 1996; Duffin et al. 2002; Oberdörster et al. 2005).

A simple quantitative method could be developed and tested using experimental data. The following equation provides an example of the concept:

$$OEL_{NP} = OEL_{FP}/[(SSA_{NP}/SSA_{FP}) \times (DF_{NP}/DF_{FP}) \times (Activity_{NP}/Activity_{FP}) \times (UF)]$$

Where OEL_{NP} is the new OEL for the nanoparticle, and OEL_{FP} is the existing OEL for the fine particle; SSA_{NP} and SSA_{FP} are the specific surface areas (m^2/g) of the nanoparticle and the fine particle material, respectively; and DF_{NP} and DF_{FP} are the respective deposition fractions of the nanoparticle or fine particle in the respiratory tract or region of the respiratory tract associated with the adverse response. The term $Activity_{NP}/Activity_{FP}$ indicates a measure of the surface reactivity (e.g., free radical activity) of the nanoparticle relative to the fine particle, in addition to that due to surface area alone (e.g., when comparing materials of similar chemical composition but different age of generation); and UF is a currently undefined factor that relates to the degree of certainty about the health protectiveness of applying an existing OEL to nanoparticles (factors not addressed in the derivation of the original OEL). Additional factors that could be considered include the variability and uncertainty with respect to these factors (e.g., variability in DF within a worker population by exercise and breathing pattern).

Even the relatively basic data needed for the equation above and for testing such an approach are often not available for many substances. Obtaining such data in toxicology studies would allow for testing hypotheses about how the chemical and physical properties influence particle toxicity, for more rigorous comparison with "benchmark" materials, and for the development of risk assessment approaches that take into account these properties and their interaction with biological systems. Direct measurement of the specific surface area, rather than estimation from aerodynamic or particle diameter, is needed to accurately determine the surface area (Stefaniak et al. 2003).

2.4. QUALITATIVE APPROACHES

2.4.1. Hazard Assessment

A hazard ranking approach is sometimes used to make "initial judgments about how much caution a particular type of nanoparticle merits" (Lux Research 2005). One evaluation used a qualitative assessment (yes, no, somewhat) of hazardous characteristics including: evidence of toxicity, reactivity compared to bulk material, toxicity of bulk material, biodegradation, agglomeration, and other factors. The approach was to assign ranks of low, medium, and high toxicity to various commercially relevant nanoparticles (Lux Research 2005). Limitations of this approach include uncertainty about whether comparable data were available among the various nanoparticles evaluated, and whether the relative rankings could change as more data, especially long-term health effects data, become available. Yet, this approach provides an example of a framework to begin to evaluate nanoparticles and provide input for risk management decision-making, such as selection of exposure controls.

2.4.2. Life Cycle Analyses

To assess the total impact of a potentially hazardous substance, an evaluation of the "life cycle" (from production to disposal) of that substance may be performed. In a recent life cycle analysis, a relative risk approach was used to evaluate the risks of nanomaterials along their life cycle compared to other manufactured materials (Robichaud et al. 2005). Five nanomaterials – SWCNT, fullerenes (also called bucky balls or C_{60}), one type of quantum dots, alumoxane nanoparticles, and nanotitanium dioxide – were selected for analysis "based on their current or near-term potential for large-scale production and commercialization." These materials were qualitatively ranked by relative risk scores calculated from factors such as toxicity, flammability, persistence in the environment, and mobility of the substance; characteristics of the production process. The authors concluded that compared to other common industrial manufacturing processes these nanomaterials

presented relatively low-environmental risk. Limitations in this analysis, which could alter the conclusions, include: (1) the risk to workers was not considered (possibly due to insufficient data), and (2) the emission estimates were mass-based, which may not accurately reflect the hazard of nanomaterials relative to bulk materials. Yet, it illustrates a framework for investigating life cycle factors that may influence risk and for identifying target areas for intervention to mitigate those risks. In addition, if such factors are considered at the outset, then opportunities exist to improve the application of controls and work practices and to validate their effectiveness.

Another approach to considering the potential hazard of nanomaterials throughout their life cycle is an "influence diagram" (Morgan 2005). The influence diagram provides a systematic evaluation of the various factors influencing exposure and toxicity of nanoparticles. It also provides a useful framework for determining the data gaps and research needs for risk assessment and risk and risk management of nanomaterials.

2.4.3. Control Banding

Control banding is not so much a risk assessment approach, as it is a risk management approach in which the available hazard or risk data are used to develop simple, practical guidance on the selection of appropriate measures for controlling exposures in the workplace (http://www.coshh-essentials.org.uk/; http://www.cdc.gov/niosh/topics/ctrlbanding/; AIHA 2006). It is intended as a tool for identifying appropriate control strategies when adequate scientific data are not available to develop specific exposures limits, or when validated sampling methods have not been developed. Inferences about the potential hazard are made based on the physical or chemical properties of the material and their potential to result in exposures (e.g., dustiness). There are four main control bands to which substances are assigned based on four broad hazard groups, including consideration of the irritant and toxic properties of the substance, and target ranges of airborne mass or number concentrations of dust or vapor. These four bands include:

1. Use of good industrial hygiene practice and general ventilation
2. Use of local exhaust ventilation
3. Enclose the process
4. Seek expert advice

Each control band has a set of control guidelines for specific processes and tasks.

At this time, control banding as presented in COSHH Essentials (http://www.coshh-essentials.org.uk/) is not considered appropriate for situations in which nanoparticles may be generated, including "hot" processes, open spray

applications, and gases (AIHA 2006). Moreover, a limitation or shortcoming of control banding is that the target ranges of exposure concentration may not provide consistent or adequate margins of safety (AIHA 2006). Since OELs have not been developed for most nanoparticles, it is difficult to determine what would be the adequate margins of safety for various nanoparticles. The possible application of a control banding approach to controlling nanoparticle exposures in the workplace is a current area of investigation.

3. Discussion

3.1. RISK ASSESSMENT APPROACHES

In this paper, we have described several approaches to assess and manage the potential risk of occupational exposure to nanoparticles, including: QRA, comparative toxicity analysis, evaluation and adjustment of existing OELs, and qualitative hazard identification and ranking approaches. The methods used will depend to a large extent on the available data.

QRA is often considered the "gold standard" because it allows for the estimation of the probability of adverse health effects associated with exposure to a given substance, as well as the estimation of variability and uncertainty in those risk estimates. Dose-response data are required for QRA. Both statistical and biologically based models can be used to evaluate the dose–response relationships and extrapolate from animals to humans. Although quantitative dose–response data are limited in humans exposed to ultrafines (nanoparticles), chronic and subchronic inhalation studies in rodents exposed to fine or ultrafine particles can be used for QRA, in combination with lung dosimetry models to account for differences in the particle deposition and clearance kinetics in humans. These analyses provide preliminary estimates of risks associated with occupational exposure to nanoparticles. Additional evaluation of these risk assessment models is needed for their application to new engineered nanoparticles, which may differ in their chemical and physical properties, and biological mechanisms.

In addition, *in vivo* dose–response data by other routes of administration (e.g., intratracheal instillation, pharyngeal aspiration) are available for both ultrafines and new engineered particles, which provide opportunities for comparative potency analyses. For example, if the short- and long-term dose–response relationships (e.g., pulmonary inflammation, fibrosis, and lung cancer) are well-characterized for certain "benchmark" particles, and additional data are available on particle characteristics and short-term dose-response with engineered particles, it may be feasible to estimate risk for the nanoparticles using the early response data as a marker for long-term disease response. Such

benchmark materials may include fine or ultrafine carbon black or titanium dioxide, crystalline silica, and asbestos or other mineral fibers. Further research is needed to develop and validate *in vitro* assays that are predictive of *in vivo* dose-response before such data could be used in hazard or risk assessment, although preliminary analyses are promising for markers of pulmonary inflammation using lung epithelial cells (Faux et al. 2003).

Adjustment of existing OELs to account for differences in the surface area, surface reactivity, and other factors may be useful as a preliminary measure to estimate OELs for nanomaterials until more data are available. This is consistent with recommendations by the U.K. Royal Society and the Royal Academy of Engineering (2004) regarding the need to review the adequacy of regulations pertaining to nanoparticle exposures in the workplace, evaluate the measurement metric (e.g., particle mass versus number), and consider setting lower occupational exposure levels for manufactured nanoparticles.

Qualitative hazard identification and ranking approaches may also provide initial information about the potential hazard of a nanomaterial, the adequacy of current OELs, and decisions on engineering controls and other risk management strategies. Such evaluations also provide an opportunity to engage decision-makers (including business owners and managers) at the beginning of the process to improve the application of controls, and to evaluate their effectiveness.

3.2. FUTURE DIRECTIONS

Given the almost limitless variety of nanomaterials, it will be virtually impossible to assess the risk of each nanomaterial individually. To help address this issue, a screening strategy for hazard identification of nanomaterials was developed (ILSI 2005). The recommendations include physicochemical characterization, *in vitro* analysis, and two-tiered *in vivo* analysis. These screening methods are intended to provide comparison of toxicity among nanoparticles and benchmark materials, identification of high potency or unusual responses, and suggest future testing needs. These guidelines were not developed to address risk assessment data needs, and as such, they recommend limited dose–response data and no chronic testing requirements. A possible approach to develop risk estimates for nanomaterials is to select representative materials from various classes of nanomaterials for more detailed investigations. Such analyses could include systematic comparison of dose–response relationships for new nanomaterials and “benchmark” materials, as discussed earlier. In addition to evaluation of lung responses to nanoparticles, data are needed to determine the disposition of nanoparticles beyond the lungs and by other routes of exposure, and to quantify dose–response relationships in various

organs and tissues. Particular attention to the dose metrics (e.g., particle mass, surface area, number) and particle characteristics (e.g., surface reactivity, solubility, shape, charge) that influence the exposure, dose, and response relationships is also needed for QRA. Such research would benefit if the experimental design is developed through collaboration between the experimental scientists and risk assessors. This would allow a coordinated approach in which both mechanistic and quantitative dose-response data are obtained in a given experiment. This is particularly valuable for longer-range studies, which are more costly and for which optimization of the study design is particularly valuable.

Risk assessment and risk management in occupational safety and health ideally are data-driven, iterative processes (Fig. 3). As new data are generated, better risk estimates can be generated with reduced uncertainty and greater protection for worker safety and health. In the meantime, risk management decisions are needed even when information on hazard and risk are limited or uncertain. According to a precautionary approach, greater uncertainty about a hazard requires greater precaution and control of exposures (Schulte and Salamanca- Buentello 2006). It has been recommended that in the absence of evidence to the contrary, nanoparticles be treated as if they are hazardous (The Royal Society and the Royal Academy of Engineering 2004). Guidelines for working safely with nanomaterials are being developed by NIOSH (http://www.cdc.gov/niosh/topics/nanotech/nano_exchange.html) and other organizations.

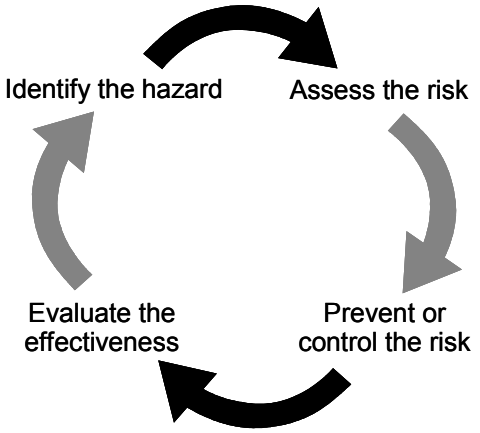

Figure 3. Feedback for continual improvement of providing a safe and healthful work environment, including in workplaces using nanomaterials.

3.3. RESEARCH NEEDS

Based on evaluation of the scientific literature and data gaps, as discussed in this paper, research needs can be identified in each of the three main categories of risk assessment, including research, assessment, and management (Fig. 1). Filling these data and information gaps would increase our understanding of the properties influencing the potential harmfulness of nanoparticles, improve the measurement and interpretation of nanoparticle exposures in the workplace, and provide for more effective control of exposures and monitoring of workers' health:

- *Workplace exposure monitoring:* measure nanoparticle aerosol concentrations by work process or task. Develop standardized measurement methods and sampling devices for nanoaerosols. Provide guidance on the most appropriate exposure metric(s) (e.g., particle number, mass, surface area).
- *Engineering controls:* evaluate the effectiveness of engineering control techniques for nanoparticles (e.g., to eliminate generation of nanoparticle aerosols), and develop new approaches as needed.
- *Personal protective equipment (PPE):* evaluate and improve, as needed, the effectiveness of PPE (including barrier materials and respirators) for reducing exposures to nanomaterials.
- *Internal dose:* determine the uptake, fate, and persistence of nanomaterials in the body, by route of exposure, including the influence of the chemical and physical properties and the agglomeration/aggregation state of the particles.
- *Key determinants of toxicity:* systematically investigate the physical and chemical properties of particles that influence their toxicity. Evaluate acute and chronic effects in the lungs and in other organs and tissues, and determine if there are *in vitro* or short-term tests that are predictive of nanoparticle toxicity. Quantify the dose–response relationships, including which dose metrics are most predictive of disease.
- *Communication and training:* develop authoritative guidance on working safely with nanoparticles; develop specific hazard information for nanomaterials on MSDS.
- *Medical surveillance and monitoring:* identify appropriate medical tests and possible biomarkers of exposure or adverse response to nanoparticles. Develop procedures to link exposure and health effects data for epidemiological studies.

4. Conclusions

Although there are many data gaps concerning risk assessment of nanomaterials, initial assessments can be based on the existing scientific literature of particles and fibers. For example, studies in animals and humans that have shown that exposure to relatively low mass concentrations of nanoparticles are associated with adverse lung responses compared to larger particles of the same or similar chemical composition. Surface area, surface reactivity, and other properties also influence toxicity, and nanoparticles vary from relatively low to high toxicity. Risk assessment methods are still being developed to estimate the risk of adverse health effects in workers exposed to nanomaterials. Based on available data, and given the uncertainties, it is prudent to reduce or eliminate worker exposures to nanoparticles using engineering controls, enclosed processes, work practices, and personal protective equipment.

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RISK ASSESSMENT RELATED TO NANOTECHNOLOGY: ENVIRONMENTAL AND POLICY-MAKING

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Abstract: Additional research on the health and environmental risks posed by nanoparticles is needed to help ensure that asbestos-like scandals will not return to haunt nanotechnology companies in the future. Nanotechnology, which concerns the study and application of materials on an ultrasmall scale, is widely perceived as one of the key technologies of the 21st century with a potential to grow into a trillion-euro industry within a decade. Fears are growing that the field could develop into a political battleground with fiery debates about environmental and ethical consequences and dangers, as has occurred in the field of biotechnology. Recent debates on issues such as genetically modified organisms (GMOs) and the related review of the European chemicals policy have sparked heated discussions among stakeholders about the analysis and assessment of the underlying risks and the way in which these environmental and health-related concerns can be managed and communicated. Questions about the definition of risk, the objectivity of scientific information and the role that risk analysis should play in policy decisions are at the heart of the debates.

Keywords: nanotechnologies, ecological risk assessment, human health risk assessment, policy-making based on risk

1. Introduction

A nanometer is one-billionth of a meter and around one-thousandth of a single human hair. Nanotechnology involves manufacturing and engineering techniques applied on an atomic nanoscale. The past few years have seen a growing interest and investment in nanotechnology.

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Industry is increasingly using nanotechnology in sectors such as health care, consumer products, information technology, and the environment.

The political debate on regulating nanotechnology is just beginning. The European Commission adopted a strategy for nanotechnologies in May 2004 and an action plan for 2005–2009 in June 2005. Both documents emphasize the need for a "safe, integrated and responsible approach to the development of nanotechnologies and nanosciences" and the importance for the "assessment of potential risks throughout the life cycle of nanotech-based products".

The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) recently adopted an opinion on "the appropriateness of existing methodologies to assess the potential risks of nanotechnologies". Delivered at the Commission's request, the report concludes that existing risk assessment methodologies require some modifications to address hazards associated with nanotechnology.

The report states that "in particular, the existing *toxicological and ecotoxicological methods* may not be sufficient to address all of the issues arising with nanoparticles". SCENIHR also points out that very little is known about the physiological responses to nanoparticles. Therefore, the conventional toxicity and ecotoxicity tests may require modification regarding hazard evaluation and the detection of nanoparticle distribution in the human body and in the environment.

The Nanoforum, a pan-European nanotechnology network funded by the European Union, has published a report on the current state of the art of the European and global debate on benefits, risks, ethical, legal, and social aspects of nanotechnology. The forum arrived at the same conclusion as SCENIHR, by stating that the most pressing issue in the current debate on nanotechnology is the need for appropriate research on the potential risks of nanoparticles on human health and environmental implications. Human health impacts of nanoparticles currently under investigation include the risk of lung and heart diseases from inhaled nanoparticles, accumulation of non-biodegradable nanoparticles in the liver, and uptake into the brain.

The Nanoforum report also highlights the importance of realistically taking into account the long-term visions and scenarios of proponents and opponents of nanotechnology, including science fiction writers, as they influence public opinion. The pro-nanos view nanotechnology as a way to produce, for example, new and stronger materials, smart medicines, and unlimited computer power. The anti-nanos point to concerns of nanoparticles ranging from accumulation in food and uptake in the body, to new methods of surveillance, such as cameras that monitor our every step to new means and methods of war, such as selective biological and genetic weapons or a new "half-drone half-human" super soldier.

2. Nanotechnology

Nanotechnology involves studying and working with matter on an ultra-small scale: 1 nm is one-millionth of a millimeter and a single human hair is around 80,000 nm in width. The technology encompasses the whole spectrum of science, touching on medicine, physics, engineering and chemistry. Nanosubstances are already included in sun creams to block ultraviolet rays, while nanoceramics are being used as bone-replacement agents. Research is expected to lead to advances in areas such as medicine, environment, manufacturing, communications, and electronics.

Described as "a new industrial revolution", nanotechnologies have the potential to produce sweeping changes to all aspects of human society. Their use might be particularly beneficial in the areas of environment, communication, health, and production. Supporters of this view state that nanomaterials can provide cleaner, safer, and more competitive production processes, as well as smarter, more durable, and more user-friendly products. They could provide innovative answers to the challenges of sustainable development, such as, fueling economic growth, while preserving the environment, and at the same time enhancing the safety, security and quality of life of European citizens. At the same time, critics warn of dangers such as terrorist use of weapons based on nanotechnology, or the so-called "grey goo" scenario, in which the biosphere is destroyed by out-of-control, self-replicating robots. Although there is no immediate evidence for such dangers, decision-makers agree that in order to gain public support, there is a strong need for an open debate about the safety of products at the nanoscale, how future advances can be monitored and controlled, and who may profit from them.

In the Communication "Towards a European Strategy for Nanotechnology", which was adopted in May 2004, the Commission specifies a series of recommendations and initiatives on how to boost European nanotechnology research and development (R&D). The Commission's main considerations are the consolidation of public and private research efforts as well as improved technology transfer to turn research findings into commercially viable products. It also addresses the need to identify and respond to concerns about safety, health and environmental risks related to nanotechnologies.

A public consultation on the communication was organized in autumn 2004. The results of the consultation revealed a strong stakeholder consensus that nanotechnology will have a significant impact on European industry and its citizens within 10 years from now. In its Communication, the Commission proposes the following key actions:

- Boost R&D investment and infrastructure
- Improve training for research personnel

- Enhance and support technology transfer in Europe
- Increase international cooperation towards a responsible approach to nanotechnology R&D globally

On June 7, 2005 the Commission adopted an action plan for 2005–2009 defining actions for the "immediate implementation of a safe, integrated and responsible strategy for Nanosciences and Nanotechnologies (N&N)". The actions included:

- Foster industrial exploitation of R&D on N&N by bringing together stakeholders to discuss best practices for commercialization, the societal, political, and psychological barriers to entrepreneurship in Europe and license arrangements between industry and R&D organizations
- Establish common standards
- Boost funding for nanotechnology in the Seventh Framework Programme (FP7), including specific support for research into the impact on human health and the environment
- Support research in nanoelectronics under the Information and Communication Technology (ICT) priority of FP7
- Foster technology platforms to implement a strategic R&D agenda for N&N
- Develop an N&N research infrastructure and poles of excellence
- Establish a dialogue with citizens and inform all stakeholders about progress and expected benefits of N&N
- Ensure that ethical principles are respected and citizens' concerns and expectations are taken into account
- Integrate risk assessment related to human health, the environment, consumers, and workers at all stages of the life cycle of the technology
- Establish a European Award for nanotechnology to highlight best practice

With a total budget of €1.43 billion for 2002–2006, Priority 3 of the European Union's 6th Framework Programme for Research and Technological Development (FP6) brings together nanotechnologies, materials science and manufacturing, as well as other technologies based on biological or environmental sciences. Over €700 million of this budget is devoted specifically to nanotechnology. The Commission's official proposal for the FP7 suggests €4.27 billion for research on N&N for 2007–2013.

In July 2002, the Commission launched "Nanoforum," a €2.7 million pan-European thematic network on nanotechnology aimed at strengthening the EU's economic competitiveness in this field. This information portal leads to the Institute of Nanotechnology, and provides a framework for raising awareness,

supporting and encouraging the adoption of nanotechnologies, and facilitating the development of new industrially orientated nanotechnology research across Europe. Nanoforum will continue operating throughout the period of the FP6 (2002–2006).

Nanologue, a Commission-funded project bringing together leading research on the social, ethical, and legal implications of nanotechnology, was launched in March 2005. This project will facilitate dialogue and produce guidance for stakeholders and developers of nanotechnology and specifically on how to secure wider benefits for both society and the economy. The project will continue until August 2006 and any interested parties may contact the project leader to participate in the dialogue.

The NanoMedicine technology platform was launched in September 2005. Its aim is to develop a Strategic Research Agenda for nanomedicine in Europe. The public debate on nanotechnology really began to take off in 2003 with several articles and publications discussing the benefits and risks of the new technology. In January 2003, the Canadian environmental Action Group on Erosion, Technology and Concentration (ETC) published a report on nano-technologies and their potential impact on society entitled *The Big Down.* Reviewing the impact, risks, and main actors in the area of nanotechnology and outlining policy recommendations, the ETC is a firm believer in the dangers of the "grey goo scenario" and warns that "in the future, mass production of unique nanomaterials and self-replicating nano-machinery pose incalculable risks. Atomtech [nanotechnology] could also mean the creation and combination of new elements and the amplification of weapons of mass destruction."

In February 2003, the University of Toronto published a paper called "*Mind the Gap Science and Ethics in Nanotechnology,*" pointing to the lack of research into the ethical, legal and social implications of nanotechnology. The study warns "as the science leaps ahead, the ethics lags behind. There is danger of derailing NT [nanotechnology] if the study of ethical, legal, and social implications does not catch up with the speed of scientific development."

A report entitled *The Social and Economic Challenges of Nanotechnology* published in July 2003 by the Economic and Social Research Council in the UK is the result of a cooperation between three Sheffield academics in social and natural sciences, and provides an assessment of the various scenarios. It concludes that the public debate focuses on the long-term possibilities of radical nanotechnology rather than the mundane applications that have arrived so far, although there is as yet no conclusion as to the practical limits to nanotechnology. One immediate issue identified by the report is whether regulatory regimes are robust enough to deal with any consequences that may arise from continued research.

Greenpeace Environmental Trust launched their report *Future Technologies, Today's Choice* in July 2003 with information on nanotechnology, artificial intelligence, and robotics, putting these emerging technologies into their technical, political, and institutional context. It calls on government and industry to thoroughly assess the environmental, medical, and ethical issues that may arise. In particular, the report demands that an in-depth analysis of environmental implications be conducted, stressing that while environmental benefits may well be achievable in some areas, a number of practices which might lead to the release of nanoparticles into the environment are a cause of major concern. These, say Greenpeace, could "constitute a whole new classes of non biodegradable pollutants."

The UK Royal Society and the Royal Academy of Engineering in November 2003 published a report, commissioned by the UK government, entitled *Nanotechnology Views of Scientists and Engineers* as part of a study into the benefits and problems of N&N. While the experts believe that nanotechnology can be used to benefit human health and the environment, a strong focus of the report is on the question of health risks and environmental dangers of nanotubes and other nanoparticles. The scientists therefore call for further studies to be carried out to assess these dangers. The report also concludes that the science fiction scenario of self-replicating "nanorobots" transforming the world into "grey goo" is likely to be physically impossible.

3. Risk-Based Policy-Making

When talking about risk-based decision-making, it is important to understand the concepts this approach is based upon. In particular, there is often some confusion over the terms of "risk" versus "hazard," as well as the concept of risk management. The following definitions are therefore essential:

Hazard is the *potential to cause harm*. A good example would be a chemical substance that, if in contact with humans, could cause serious health problems.

Risk on the other hand is the *likelihood* of harm to actually occur. This normally depends on the degree of exposure to a given hazard. For example, a very low exposure to a highly hazardous chemical may result in a low risk, while a high exposure to a substance of very low hazard may result in a moderate or even high risk. In other words, for there to be a risk, there must be both the hazard and the exposure to the hazard.

Risk management describes the process of weighing all the *policy options* including consultation with all the stakeholders. By studying the actual risks, the policy-makers decide on how to implement their decision, evaluate the results, and address the perception of the risk to the public.

Risk perception refers to the way the public and other stakeholders perceive any given risk. This *can be quite different from the scientific evidence* provided, such as in the case of GMOs in human food. Understanding the risk perception is crucial as it can make debate highly charged and helps determine the best ways to communicate the risk.

Risk communication is key to gain stakeholder acceptance of policy decisions. It may include *economic, social, and ethical values,* as well as the scientific facts. Policy-makers used to take a top-down approach to risk communication (from regulator to public), whereas a more modern approach encourages public and stakeholders to participate actively in the communication process through public consultations, hearings, etc.

Precautionary principle: In 2000, the Commission published a communication on the *precautionary principle*, stating that this covered "cases where scientific evidence is insufficient, inconclusive or uncertain and preliminary scientific evaluation indicates that there are reasonable grounds for concern that the potentially dangerous effects on the environment, human, animal or plant health may be inconsistent with the high level of protection chosen by the EU." Applying the precautionary principle is a risk-management policy-decision.

In its 2001 White Paper on European Governance, the Commission recognized that scientific and other expert advice played an increasingly significant role in decision-making. Expert advice particularly serves to anticipate and identify potential problems and uncertainties, helps in the decision process and ensures good risk communication.

The White Paper points out that recent food scares such as the one generated by BSE (mad cow disease) have undermined public confidence in risk-based policy-making. Furthermore, it recognizes that this problem is worsened by the "opacity of the Union's system of expert committees or the lack of information about how they work," making it unclear who is making the decisions, policy-makers or experts. There is also more and more mistrust in the independence of expert advice given to decision makers.

Questions such as "What is risk?," "Who defines it?" and "Who makes the decisions?" become even more pertinent as the EU is committed to applying the precautionary principle and to conducting thorough risk analysis and risk management. In December 2002, the Commission published a Communication on principles and guidelines on the collection and use of expertise, which stipulates that all gathering of expert advice should be underpinned by quality, openness, and effectiveness.

There has been widespread debate on the advantages and disadvantages of risk analysis as a useful tool for policy decisions. In particular, stakeholders, including industry, NGOs, and academia are often at odds as to how risk

analysis should be used and how much influence it should have on the decisions. One of the main points of debate concerns the objectivity of the consulting experts.

The scientific community and industry often argue in favor of a strictly risk-based policy-making, saying that risk analysis is the only "objective scientific basis", which can lead to more "rational" decisions. According to the proponents of this approach, problems and limits of risk analysis can be overcome through thorough data collection and research, as well as strict guidelines for the consistent conduct and presentation of the results. Others, mainly environmentalists, consumer interest groups, and other NGOs, point to the danger that risk analysis tends to oversimplify the problems faced by policy-makers by focusing on one hazard and one effect at the time or on problems that are well understood. They criticize that because risk-assessment methods tend to be very complex, they can be easily manipulated for political purposes. As a result, the decision-making process will be less democratic.

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NEEDS FOR REGULATIONS, TRAINING, AND EDUCATION FOR HEALTH PROTECTION AND ENVIRONMENTAL SECURITY OF NANOTECHNOLOGIES

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Abstract: This paper discusses the literature, definitions, possible applications, future benefits, and arising concerns related to nanotechnology. Assuring safe nanotechnology is a society demand. Science has to provide valuable guidance to the safe handling of nanoparticles and other safe approaches to nanotechnology, but government bodies must approve and implement human health and environment protection regulations. It will allow new products resulting from nanoscience to be introduced and used safely. It should be acknowledged that it will not be possible to undertake full toxicity testing for all nanomaterials, and therefore there is a necessity in short-term strategies to address potential toxicity. Researchers should refine these strategies as data accumulate. There is a need for a deeper understanding of the biological responses of a selected set of nanomaterials, in order to ensure that the data contribute to the broader understanding of the toxicity of nanomaterials. There is a need for a minimum standard physical characterization of the materials being tested and for internationally harmonized standard reference nanomaterials. Exposure data should be combined with medical screening data to facilitate epidemiological studies on nanomaterials. There is a need for standard, validated test methods for monitoring nanoparticles and in particular at the workplace.

Keywords: nanotechnology, nanoparticles, general benefits, potential risks, toxicology, environmental safety, health safety

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1. Introduction

Nanotechnology, the manipulation of matter on a near-atomic scale to produce new structures, is a new industrial technology. The field of nanotechnology is advancing rapidly and will likely revolutionize the global industry. As with any new technology, we are faced with many unknowns, all of which raise questions concerning health, occupational safety, and environment. It is imperative that the scientific community come together to advance individual and collective understanding of the nature of nanotechnology and its implications in human health and environment.

2. Definitions and Essence

The definitions of nanotechnology are as diverse as its applications. The term "nanotechnology" was defined by Norio Taniguchi in a 1974 paper (Taniguchi, 1974) as follows: '"Nanotechnology' mainly consists of the processing of separation, consolidation, and deformation of materials by one atom or one molecule". In the 1980s the basic idea of this definition was explored in much more depth by Dr. Eric Drexler, who promoted the technological significance of nanoscale phenomena and devices through talks and books, and so the term acquired its current sense. One popular definition is that nanotechnology is the range of technologies, techniques and processes that involve the manipulation of matter at the smallest scale (from 1 to 100 nm).

The USA's National Nanotechnology Initiative considers that "Nanotechnology is the understanding and control of matter at dimensions of roughly 1 to 100 nanometers, where unique phenomena enable novel applications," and nanoscience is used to describe the interdisciplinary fields of science devoted to the study the nanoscale phenomena employed in nanotechnology (What 2006). Many US regulatory agencies use some form of the following definition for "nanotechnology": Research and technology or development of matter that involve all of the following:

1. The existence of materials or products at the atomic, molecular or macromolecular levels, where at least one dimension that affects the functional behavior of the drug/device product is in the length–scale range of approximately 1–100 nm
2. The creation and use of structures, devices, and systems that have novel properties and functions because of their small size
3. The ability to control or manipulate the product on the atomic scale

One of the basic definitions is: engineering of functional systems at the molecular scale. This covers current work and concepts that are more advanced.

In its original sense, "nanotechnology" refers to the projected ability to construct items from the bottom up, using techniques and tools being developed today to make complete, high-performance products (Nanotechnology Basics 2005).

Nanotechnology is also considered the engineering of tiny machines, using techniques and tools being developed today to make novel, highly advanced products. Ultimately, nanotechnology should enable control of matter at the nanometer scale, using mechanochemistry. If this envisioned molecular machinery is created, it will result in a manufacturing revolution (Nanotechnology 2002). It also can have serious economic, social, environmental, and military implications. George Robillard has a more focused definition: "The core of nanotechnology consists of systems in the size range of nanometers" (Malsch 2002).

More broadly, nanotechnology includes the many techniques used to create structures at a size scale below 100 nm, including those used for fabrication of nanowires, those used in semiconductor fabrication such as deep ultraviolet lithography, electron-beam lithography, focused ion-beam machining, and further including molecular self-assembly techniques such as those employing diblock copolymers. However, all of these techniques preceded the nanotech era, and are extensions in the development of scientific advancements rather than techniques which were devised with the sole purpose of creating nanotechnology or which were results of nanotechnology research.

The term nanotechnology is sometimes confused with the more specific molecular nanotechnology, a proposed form of advanced nanotechnology based on productive nanosystems. Molecular nanotechnology would fabricate precise structures using mechanosynthesis to perform molecular manufacturing. Molecular manufacturing is the use of programmable chemistry to build exponential manufacturing systems and high-performance products. Molecular manufacturing can be built into a self-contained, tabletop factory that makes cheap products efficiently at the molecular scale. The potential benefits of such a technology would be immense.

3. General Benefits

Advanced nanotechnology can be very beneficial. Nanotechnology is already having a considerable impact on the field of electronics, of medicine, etc. The new products will be more powerful and will be developed faster. Electrical power can be converted to motion, and vice versa, with minimal power loss by using more compact equipment. Computers can be a billion times smaller and use a million times less power. Materials can be about 100 times stronger than steel. This means that most human-scale products would consist almost entirely of empty space, reducing material requirements and cost. Most of the rest of the product would be structural, easy to design. Even the simplest products could

be software-controlled at no extra hardware cost. Manufacturing of prototypes would be quite rapid. Because manufacturing and prototyping are the same process, a successful prototype design could immediately be distributed for widespread use. A designer working with a few basic pre developed blocks could design, build, and test a simple product in short time. Products with complex interfaces to humans or to their surroundings – information appliances, automobiles, aerospace hardware, medical devices – would be limited by the time required to develop their software and test their functionality. In some fields the high time and money cost of manufacture slows other parts of the development cycle (Center 2006).

Manufacturing with nanotechnology can solve many of the world's current problems. Water shortage is a serious and growing problem. Most water is used for industry and agriculture; both of these requirements would be greatly reduced by products made by molecular manufacturing. Computers and display devices would become stunningly cheap. Electrical power is still not available in many areas. The efficient, cheap building of light, strong structures, electrical equipment, and power-storage devices would allow the use of solar thermal power as a primary and abundant energy source. Environmental degradation is a serious problem worldwide. High-tech products can allow people to live with much less environmental impact. Molecular manufacturing can be self-contained and clean; a single packing crate or suitcase could contain all equipment required for a village-scale industrial revolution. Advanced productive nanotechnology will enable the fabrication of large, complex products cleanly, efficiently, and at low cost (Crucial 2006). Among the feasible products of advanced productive nanosystems will be:

- Desktop computers with billions of processors
- Inexpensive, efficient solar-energy systems
- Medical devices able to destroy pathogens and repair tissues
- Materials 100 times stronger than steel
- Superior military systems
- Molecular manufacturing systems, etc.

The problem is to what extent molecular-manufacturing technologies are realistic. Phoenix et al. (2006) considers that, first, mechanical operations can reliably be carried out at the nanoscale, and second, that handling of individual molecules can be scaled up to produce useful quantities of product. On closer examination, these ideas appear to be supportable. The development of molecular manufacturing can be an incremental process from today's capabilities. Although the most exciting results rely on the most advanced and integrated

capabilities, even the earliest products of basic molecular manufacturing could be useful for basic research and for components such as sensors.

4. Benefits for Health and Medical Practice

Nanotechnology can impact the practice of medicine in many ways (Center 2006) Medicine is highly complex, so it will take some time for the full benefits to be achieved, but many benefits will occur almost immediately. The tools of medicine probably will become cheaper and more powerful. Research and diagnosis will be far more efficient, allowing rapid response to new diseases, including engineered diseases. Small, cheap, numerous sensors, computers, and other implantable devices may allow continuous health monitoring and semi-automated treatment. Some new kinds of treatment will become possible. As the practice of medicine becomes cheaper and less uncertain, it can become available to more people.

Malsch et al. (2002) considers that three applications of nanotechnology are particularly suited to biomedicine: diagnostic techniques, drugs, and prostheses and implants. Interest is booming in biomedical applications for use outside the body, such as diagnostic sensors and "lab-on-a-chip" techniques, which are suitable for analyzing blood and other samples.

Research and diagnosis will become more efficient (Medical 2006). With real-time monitoring of the body's systems, it will be possible to detect undesired effects far earlier, allowing a more aggressive and experimental approach to treatment. Researchers will be able to gather far more data and process it with computers millions of times more powerful. The result will be a detailed model of the body's systems and processes, and the ability to predict the effects of any disease or treatment. Diagnosis will also be far easier and more informative. Trustworthy diagnosis will make medicine far more efficient, and also reduce the risk of malpractice.

It will be increasingly important to have a technology base that can detect new diseases even before symptoms appear, and create a cure in a matter of days. Molecular nanotechnology will enable such a rapid response. With complete genomes and proteomes for humans and for all known pathogens, plus cheap, highly parallel DNA and protein analysis and sufficient computer resources, it will be possible to spot any new pathogen almost immediately.

Health improvement and life extension do not depend on molecular nanotechnology, but molecular nanotechnology will certainly make them accessible to more people. Any treatment that can be automated can be applied to any number of people at low cost. New therapeutic techniques will allow the treatment of more types of diseases.

Genetic therapy holds great promise for treating several serious health problems. However, the current state of the art can also cause problems, including cancer. Eventually, we may hope that molecular nanotechnology will be able to directly edit the molecular nanotechnology of living cells in the body. Microsurgical techniques could allow the implantation of modified cells directly into the target tissues.

Many organs in the body perform fairly simple functions. Already, sophisticated machinery can replace lung function for hours, heart function for months, and kidney function for years. Since molecular nanotechnology can build machines smaller than cells, many other organs will be candidates for replacement or augmentation, including skin, muscles, various digestive organs, and some sensory functions.

5. Potential Risks

The potential benefits of molecular manufacturing are immense, but so are the dangers (Dangers 2006a). Molecular nanotechnology creates many new risks, including severe ones which could be very dangerous. As with any new technology, we are faced with many unknowns, all of which raise questions concerning health, occupational safety, and environment. The concept of risk involves two factors – hazard and exposure.

Molecular nanotechnology will be a significant breakthrough, comparable perhaps to the Industrial Revolution – but compressed into a few years. This has the potential to disrupt many aspects of society and politics. A European Commission – National Science Foundation workshop held early this year (Report 2004) discussed the societal aspects of nanotechnology. Participants concluded, "Nanobiotechnology could dramatically improve public health, but there is concern that technical developments could cause unforeseen adverse effects.…" Governments must stimulate scientists to monitor possible health risks of nanomaterials that may affect environment and accumulate in the body.

Center for Responsible Nanotechnoloigy (Dangers 2006b) has identified several separate and severe risks. The first step in understanding the dangers is to identify them. Several separate and severe risks were listed and described. Although probably incomplete, the list is already worrisome:

- Economic disruption from an abundance of cheap products
- Economic oppression from artificially inflated prices
- Personal risk from criminal or terrorist use
- Personal or social risk from abusive restrictions
- Social disruption from new products/lifestyles

- Unstable arms race
- Collective environmental damage from unregulated products
- Free-range self-replicators (grey goo)
- Black market in nanotech (increases other risks)
- Competing nanotech programs (increases other risks)
- Attempted relinquishment (increases other risks)

Nanotechnology applications have not been marketed long enough for claims to be corroborated about risks to human health or the environment. Still, small nanoparticles can enter the human body through pores and may accumulate in cells. The health effects of such nanoparticles are unknown. Historical experience with unintended consequences of technologies, such as drug resistance to antibiotics or the persistence of chemicals such as dioxin or DDT in the environment, teaches us to take precautions (Malsch et al. 2002).

The OECD's Environment, Health and Safety Programme 13 points out that nanotechnology is not specific to one industrial sector but will impact many, for example, electronics and computing, the chemicals industry, environmental technologies, medicine, cosmetics, foods, the military and the energy sector. Nanomaterials are not just for the future. There are already a number of products on the market which incorporate them, ranging from lotions, creams and shampoos in the cosmetics sector to self-cleaning glass that is coated with titanium oxide nanomaterials. For example, buckyballs are considered especially promising for applications in drug delivery and cosmetics as well as fuel cells and solar cells.

The latest investigations show that some nanoparticles, being released into the environment as pollutants, may cause toxic effect on certain species (Nano's). More of that, nanoparticles could begin to be accumulate throughout the food chain, affecting not just hydrobionts, but plants and other animals, including people (Sampson 2004). It is known that nanoparticles can cross the blood/brain barrier in humans. Researchers also found chemical markers in the liver indicating inflammation, which suggested a full-body response to the buckyball exposure.

It has to be taken into account that in nanotechnology the properties of materials become markedly different on the number of atoms used (hundreds or even tens). The different properties present at such small scales means that man-made nanodevices will probably bear much stronger resemblance to nature's nanodevices: cells, viruses, and prions.

Already there is some evidence of possible baleful effects of some nanotechnology or nanoparticles. For example:

- Titanium dioxide/zinc oxide nanoparticles from sunscreen are found to cause free radicals in skin cells, damaging DNA (Dunford 1997).
- Engineered nanoparticles accumulate in the organs of lab animals and are taken up by cells. This demonstrates that nanomaterials may enter into the food chain (Brown 2002).
- Studies on effects of nanotubes on the lungs of rats produced more toxic response than quartz dust. That means nanotubes can be highly toxic (Hogan 2003).
- The smaller the particle, the higher its likely toxicity; nanoparticles have various routes of penetration into the body and across membranes such as the blood/brain barrier. There is a potentially hazardous process (Howard 2003).
- Nanoparticles can move across the placenta from mother to fetus (Wootliff, 2004).
- Cadmium selenide nanoparticles (quantum dots) can break down in the human body potentially causing cadmium poisoning, etc. (Mullins 2004).

These toxic warnings highlight the need for a moratorium on the release of new nanomaterials in environment. Presumably, the potential of safe applications of nanoparticles still exists, but that commercialization should proceed cautiously until scientific toxicological data catch up to the technology.

6. The Approaches to Assessment of Exposure to the Nanotechnology

We should have in mind that nanotechnology is a way for developing a quite new, unknown earth compounds which could result in unforeseen effects on health or on environment. Up to now the knowledge about the danger and safety of nanoparticles and nanotechnology is still at an early stage. The above- mentioned evidences of possible hazard (Dunford 1997; Brown 2002; Hogan 2003; Howard 2003; Wootliff 2004; Mullins 2004) have a solitary character yet.

Control of risks from exposure to dangerous factors requires first of all a scientific, ideally quantitative, assessment of potential effects at a given level (risk assessment) (IPCS 1999). At the early stage of a safety evaluation, consideration must be given to whether enough information is available on how much of the nanoparticles will be present in foods, drinking water, or air. Further, it will be important to know which section of the population may be most exposed to nanoparticles and at what level. Exposure is used as one of the aspects to be taken into account when setting priorities for testing. In the laboratory scientists observe that for most toxic effects there is an exposure

dose, or threshold, below which no adverse effects are seen. If a general threshold, or several thresholds, could be determined for the nanoparticles, below which exposure did not raise safety concerns for humans, then this could be a useful tool, among others, in deciding on the need for toxicity testing. This concept has become known as the threshold of toxicological concern.

The use of such a tool would have benefits, not only for industry and regulatory authorities, but also for consumers, because it would enable the world's limited resources for toxicity testing and safety evaluation to be focused on nanoparticles that may pose a real threat to human health.

The system for safety evaluation of nanoparticles is largely based on both the precautionary principle and on a case-by-case approach. Ideally, for a full assessment of any harmful chemical compound a range of laboratory toxicity tests are needed (see Table 1). Scientists first assemble all the information they have on a chemical compound and make a judgment on the likely level of concern. The second, it should be taken into consideration that the health effects of many nanoparticles are unknown – and this teaches us to be cautious.

TABLE 1. Laboratory toxity tests. (From Barlow, S., Threshold of the Toxicological Concern, ILSI Europe, 2005, 32p.)

BOX 1.	
Type of laboratory toxicity test	What it can reveal
Sub-chronic toxicity	Adverse effects on structure and function in any part of the body following repeated daily exposure for up to 10% of lifetime
Chronic toxicity	Adverse effects on structure and function in any part of the body following repeated daily exposure over a substantial part of lifetime
Carcinogenicity	Cancer
Genotoxicity	Damage to the inherited genetic material inside cells (DNA)
Reproductive toxicity	Adverse effects on fertility and reproduction
Development toxicity	Adverse effects on the embryo and fetus
Immunotoxicity	Adverse effects on the structure and function of the immune system, or in reaction to immune challenge
Neurotoxicity	Adverse effects on the structure and function of the nervous system and behavior

In the initial stages, the information available may be limited to knowledge of the chemical's structure, where it occurs and what degree of human exposure can be anticipated. The potential sources of exposure, the use of products (in particular dispersive uses), degradation of the product and disposal must be taken into consideration. Also researchers have to pay attention to the life cycle

of the product when considering human exposure, to all routes of exposure (inhalation, dermal, ingestion, injection) and other factors which may influence toxic effects).

If the data available cover all or most of the above types of tests, then a comprehensive safety evaluation can be conducted. If a noncritical piece of information is not available, those conducting the safety evaluation can use scientific judgment to make allowances for the missing data. If the missing data are consider critical to the safety evaluation, then more tests must be conducted.

7. Health and Environmental Safety Issues and Needs for Training and Education

Today assuring safe nanotechnology is a society demand. Science has to provide guidances to the safe handling of nanoparticles and other safe approaches to nanotechnology, but government bodies must approve and implement human health and environmental protection regulations into programs. It will allow new products resulting from nanoscience to be introduced and used safely. According to the US National Nanotechnology Initiative vision, there are three broad areas of research toward understanding environmental, health and safety impacts of nanoscale materials. They are:

1. Basic research to expand knowledge and further understanding of how nanomaterials behave, including in the environment and in the human body
2. Research to develop instrumentation and methods for measuring, characterizing, and testing nanomaterials and for monitoring exposure
3. Research toward assessing safety of chemicals, food, drugs and medical devices, among other items (NNI)

It is acknowledged that it will not be possible to undertake full toxicity testing for all nanomaterials, and therefore there is a necessity in short-term strategies to address potential toxicity. Researchers should refine these strategies as data accumulate.

There is a need for a deeper understanding of the biological responses to a selected set of nanomaterials, in order to ensure that the data will contribute to a broader knowledge on nanomaterial toxicity.

A tiered approach and/or decision tree should be developed to determine the most appropriate testing regime for nanomaterial toxicity. There is a need for a minimum standard physical characterization of the materials being tested and for internationally harmonized standard reference nanomaterials.

The potential range in variants for a given nanomaterial is an additional challenge for toxicity testing.

Exposure data should be combined with medical screening data to facilitate epidemiological studies on nanomaterials.

There is a need for standard, validated test methods for monitoring nanoparticles and in particular at the workplace. The long-term goal would be to develop sampling methods. Also critical parameters should be identified, depending on the material.

The activities which governmental bodies must address are the following:

- Raise awareness of the environmental safety and health issues associated with the rapidly changing science and applications of nanotechnology.
- Information exchange among research and regulatory agencies and bringing together experts that can both identify the research needed in support of regulatory decision-making and implement those priorities into the research and development program.
- Extension of education-related activities such as the bringing up-to-date undergraduate, postgraduate, and refreshing courses programs, development of materials for schools, undergraduate programs, technical training, and public outreach.

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BIOETHICS AND LEGAL ASPECTS OF POTENTIAL HEALTH AND ENVIRONMENTAL RISKS OF NANOTECHNOLOGY

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Abstract: The purpose of this review is to describe the use of nanotechnology in the field of medicine with regards to future perspectives and ethical considerations arising in the context of their implication on health and the environment. Legal regulations aimed to achieve maximum possible risk reduction are summarized.

Keywords: nanotechnology, bioethics, legal regulation, health and environmental risks

1. Introduction

As developments in biotechnology take place, decisions must be made not only on economic and scientific considerations, but also on ethical ones. The advent of nanotechnology raises the question, if and how ethical thinking could be integrated within scientific research into nanotechnology opportunities. That ethics will play a role in the forming of nanotechnology is without a doubt. The question is what role it should play?

Having witnessed the emergence of bioethics in the wake of biotechnology, the current interest in the ethical questions related to nanotechnology brings back memories. In a recently published draft of a Danish governmental plan for the development of nanotechnology it is suggested that research in "nanoethics" is integrated into the development of nanoscience and nanotechnology much in the same way bioethics is integrated into the development of biotechnology. The reasoning behind the current cry for nanoethics, however, can be found in most national and international reports on the potential of nanotechnology. In almost all reports, biotechnology is viewed as a science of having not paid sufficient attention to the ethical questions that the technology might give rise to (Ministry 2004a).

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Several interdisciplinary studies between ethics, sociology, and social psychology have been undertaken to examine the relationship between the academic-ethical debate and public attitudes towards biotechnology. The results show that, to a large degree, ethics answers the philosophers' questions while the problems of the general public go unanswered or are as seen as irrational, metaphysically founded, or stemming from a lack of knowledge (Lassen 2002). Two recent studies conducted in Denmark and Great Britain, explored public perceptions of nanotechnology. These studies provide a basis for evaluating the problems that are most prominent in the eye of the general public according to the available sociological data. Such studies can help ensure that the ethical concerns considered are relevant to the general public (Royal Society 2004).

The goal of our research conducted in 2005 was to estimate the knowledge of nanotechnology and related issues of medical students coming from more than 10 countries of Southeast Asia and Africa to Russia. We interviewed 450 medical students in their 2nd, 4th and 6th years of study. We detected a low percentage of students who could clearly define "nanotechnology" (15% among the junior students, 25% among the 4th year students, and about 35% among the graduates). The difference between students who took different courses was statistically significant ($p < 0.05$). We then arranged a lecture course consisting of 12 academic hours on the use of nanotechnologies in the field of medicine. After these lectures we again interviewed the students and found about 80% of students properly characterized the potential risks of nanotechnology in the health and environment science. When we asked about ethical issues in the use of nanotechnology, almost all the students agreed that understanding bioethics is limiting in nanotechnology and that legal regulation of nanotechnology and nanoresearch is underdeveloped in their countries. It was noted that the majority of students wanted more knowledge about nanotechnology and its future implications in health care. The development in medical, information, communication, or agricultural technology challenges the ethical standards that should be at the root of our medical, social, and educational systems.

2. Applications of Nanotechnology in Healthcare

In the context of their implication on medicine and health care, nanotechnologies can be divided broadly into pharmaceuticals and medical devices. Nanomaterials are used in several key areas, specifically diagnostics, drug discovery and delivery, surgery, tissue engineering, and implants (Nanotechnology in Medicine, Nanotechnology and Health).

Nanomedicine can be defined as: (1) the comprehensive monitoring, control, construction, repair, defense, and improvement of human biological systems,

working from the molecular level, using engineered nanodevices and nanostructures; (2) the science and technology of diagnosing, treating, and preventing disease and traumatic injury, of relieving pain, and of preserving and improving human health, using molecular tools and molecular knowledge of the human body and; (3) the employment of molecular devices and knowledge to address medical problems and improve human health at the molecular scale (Freitas 1999).

Nanotechnology is already being used within the health care market as the following examples illustrate:

1. Atomic force microscope (AFM) technology, which is being used to create smaller and more sensitive microarrays for use in diagnostics and drug discovery. AFMs can also be used to prepare nanostructure surfaces to make them more biocompatible.
2. Nanoparticles, such as fullerenes (molecules based on a 60 carbon atom cage) and quantum dots (complexes of semi-conductor material that have unique fluorescent properties) are being exploited in many areas including imaging (e.g., enhancement of magnetic resonance imaging [MRI] and ultrasound) and drug delivery (e.g., a modified fullerene is entering clinical trials as an anti-HIV agent) (Porod et al. 1999) Formulating drugs with nanoparticles can also improve their solubility, increase their resistance to stomach acid and enzymes to allow for better uptake from the small intestine, and allow for better-controlled drug release. Nanotubes represent another mechanism for drug delivery, both as a "container" and potentially a system for "nano-injection" into cells.
3. Nanocomposites of titanium alloys are used to improve the biocompatibility and longevity of surgical devices and implants.
4. Nanostructuring surfaces can improve cell adhesion (e.g., etching surfaces with nanoscale grooves or using instruments such as an AFM to imprint surfaces with cell-attachment molecules), and direct cells to grow into defined structures. By incorporating biodegradable polymers to act as scaffoldings, these structures can be assembled into 3D "tissues." Nanostructuring can also be used to provide an antimicrobial coating on implants (Merz 1986; Naughton 1999).

What does the future hold? Nanotechnologies will allow us to rapidly sequence an individual's DNA (nanosequencing) and thereby determine genetic susceptibility to disease, drug intolerances and drug metabolism rates. We will be able to target molecules to individual cells within the body for drug delivery or imaging purposes. Patient illnesses will be diagnosed more rapidly through advancements in "lab-on-a-chip" devices, and at the same time a patient's vital

signs could be closely monitored through similar devices. Damaged body parts could be replaced through advances in tissue engineering with physiological tissues and organs grown in the clinic in bioreactors and improved implants will allow patients to regain sight and hearing (Casey 1978; Malinow 2000; Nanotechnology and Health).

3. Bioethics and Nanotechnology

Any technology has the potential for misuse. Studies on the benefits to society of the application of any technology must incorporate evaluation of risk. Many of the ethical questions that are raised by nanotechnology are closely linked to the related disciplines of biology and biotechnology. Public anxieties are focused mainly on fears that the introduction of nanotechnology could lead to an invasion of privacy as a result of genetic data banks, the prospect of runaway proliferation of self-replicating systems and the possible toxic nature of nanoparticles dispersed in the environment.

What will be the short-term impact of nanotechnology, such as in the next 2–3 years? Nanobiotechnology is a rapidly advancing frontier which has already catalyzed an explosion of entirely new industries in health care, medicine, food and nutrition, environmental management, chemical synthesis, and agriculture. Advances in nanoanalytical tools and engineered nanoscale systems are converging with the rapid progress made in genomics, combinatorial chemistry, high-throughput screening and sequencing, drug discovery, microfluidics, and bioinformatics. Nanobiotechnology will also bring tremendous advances in the early detection of diseases and their treatments, and in our fundamental understanding of pathogenic pathways. New nanoanalytical tools are pushing detection limits down to the single-molecule level, which is scientifically a huge success but could be a regulatory problem. Ultrasensitive detection of toxins and pollutants will alarm the public. The public will demand that accurate and scientifically defendable health risk thresholds be redefined, which will not be a trivial task. Furthermore, new DNA-chip technology will be able to identify genetic predispositions at affordable cost. The ethical implications of predictive technology to assess the mental and physical health of patients are far-reaching and are being heavily discussed in conjunction with the human genome project (Whittaker 2005).

On a short-term perspective nanomedicine faces ethical questions that arise mainly from the knowledge gaps concerning the risks of interventions using nanomedical products, nanocosmetic products and nanodelivery vehicles. In general, due to the present lack of knowledge on the health and environmental consequences of nanoengineered materials, the precautionary principle, i.e., the responsibility to take preventive action to avoid harm to human health or environment in a situation where knowledge gaps prevail, should be applied

This is particularly true in the case of the invasive use of nanotechnologies in the human body (Whittaker 2005; Nanotechnology and Nanoscience[1]). The dangers of affecting the human brain with nanomaterials are among the most controversial ethical aspects, particularly if such interventions are beyond a healing perspective. The preservation of human identity should be respected in all such interventions, as well as in research projects dealing with them (Tramontin 2000).

In the medium-term perspective (5–15 years) there are different considerations. The shortage of organ transplants is already a major problem that is likely to worsen as the population rapidly ages. Efforts are, thus, underway to develop synthetic organs. Advances in nanoengineered materials combined with a molecular-scale understanding of wound-healing and tissue- repair processes will be key to integrating engineered biomaterials into biological tissue and to engineering tissue and organs that will replace vital functions of failing organs. The first artificial skin has received Food and Drug Administration (FDA) approval in the USA. Society will also see major advances in treating the loss or partial loss of auditory, visual, and sensory functions through the introduction of novel micro- and nanoengineered-electronic devices. Some of these may be hidden in goggles, behind the ear, or in modern-interactive clothing; others may be directly implanted. Cochlear implants that record a broad spectrum of frequencies are already available. Visual image enhancement or processing implants may be feasible within a decade (Nanotechnology and Nanoscience[1, 2]).

Thus, on a middle term perspective, nanodevices and nanomedical products will be used in all medical fields. This raises ethical questions of responsibility at the local and global levels. Questions on data protection and privacy arise, as in the case of genetic testing. Nanodiagnostic tests will raise the question of healing expectations that in many cases will not be fulfilled. The gap between diagnostic and healing possibilities will affect the relationship between the physician and the patient concerning informed consent, as well as the right of the patient to know. Nanomedical implants, drugs, and treatments will raise the question of justice and fairness in the health-care system as well as between rich and poor societies. It is unlikely that such drugs or treatments will be cheaper than the ones used today. Finally, in the long-term perspective (20 years) electronic devices will be implanted into the brain to enhance or compensate for lost brain function. Major progress has already been made in recording single neurons and their stimulation, and in culturing nerve cells on microelectronic devices (Bai 2000). It seems likely that technology will be able to control at least a few simple brain functions by the use of brain implants. While this will allow for a marked improvement in life quality for some patients, various ethical issues will have to be addressed (Jacobs 2000; Simos

2000). It will be important to regulate legally and ethically experimentation, fabrication, and usage of brain implants. However, the implications of such implants reach much further than safety and ethics. For example, current laws consider whether crimes are conducted under the influence of drugs. In the future, one may have to ask whether a person's state of mind has been impacted by the influence of externally addressable brain implants and if so, who is responsible for their actions. Hence, in the long term, nanotechnology envisages not only the creation of autonomous nanomachines to be used inside the human body but the enhancement and even transformation of the human body and human identity, particularly in case they were used to modify the human brain (Kurzweil 1999; Smalley 2000; Whittaker 2005).

Professor Göran Hermerén of Lund University, Sweden, in his presentation at the EuroNanoForum conference discussed ethical consideration of nanotechnology in three main aspects:

- What are the risks (based on what we know and what can be deduced)?
- How should this knowledge be shared (freely or only to selected groups)?
- How do we achieve public acceptance and ensure that access to nanotechnologies is equitable?

It is essential that a mechanism for assessing risk be established that takes into account the relative frequencies of risk and the gaps in our knowledge. It is important that risk assessments are initiated early to avoid negative impact of nanomaterials on health and environment. In any study of potential risks it is important to remember that any risks must be balanced with benefits, and that risks may only be present in specific contexts. In certain circumstances the action, itself, may lead to a worse outcome, such as forcing the development of technologies outside established regulatory bodies (Ministry 2004b).

However, when discussing the development of nanotechnology and necessity to conduct high-quality research there are two ethical questions: (1) who participates in the experiment; and (2) how is it done? The general principles of ethical research are as follows: essentiality; voluntarism, informed consent, community agreement; non-exploitation; privacy and confidentiality; precaution and risk minimization; professional competence; accountability and transparency; maximization of public interest and distributive justice; institutional arrangements; public domain; totality of responsibility; and compliance. In the case of developing countries the people with low socio-economic status are often used as the research subjects. Usually, researchers provide incentives to people who are willing accept payment for their participation (European Group 2002).

When we talk about research on risk assessment of nanotechnologies, there are plenty of questions. Does nanotechnology offer any capabilities that

will allow us to avoid animal testing in the future? What governments have jurisdiction over research, patents, etc.? What ethical/deontological standards should be applied/considered? Will nanotechnology-based devices cause harm to patients? If so, what standards will be used to ascertain medical malpractice?

What are the questions we should ask about clinical trials of nanodevices, nanodiagnostics, etc.? Does this question imply that nanomedicine should be somehow restricted to academic medical centers or some other regulated institution or should the market decide? Will there be limits on the liability of researchers and their employers? Who will cover the cost?

Who would be best to conduct comprehensive risk assessment? If developers and researchers are wrong in their assessment of risks, who might suffer and how much? What is the most appropriate medium for experimentation in early stage nanodevice research? White paper only? Computer model? Physical model? Animal? Human? Who will decide these questions? Is a nanomedical treatment a drug or a device? What and whose rules apply? Might an increasingly sophisticated nanomedicine capability change the way society looks at "risky" behaviors? What will constitute "informed consent"?

We will come to understand that there will be unintended consequences, both good and bad. Should we allow (or how can we avoid) political or religious interference in research funding? In addition, it is important to take account of the viewpoints of different cultures and populations that have the potential to be radically different. What may be an acceptable risk to one group may be totally unacceptable to another (Nardi 2001). In this context one must account for public perception following media exposure, i.e., if there is a risk assessment does it mean that there is something wrong? It is wrong to assume that people will automatically respond with suspicion. This will certainly result if the reason behind the risk assessments is shrouded in secrecy. A certain amount of honesty and transparency can provide support from the general public (Whittaker 2005).

Regarding sharing of information, acceptance of new technologies necessitates an understanding of not only the science behind them, but also the value of this technology to society. Therefore the public must be given sufficient information to make an informed decision. This information should be correct, should not be misleading and must address public concerns, in order to avoid deeply polarized debates. We know that publication bias can occur. Thus, it is important to present to the public and publish not only positive research results but also negative results.

There are a number of issues that will affect public acceptance and again these may vary in importance between different societies and populations. Developments in nanotechnology have the potential to vastly improve the level

of patient care and therapy and to provide a safer environment for all in society. While ostensibly these should improve disease or injury prognosis, and reduce crime, there are questions of invasion of privacy and sharing of information.

The maintenance of human dignity is firmly rooted in all societies, with most establishing systems to protect vulnerable members such as children, the elderly, and the infirmed. In all circumstances it should be recognized that individuals have the right to determine what information about them can be disclosed to third parties, and what information can be communicated to them by third parties. As such, there must be regulations protecting the individual (especially those most vulnerable) from misuse of nanomaterials, which must be established at the onset and must ensure that mental and physical integrity are not imposed upon without informed consent (Ministry 2004b; Nanomedicine).

Nanotechnology may have the potential to improve the quality of life, but it can only do so with public acceptance of its validity and value. What must be remembered is that different societies, demographic groups and individuals may have quite different religious and philosophical beliefs, and physical needs. As a result, it is clear that within any society there will be a spectrum of feelings pertaining to advances in nanotechnology and their impact on such aspects as: personal integrity, safety and security, the well-being of future generations, health, economic growth, environmental impacts and freedom to conduct research (Roco 1999).

The financial costs of technologies will need to be explained carefully to the public in terms of priorities, associated costs, future markets, and how such investments compare with other technologies. Those individuals involved with the research, development, and ultimate commercialization of nanotechnologies are may have different agendas for the use of a new technology. However this can lead to controversy over data access, patents and publications. Finally the public will want to know who will have access to the new technology and if it will widen the gap between developed and developing countries (Ministry 2004b).

The major bioethical concerns arise around the long-term perspectives of nanotechnologies' like the following:

1. Nanosystems may help solve problems of disease and aging, pollution and scarcity, overpopulation and starvation, and could create revolutionary changes unlike any ever seen. However there are definite ethical concerns if people control most diseases and live very long lives. Will it bring real benefits to mankind or will it play against the laws of nature?
2. The transition from a pre-nano to a post-nano world could be very traumatic and could exacerbate the problems between "the haves and the have-nots."

Have-nots do not easily obtain access to new technologies; the difference between the lives of the nano-rich and the nano-poor will likely be striking.

3. Potential harmful uses, intentional and unintentional, need to be studied well in advance. These include nanoweapons, intelligence-gathering devices, artificial viruses to which humans have no immunity, etc. (Wikipedia).

We also need to highlight the question of manipulation of the mind. Advances in neurosciences are pointing to ways in which it might be possible to manipulate the human brain to control behavior, personality and motivation. Commercial uses have already been seen in the areas of advertising using subliminal visual or auditory cues. Can or should we consider the replication of brains? Whilst this in itself is ethically highly questionable, leading to learning without consent, possible future uses pose even more serious ethical questions (Whittaker 2005; Wikipedia; Zyrex).

One of the main applications of nanotechnologies is in the use of integrated-nanoscale sensors that could monitor the condition of a living organism, the environment, or components of the nutrient supply, sampling a range of conditions with a high degree of sensitivity. For example, the development of tiny sensors is anticipated that could be placed in the human body through implantation or injection into the bloodstream. These sensors could measure the chemistry and biochemistry of the host, collecting unprecedented quantities of data, and might even be able to broadcast this information to remote receivers using wireless techniques. Considerable progress in this field is currently underway (Lee 1998). To cite a few examples of the immense effort that is unfolding in this area, biocompatible sensors are being developed with potential for use *in vivo*, hybrid nanoelectromechanical (NEMS) devices powered by bimolecular motors are being developed for application to biosensors and self assembling, subcellular NEMS devices, a transcutaneous power source is being investigated to drive a totally implantable artificial heart, and highly luminescent semiconductor-quantum dots have been coupled to biomolecules for use in ultra-sensitive biological detection (Bachand 2000; Chan 1998; Chen 1999; Matsuki 1996). Research is underway to power implantable devices remotely and to transmit information between them and external data stations (Dudenbostel 1997; Matsuki 1996; Von Arx 1997).

So the data derived from such sensors can be used for the highly desirable monitoring and treatment of a patient's condition, or for forms of manipulation that may be unwanted by the patient. Ethical theologians speak of "convergences" of unrelated events that converge to a totally unexpected and sometimes undesired result. A striking example of a desirable convergence in the physical sciences was the unrelated but nearly simultaneous development of semiconductor lasers and optical fibers during the early 1970s (Pollock 1995).

At the same time that these rather incredible research advances are taking place, there are many who would like to take advantage of the resulting diagnostic information in ways that might not be beneficial to the patient. For example, those responsible for managing medical insurance would find these data extremely valuable, leading to a convergence which might result in more affordable medical treatment, but, at the same time, in the underinsurance of the patients who have more chance to develop severe diseases. The development of these new technologies inexorably lead to fundamental questions such as "Who has access to this medical diagnostic information?", "To what uses may it be applied?", and, once cures are developed, "Who has access to extremely costly cures?"

Advances in medical biotechnology, whether in the form of "biologic" drugs emanating from the greater understanding of molecular medicine or of cell-based therapies emerging from stem cell research, are likely to be expensive. The expectation that these will be provided on demand for all citizens will impose major strains on already economically struggling health services. The principles of justice and solidarity demand that developments in medical therapy should become available to all that might benefit from them. This is a challenge that we are already facing and one to which there are no easy answers. The problem is compounded by the fact that ill health correlates with levels of poverty and there is a strong ethical case to be made for addressing this situation. It is important to stress that advances in medicine should come not only from high-tech research that benefit the already more advantaged, but also from the medical needs of the poorer members of society.

Much of the criticism of enhancement technologies has focused on the potential for increased discrimination against women, people of color, the poor, the differently enabled, or "unenhanced" humans. Some bioethicists have proposed a global treaty to ban enhancement technologies as "crimes against humanity". Defenders of enhancement argue that the use of biotechnologies is a fundamental human right, inseparable from the defense of bodily autonomy, reproductive freedom, free expression, and cognitive liberty. While acknowledging real risks from genetic, prosthetic, and cognitive enhancement, defenders of enhancement believe that bans on the consensual use of new technologies would be an even greater threat to human rights.

Health care, disability, and reproductive rights activists have argued that access to technology empowers full and equal participation in society. On the same grounds, a generalized right to "technological empowerment" might connect defenders of enhancement technologies with disability activists, reproductive rights activists with would-be parents seeking fertility treatments, the transgendered with aesthetic body modifiers, drug policy reformers and antiaging researchers with advocates for dignity in dying (Stix 1996; White

1986). Yet, what, if any, limits should be considered to human enhancement? On what grounds can citizens be prevented from modifying their own genes or brains? How far should reproductive rights be extended? Might enhancement reduce the diversity of humanity in the name of optimal health? Or, conversely, might enhancements inspire such an unprecedented diversity of human beings that they strain the limits of liberal tolerance and social solidarity? Can we exercise full freedom of thought if we cannot exercise control over our own brains using safe, available technologies? Can we ensure that enhancement technologies are safe and equitably distributed? When are regulatory efforts simply covert, illiberal value judgments? Between the ideological extremes of absolute prohibition and total laissez-faire that dominate popular discussions of human enhancement there are many competing agendas, hopes, and fears. How can the language of human rights guide us in framing the critical issues? How will enhancement technologies transform the demands we make of human rights? (Ministry 2004a, b).

4. Legal Regulatory Considerations of Nanotechnology

Technology transfer from pure research to commercial applications requires appropriate regulatory procedures to ensure that the products are properly approved and certified for use. In order to avoid the possible negative consequences of the use of nanotechnology, there should be created proper legal basis for its regulation. Maximum use should be made of existing legislation. However, the particular nature and unique characteristics of nanotechnologies require the reexamination of existing legislation and, if needed, its revision. In particular, the visibility of nanotechnologies to the human and environmental health and safety regulatory eye should be guaranteed. A proactive approach should be taken. Advancing knowledge about science and society through national, as well as international collaborations with EU Third Countries should play a key role in furthering action in this direction (Meili 2006).

Specifically, there is case for reexamining and, if justified, for lowering the current 1 ton per annum threshold in existing national regulatory frameworks on the registration and documentation of manufactured and imported, intermediate, and finished engineered nanomaterials and products of nanotechnologies. In general terms, regulation requires assessment of hazard (the intrinsic harmfulness of the material) and assessment of the likelihood or duration of exposure. These factors are used to establish the risk to the environment or human population. The overall aim in risk management is to eliminate risks or (in practice) reduce them to acceptable levels. Where possible, this process is guided by factual evidence, usually obtained from

toxicological, environmental, or epidemiological studies. The precautionary principle comes into play when there is a lack of full scientific certainty about the threat of harm from the substance. An assumption will then have to be made about the potential hazard on the basis of such evidence as is available (for example by analogy with materials of known toxicity) and the best available judgments about the hazard-inducing properties of the substance

The need to control the use of hazardous substances to prevent harm to the public or the environment is not new. Only those substances that imply the most serious risks to health or to the environment, for example certain carcinogens, are usually banned. There is already extensive national and European legislation covering different aspects of hazardous substance use. In addition, several international agreements have been developed that are aimed at controlling hazardous agents at the global level. When it is determined that controls are necessary, several regulatory options are available. For example:

- Workplace controls
- Classification and labeling measures
- Control of emissions to air, water and land
- Waste disposal restrictions
- Marketing and use of restrictions
- Prohibition

All these options can be written into legislation. Regulatory measures are not static; the regulator agencies collaborate with industry in seeking to identify further measures that are reasonably practicable to reduce risks (Royal Society of Engineering 2004). Regulation operates under a broad framework. Current frameworks already in place cover a wide range of products and processes, such as chemicals, cosmetics, and medicines, which represent some of the major areas that nanomaterials are likely to impact. The existing regulatory framework for the medical device industry in Europe is based on three European Commission Directives. These are the Medical Devices Directive, the Active Implantable Medical Devices Directive, and the In Vitro Diagnostic Directive. Within the scope of these current regulations, no distinction is made between medical devices and diagnostic systems based on conventional technology and those based on nanotechnology. Technological progress needs to be closely linked to the reexamination of the existing laws, regulations, and standards so that revisions can be made where appropriate.

Currently, the most likely place of exposure to nanoparticles and nanotubes is the workplace, including academic research laboratories. The Health and Safety at Work, etc. Act (1974) sets out the responsibilities for health and safety that employers have towards employees and members of the public, and

employees have to themselves and to each other. Detailed regulations that build on this Act allow these general responsibilities to be expanded and adapted in the light of technological developments and the identification of new risks. Responsibility for health and safety in the work place rests primarily with the employer, whereas the health and safety executive (HSE) is responsible for developing detailed standards and ensuring compliance, containment in normal use, and because of isolated events arising from human error or equipment failure. Minimizing these possibilities is an essential part of risk management (Meili 2006; Royal Society of Engineering 2004).

Concern has been expressed about the potential risk (particularly through inhalation) to workers involved in the production and use of manufactured nanoparticles and nanotubes. Personal exposure, through inhalation, is regulated by requiring compliance with occupational exposure limits (OELs) for individual substances. The OELs are separately specified and are reviewed and adapted in the light of new knowledge through a process that involves the regulator, industry, employees, and the public interest.

Some materials, such as carbon black and titanium dioxide, are being produced by industry either as micrometer-sized or as nano-sized particles. These materials, previously regarded as harmless in their larger forms, may present different toxicological characteristics in their nanoparticulate forms. At present, the regulatory standards are based on the mass of inhaled particles and are derived from a consideration of larger-size distributions. If these mass-based standards were to be applied to nanoparticle materials, it would imply the relative safety of inhaling vast numbers of nanoparticles. There is now experimental toxicological evidence that this will not be a valid assumption.

In many cases it is expected that high standards of containment will be used to prevent the release in workplaces of nanoparticles and high standards of occupational hygiene will be in place. However, releases can and does occur, both because of leakage from containment during routine use and because of isolated events arising from human error or equipment failure. Minimizing these possibilities is an essential part of risk management.

It is important to assure whether current methods are adequate to assess and control the exposures of individuals in laboratories and workplaces where nanotubes and other nanofibers may become airborne, and whether regulation based on electron microscopy rather than phase-contrast optical microscopy is necessary. There will be a need for interim guidance to ensure as far as possible the safety of workers in academic laboratories and industry.

The chemicals industry is likely to be the major producer of nanomaterials, currently in the form of bulk nanoparticles such as titanium dioxide and eventually more advanced functional materials as research and development progresses. Although nanomaterials currently account for only a tiny fraction of

the total quantity of chemicals manufactured, production is expected to increase in the coming years, albeit probably not reaching the levels of larger particulate chemicals currently produced.

Nanoparticles (particularly at the smaller end of the scale) often have different or enhanced properties compared with those same chemicals in a larger form. It is not yet known to what extent the new or enhanced properties of nanomaterials will be different in their toxicity but there is evidence that some nanosubstances, because of their greater surface area, will be more toxic. Whether this increased toxicity poses a risk to human health will depend on the mode of exposure and whether the particles are coated.

Existing substances that are produced in the form of nanoparticles are not defined as new chemicals and the threshold levels do not recognize the fact that substances in nanoparticle form may have different health and environmental impacts per unit mass (NSET 2000; Poldrack 2000). Some manufacturers of consumer products, particularly cosmetics, may utilize the advantages derived from including nanoparticulate materials in their products to give improved or additional functionality. Here the nanoparticles will essentially be free rather than fixed, although their reactivity and, thus, toxicity may be influenced by coatings.

Research is being undertaken to introduce nanomaterials into medical diagnosis and treatment. Although such materials would be subject to the stringent regulatory regime that governs all new interventions in medicine, the particular properties of nanoparticles suggest the possibility of unforeseen toxicity if introduced into the body in large numbers. Particle size and chemistry are taken into account in investigating possible adverse side effects of medicines.

Finally, waste management is already considered as part of the product life cycle. In the EU-extended producer responsibility is mandated through Directives of which those applying to Waste Electrical and Electronic Equipment (WEEE) and End-of-Life Vehicles (ELVs) already cover two of the leading potential engineering applications of nanotechnologies. Take-back Directives require the industry to take responsibility for recovering used products and for recycling materials or reusing components. In addition to ensuring that such products do not enter the waste stream, the take-back principle is intended to encourage design for disassembly, reuse and recycling.

The objective of minimizing human and environmental exposure to free nanoparticles and nanotubes at all stages of the life cycle should also form an integral part of the innovation and design process (Royal Society of Engineering 2004).

At present, very few studies have been published on the potential adverse effects that nanoparticles or nanotubes may have on humans, and only one

to our knowledge on environmental effects. It is necessary to develop internationally agreed protocols and models for investigating the routes of exposure and toxicology to human and nonhuman organisms of nanoparticles and nanotubes in the indoor and outdoor environment, including investigation of bioaccumulation. As it will not be possible to test the toxicity of all sizes of nanoparticles with all possible coatings, there is a need for models to be developed so that results can be extrapolated and the amount of testing reduced. Establishment of a centre to undertake research to address these knowledge gaps and to provide advice to regulators can be very helpful.

Roughly spherical nanoparticles present a regulatory problem that is far removed from the high technology of laboratory nanoscience. Such particles are not only present in urban air but are also generated in very large numbers by such day-to-day activities as cooking. In industry, welding, soldering, and burning operations also generate nanoparticles, and these are currently regulated on a mass basis. The specific production of useful, rather than polluting, nanoparticles of titanium and zinc oxides for paints, cosmetics, and colorants involves rather few occupationally exposed individuals compared with these. Nevertheless, workers are exposed to such materials and it is questionable whether regulation by mass or by another metric reflecting surface area or number is the more appropriate. A decision on this can only be made on the basis of good epidemiological studies, comparing different measurement metrics in relation to health outcomes, combined with toxicology studies (Royal Society of Engineering 2004).

All studies would have to take account the background and complex mixture of nanoparticles normally found in outdoor and indoor air. These background levels are likely to obscure any small release of manufactured particles from production or other processes save when using pollution-free clean room technology. There is a need for the development of practical instruments to measure the size and surface area of industrial and ambient aerosols in the nanometer range, where particles may have aggregated into irregular shapes and there may be a background of nanoparticles. Precautionary principles should be incorporated within a structured approach to the analysis of potential risks of nanotechnology (Meili 2006; Royal Society of Engineering 2004).

Most importantly, a multidisciplinary approach is critical to a satisfactory understanding of the social, ethical, and legal implications of nanotechnology. Action must be taken to address fears that the introduction of nanotechnology may lead to invasion of the individual's right to privacy or to the uncontrollable propagation of self-replicating systems in the environment. Important legal issues arise concerning the rights to ownership of biological molecules and genes by individual organizations (Feynman 1961; Tushman 1996).

Implementation of ethical thinking into the further development of nanotechnology will be beneficial both to society and to nanotechnology itself. Society will benefit because early ethical reflection creates opportunities to influence the development of a technology that will play a key role in the formation of the future. Furthermore, nanotechnology itself will gain as public concerns will be discovered earlier, so that the technology can be developed along lines that are ethically acceptable to both individuals and societies at large. The general public will be perceived as a part of the process and not just consumers of the end products and the development of applications that are seen as unethical can be thoroughly discussed and perhaps halted (Nanotechnology and Nanoscience[1,2]).

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NANOTECHNOLOGY – THE FRAME OF WORKER TRAINING, PUBLIC EDUCATION, AND PARTICIPATION

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Abstract: Nanotechnology is a rapidly progressing field of scientific research and technology development. Nanomaterials possess unique electrical, mechanical, and thermal properties, with potential wide applications in the electronics, computer, aerospace, and other industries. They may be used in an array of manufacturing sectors, including sporting goods, cosmetics, clothing, transistors, and biomedical products. The increase in nanomaterial manufacture and application will make likely the exposure to workers, consumers, medical staff members, and the general population. Therefore, extensive toxicological research and risk assessment is needed. Relevant workplace regulation regarding exposure should also be considered in the light of our current knowledge. International documents for safety of nanotechnology and nanomaterials should be elaborated. Occupational health and medical personnel should be aware of the potential use of nanomaterials in their workplaces and the emerging information on methods to protect workers' health. The following levels of public participation are discussed: no participation, information, consultation, and empowerment. The scope of development of prevention measures for nanotechnology so far is narrow. The difficulty is that principles of safety precautious need to be developed before the solid scientific data of toxicity related to nanotechnology are established.

Keywords: nanotechnology, toxicology, workers exposure, training, regulation, prevention, public participation

1. Introduction

Nanotechnology is a rapidly advancing science research and technology development. Carbon nanotubes (CNTs) are the most used nanomaterial, due to their

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numerous novel and useful properties (Churg and Bauer 2000). They possess unique electrical, mechanical, and thermal properties, with potential wide applications in the electronics, computer, aerospace, and other industries, as well as medicine and consumer products.

There is increasing need for evaluation of existing knowledge of adverse effects to workers, consumers, and general population. Evaluation the potential hazards of the technology and its products is an emerging area in toxicology and health-risk assessment.

Training of workers, education, information, and active public participation should go together with the technology progress in order to avoid and prevent the potential adverse effects.

2. Nanotoxicity

A serious lack of information about the human health and environmental implications of nanomaterials exists, but scientists agree that the potential effects of nanomaterials on working populations and the environment should be under a serious consideration (Lam et al. 2006; Shvedova et al. 2005; Donaldson et al. 2006).

Significance of particle parameters in the evaluation of exposure–dose–response relationships of inhaled particles is underlined by Oberdorster et al. (1996). Pulmonary effects of particles, including of man-made organic fibers have been reported (Oberdorster et al. 2000; Warheit et al. 2001).

Nanoparticles are so small that they may interact with other substances at the atomic or subatomic level (McCauley and McCauley 2005). They do not behave like solids, liquids, or gases, (Arble 2004) and have unique mechanical and electronic properties.

The available peer-reviewed literature suggests that CNTs may have unusual toxicity properties. In particular CNTs seem to have a special ability to stimulate granuloma formation and fibrogenesis. In several studies it has been shown that CNTs have more adverse effects than the same mass of carbon black and quartz, the latter a commonly used benchmark of particle toxicity. Studies also show that CNTs may exhibit some of their effects through oxidative stress and inflammation. CNTs represent a group of nanomaterials which production as well as use is growing and therefore further research into their toxicology and safe use is warranted (Donaldson et al. 2006).

3. Workers Protection

Occupational safety and health encompasses four core disciplines: industrial hygiene, occupational safety, occupational medicine, and occupational health nursing (Morris 1994). These specialties should develop in response to the growing demands of the workplace including the development of nanotechnologies.

3.1. INTERNATIONAL DOCUMENTS

There is a need for evaluated information that should be available for the workers medical services. The World Health Organization, United Nations Environment Programme (UNEP) and others have long-lasting experience for preparation of information document at different levels.

- Screening Information Data Set (SIDS) for the substances with limited toxicological data.
- International Chemical Safety Cards (ICSC)
- Concise International Chemical Assessment Documents (CICADs)
- Environmental Health Criteria Documents (EHC)

Nanoparticle toxicology data might be sufficient for the preparation of the first document – SIDS, which practically provides the possibility for development of the more detailed document – ICSC.

3.1.1. Training

Worker training is regulated in almost all developed countries with special curricula which are updated periodically to include the new technologies, as it should be the case with the nanotechnology.

3.1.2. Legislation Measures

Relevant workplace regulation regarding exposure also should be considered in the light of our preliminary toxicological knowledge, for example CNTs. CNTs could have features of both nanoparticles and conventional fibers and so the current paradigm for fiber toxicology, which is based on mineral fibers and synthetic vitreous fibers may be used.

4. Protection of Medical Staff

The development of nanomaterials for medicine, similar to genetic tests and other molecular technology (Geppert and Roberts 2005), may have immediate and wide relevance to medical workers. This new technological progress have the potential to provide improved workplace safety and protect workers' health and to help considerably the patient health care, but they also carry the risk. Ethical safeguards are necessary if the benefits are to outweigh the adverse consequences in the workplace. The progress of nanotechnology in medicine should not be permitted to shift the responsibility for a safe working environment from the employer to the employee. Occupational physicians and clinicians treating workers should be aware of potential nanoparticle exposure.

There is an urgent need for education and research to expand and implement the recommendations of major governmental and professional policy statements.

4.1. NURSES EDUCATION

Occupational health nurses should be aware of the potential use of nanotechnology in their workplaces and stay aware of emerging information on the methods to protect worker's health.

It is proposed to review and redesign nursing curricula in relation to nursing informatics (Booth 2006). Recommendations include increased information literacy education, interdisciplinary collaboration, and client-centered technologies. Recommendations for faculty development in nursing informatics are also provided. The proliferation of teaching strategies using the Internet and other educational technology innovations has created new employment issues in higher education. Link and Scholtz (2000) discuss forces influencing the increasing use of online instruction, factors to consider before venturing into the virtual academy, and the faculty role in shaping policy related to distributed education in their institutions and academia in general.

Neuman (2006) discusses the explosion of technology and its impact on nursing education in the face of a nurse educator shortage. An attempt is made to answer the following questions: What incremental changes in technology do we have now? How do we envision technology being used in the future? Four scenarios of nontraditional approaches to nursing education are presented. They touch on the delivery of education with increased technology and universal access; the teacher as educator/mentor/coach; the product, including testing, outcomes, competencies, and process; and attracting and keeping human attention. The final section focuses on issues to consider as nurse leaders and educators bring nursing education into the future.

The employee empowerment model presented by Chang et al. (2006) may be used as a guide to design empowerment education curricula for public health nurses.

5. Public Education, Information and Participation

Why public education?

Hazardous substances are ubiquitous in the environment and common in industrialized societies. Serious harm can occur with sufficient exposures under certain conditions. However, much harm can be avoided if hazardous substances are handled with respect and appreciation for their use and potential (Arble 2004). Thus, nanoparticles are small-scale substances (<100 nm) with

unique properties which should be considered during evaluation of the exposure and health risk. Characterization of airborne particles indicates that exposures will depend on particle behavior (e.g., disperse or aggregate) and that accurate, portable, and cost-effective measurement techniques are essential for evaluation of the exposure. Under many conditions, dermal penetration of nanoparticles may be limited for consumer products such as sunscreens. Additional studies are needed for complete characterization of the penetration of nanoparticles into the skin, which should consider multiple factors such as potential photooxidation and skin conditions.

Carbon nanotubes apparently have greater pulmonary toxicity (inflammation, granuloma) in mice than fine-scale carbon graphite, and their metal content may affect toxicity. Studies on titarium dioxide (TiO_2) and quartz illustrate the complex relationship between toxicity and particle characteristics, including surface coatings, which make generalizations (e.g., smaller particles are always more toxic) incorrect for some substances. These recent toxicity and exposure data, combined with therapeutic and other related literature, are beginning to shape risk assessments that will be used to regulate the use of nanomaterials in consumer products (Tsuji et al. 2005).

Nanotechnology is developing very quickly, and is in many respects leading the world in this convergence of nanoscale-engineering techniques. The public health community must start to think about the public-health impacts of nanotechnology over the next years. The responsibility for the benefits and the harms of nanotechnology lies with government, corporations and the business community, scientists, and specialists in all related fields, and the public.

There are many questions of public health which are not yet being asked about nanotechnology. If nanoparticles are to be used in cosmetics, food production, and packaging, how will they react or interact with the human skin and organs? What chemical-toxic effects on life might occur from the nanoparticles in car tires and vehicle plastic mouldings, when they are disposed by incineration? Will they pass into the soil and groundwater and enter into the food chain? It is now an urgent ethical demand, based on the precautionary principle that the governments in the world should take an intergovernmental initiative to intervene in the further development, production and marketing of nanotechnological products with precautionary research and regulation (Matsudai and Hunt 2005).

Bryan (2003) reviewed the relevant methods for public participation and challenges for society in dealing with potentially highly disruptive technology.

The following terms of levels of participation are discussed:

- **No participation** – secrecy, silence, overconfidence in the potential for containment and accident prevention.
- **Information** – public-relation techniques including websites, newspapers and magazines. Institutions, governmental agencies, and private organizations distribute information with frequently asked questions, advertising and other methods.
- **Consultation** – public hearings, focus groups – facilitate better understanding of the problems by public and decision-makers. In case of nanotechnology the problem is: novelty, invisibility, involuntary exposure.
- **Involvement and collaboration** – workshops which engage representative range of concerned people. Negotiated rule making by regulatory agencies together with stakeholders.
- **Empowerment** – partnership, delegated authority, joint decision by concerned groups, and government in standards settings.

The scope of public participation in nanotechnology research and development so far has been narrow. The difficulty is that principles of safety precautious need to be developed before the solid scientific confirmation of toxicity related to nanotechnology is established. On the other hand there is a danger of derailing nanotehnology if the study of ethical, legal, and social implications does not go with the speed of scientific development (Mnyusiwalla 2003).

6. Conclusion

Testing strategies to establish the effects and safety of nanoparticles and nanoproducts should be adopted and implemented.

The urgent needs are:

- Methods for measuring worker's environment and ambient air should be developed and validated.
- Minimum testing for characterization of nanomaterial toxicity in inhalatory exposure.
- Dermal exposure.
- Potential hazards for local and systemic effects.

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USE OF MEMBRANE FILTRATION FOR WATER TREATMENT WITH EXAMPLES FROM THE REPUBLIC OF MACEDONIA

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Abstract: The membrane processes of most significance in water treatment are reverse osmosis, ultrafiltration, and nanofiltration. These processes have traditionally been applied to the production of water for industrial or pharmaceutical applications but are now being applied to the treatment of drinking water. During the last two decades, various applications and improvements in membrane filtration have been made. The most widespread application is in seawater desalinization. Up to 100 desalinization plants are in operation all over the world. Capacity now exceeds 1,000 m^3/h. Other applications are connected with potable water treatment, ultra-filtrated water used in the chemical and pharmaceutical industries, and wastewater treatment of industrial and municipal water. Membrane filtration is a part of membrane technology that the Fluid Project Company has successfully applied in the field of potable, ultra-pure and waste water treatment in the Republic of Macedonia in last decade.

Keywords: membrane filtration, water treatment, reverse osmosis, nanofiltration, ultra-filtration

1. Introduction

The membrane processes of most significance in water treatment are reverse osmosis, ultrafiltration, microfiltration, and nanofiltration. These processes have traditionally been applied to the production of water for industrial or pharmaceutical applications but are now being applied for the treatment of drinking water.

If two solutions are separated by a semipermeable membrane (i.e., a membrane that allows the passage of the solvent but not of the solute), the solvent will naturally pass from the lower-concentration to the higher-concentration

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solution. This process is known as osmosis. It is possible, however, to force the flow of solvent in the opposite direction, from the higher to the lower concentration, by increasing the pressure on the higher-concentration solution. The required pressure differential is known as the osmotic pressure, and the process is known as reverse osmosis. Reverse osmosis results in the production of a treated water stream and a relatively concentrated waste stream. Typical operating pressures are in the range 15–50 bar, depending on the application. Reverse osmosis rejects monovalent ions and organics of molecular weight greater than about 50 (membrane pore sizes are less than 0.002 μm). The most common application of reverse osmosis is desalination of brackish water and seawater.

2. Methodoligcal Issues

Nanofiltration uses a membrane with properties between those of reverse osmosis and ultrafiltration membranes; pore sizes are typically 0.001–0.01 μm. Nanofiltration membranes allow monovalent ions, such as sodium or potassium, to pass but reject a high proportion of divalent ions, such as calcium and magnesium and organic molecules of molecular weight greater than 200. Operating pressures are typically about 5 bar. Nanofiltration may be effective for the removal of color and organic compounds.

Ultrafiltration is similar in principle to reverse osmosis, but the membranes have much larger pore sizes (typically 0.002–0.03 μm) and operate at lower pressures. Ultrafiltration membranes reject organic molecules of molecular weight above about 800 and usually operate at pressures less than 5 bar.

Microfiltration is a direct extension of conventional filtration into the submicrometre range. Microfiltration membranes have pore sizes typically in the range 0.01–12 μm and do not separate molecules but reject colloidal and suspended material at operating pressures of 1–2 bar. Microfiltration is capable of sieving out particles greater than 0.05mm. It has been used for water treatment in combination with coagulation or PAC to remove dissolved organic carbon and to improve permeates flux (WHO 2004). The aim of this review is to present available methods used for water treatment in the world with particular reference to the Republic of Macedonia.

Membrane separation technology has significantly evolved over the last decennia. The main force of membrane technology is that it works without the addition of chemicals, with a relatively low-energy use and well-arranged process conduction. Membrane technology is a generic term for number of different, very characteristic separation processes. These processes are similar, as each of them uses a membrane. Membranes are used more and more often for processing groundwater, surface water, or wastewater are now competitive with conventional techniques. The membrane separation process is based on the presence of semipermeable membranes.

The principle is quite simple: the membrane acts as a very specific filter that will let water flow through, while it catches suspended solids and other substances. There are various methods to enable substances to penetrate a membrane. Examples of these methods are the application of high pressure, the maintenance of a concentration gradient on both sides of the membrane, and the introduction of an electric potential. Membranes constitute a selective wall. Certain substances can pass through the membrane, while other substances are caught.

The novel aspect of membrane filtration is that it can be used as an alternative for standard processes of flocculation, coagulation, sediment purification techniques, adsorption, extraction, and distillation (www.diet-nutrition-health.com). There are two factors that determine the effectiveness of a membrane filtration process: selectivity and productivity. Selectivity is expected as a parameter called retention or separation factor. This factor is measured in $l/m^2 \times h$/unit. Productivity is expressed as a parameter called flux. This is measured by $l/m^2 \times h$/unit also. Selectivity and productivity are membrane-dependent.

Membrane filtration can be divided between: micro- and ultrafiltration on one hand and: nanofiltration and reverse osmosis (RO or hyper-filtration) on the other hand. When membrane filtration is used for the removal of large particles, microfiltration and ultrafiltration are applied. Because of the open character of the membranes, the productivity is high while the pressure difference is low. When salts need to be removed from water, nanofiltration, and RO are applied. Nanofiltration and RO membranes do not behave according to the principle of pores; separation takes place by diffusion through the membrane. The pressure that is required to perform nanofiltration and RO is much higher than the pressure required for micro- and ultrafiltration, while productivity is much lower.

3. Treatment Achievability

The ability to achieve a guideline value within a drinking-water supply depends on a number of factors, including:

- The concentration of the chemical in the raw water
- Control measures employed throughout the drinking-water system
- Nature of the raw water (groundwater or surface water, presence of natural background and other components)
- Treatment processes already installed

If a guideline value cannot be met with the existing system, then additional treatment may need to be considered, or water should be obtained from

alternative sources. The cost of achieving a guideline value will depend on the complexity of any additional treatment or other control measures required. It is not possible to provide general quantitative information on the cost of achieving individual guideline values. Treatment costs (capital and operating expenses) will depend not only on the factors identified above, but also on issues such as plant throughput; local costs for labor, civil and mechanical works, chemicals and electricity, life expectancy of the plant, and so on.

A qualitative ranking of treatment processes based on their degree of technical complexity is given in Table 1. The higher the ranking, the more complex is the process in terms of plant and/or operation. In general, higher rankings are also associated with higher costs.

TABLE 1. Ranking of technical complexity and cost of water-treatment processes.

Ranking	Examples of treatment processes
1	Simple chlorination
	Plain filtration (rapid sand, slow sand)
2	Prechlorination plus filtration
	Aeration
3	Chemical coagulation
	Process optimization for control of DBPs
4	Granular activated carbon (GAC) treatment
	Ion exchange
5	Ozonation
6	Advanced-oxidation processes
	Membrane treatment

Table 2 summarizes the treatment processes that are capable of removing chemical contaminants of health significance. The table includes only those chemicals for which some treatment data are available. This table is provided to help inform decisions regarding the ability of existing treatment to meet guidelines and what additional treatment might need to be installed. It has been compiled on the basis of published literature, which includes mainly laboratory experiments, some pilot-plant investigations and relatively few full-scale studies of water-treatment processes. Consequently:

- Many of the treatments outlined are designed for larger treatment plants and may not necessarily be appropriate for smaller treatment plants or individual type treatment. In these cases, the choice of technology must be made on a case-by-case basis.

- The information is probably "best case," since the data would have been obtained under laboratory conditions or with a carefully controlled plant for the purposes of experimentation.
- Actual process performance will depend on the concentration of the chemical in the raw water and on general raw water quality. For example, chlorination and removal of organic chemicals and pesticides using activated carbon or ozonation will be impaired if there is a high concentration of natural organic matter.

TABLE 2. Treatment achievability for naturally occurring chemicals, for which guideline values have been established.

	Chlorination	Coagulation	Ion exchange	Precipitation softening	Activated alumina	Activated carbon	Ozonation	Membranes
Arsenic		+++	+++	+++	+++			+++
		<0.005	<0.005	<0.005	<0.005			<0.005
Fluoride		++			+++			+++
					<1			<1
Manganese	+++	++					+++	+++
	<0.05						<0.05	<0.05
Selenium		++	+++		+++			+++
			<0.01		<0.01			<0.01
Uranium		++	+++	++	+++			
			<0.001		<0.001			

Symbols are as follows:

++ 50% or more removal

+++ 80% or more removal

The table includes only those chemicals for which some treatment data are available. A blank entry in the table indicates either that the process is completely ineffective or that there are no data on the effectiveness of the process.

For the most effective process(es), the table indicates the concentration of the chemical, in mg/litre, that should be achievable.

- For many contaminants, several different processes could be appropriate, and the process selected should be made on the basis of technical complexity and cost, taking into account local circumstances. For example, membrane processes can remove a broad spectrum of chemicals, but simpler and cheaper alternatives are effective for the removal of most chemicals.
- It is normal practice to use a series of unit processes to achieve desired water quality objectives (e.g., coagulation, sedimentation, filtration, GAC,

chlorination). Each of these may contribute to the removal of chemicals. It may be technically and economically advantageous to use a combination of processes (e.g., ozonation plus GAC) to remove particular chemicals.

- The effectiveness of potential processes should be assessed using laboratory or pilot plant tests on the actual raw water concerned. These tests should be of sufficient duration to identify potential seasonal or other temporal variations in contaminant concentrations and process performance (WHO, 2004).

RO is the most stringent membrane process in liquid/liquid separation. Water is in principle the only material passing through the membrane; essentially all dissolved and suspended material is rejected. The more open types of RO membranes are sometimes confused with nanofiltration (NF).

True NF rejects only ions with more than one negative charge, such as sulfate or phosphate, while passing single-charged ions. NF also rejects uncharged, dissolved materials and positively charged ions according to the size and shape of the molecule in question. Finally, the rejection of sodium chloride with NF varies from 0–50% depending on the feed concentration.

In contrast, "loose RO" is a RO membrane with reduced salt rejection. This effect has proven desirable for a number of applications where moderate salt removal is acceptable since operating pressures and power consumption are significantly lowered. So, in exchange for less than complete salt removal, costs are reduced.

TABLE 3. Comparing four membrane processes.

	Reverse osmosis	Nanofiltration	Ultrafiltration	Micro filtration
Membrane	Asymmetrical	Asymmetrical	Asymmetrical	Symmetrical Asymmetrical
Thickness	150 μm	150 μm	150–250 μm	10–150 μm
Thin film	1 μm	1 μm	1 μm	
Pore size	<0.002 μm	<0.002 μm	0.2–0.02 μm	4–0.02 μm
Rejection of	HMWC, LMWC sodium chloride glucose amino acids	HMWC mono-, di-, and oligosaccharides polyvalent negative ions	Macromolecules, proteins, polysaccharides, Vibro anguillarum (virA)	Particles, clay bacteria
Membrane material(s)	CA Thin film	CA Thin film	Ceramic PSO, PVDF, CA thin film	Ceramic PP, PSO, PVDF
Membrane Module	Tubular spiral wound plate-and-frame	Tubular spiral wound plate-and-frame	Tubular hollow fiber spiral wound plate-and-frame	Tubular hollow fiber
Operating pressure	15–150 bar	5–35 bar	1–10 bar	<2 bar

Ultrafiltration (UF) is a process where the HMWC, such as protein, and suspended solids are rejected, while all LMWC pass through the membrane freely. There is consequently no rejection of mono- and di-saccharides, salts, amino acids, organics, inorganic acids, or sodium hydroxide.

Microfiltration (MF) is a process where ideally only suspended solids are rejected, while even proteins pass the membrane freely. There is, however, quite a gap between real life and this ideal situation (Table 3).

Vast arrays of products are being treated using membranes, but over 80% of all membranes having ever been sold are used for water desalination. The better portions of the remaining 20% are used for dairy processing, while the remaining membranes are sold for use with different liquids. Some liquids are waste products, while others are very expensive pharmaceutical products. Table 4 lists some typical applications, the shaded area representing the main product. Note that permeate as well as the concentrate can be the desired product, and they can be used simultaneously.

TABLE 4. Type of membrane process for several products.

		Permeate	Concentrate
RO	Dyeing effluent	clean water	BOD, salt, chemicals, waste products
	Water	low-salinity water	salty water
	Whey	low-BOD permeate	whey concentrate
NF	Antibiotics	salty waste product	desalted, concentrated antibiotics
	Dyeing effluent	clean, salty water	BOD/COD, color
	Water	softened water	waste product
	Whey	salty waste water	desalted whey concentrate
UF	Antibiotics	clarified fermentation broth	waste product
	Bio-gas waste	clarified liquid for discharge	microbes to be recycled
	Carrageenan	waste product	concentrated carrageenan
	Enzymes	waste product	high-value product
	Milk	lactose solution	protein concentrate for cheese production
	Oil emulsion	oil-free water (<10 ppm)	highly concentrated oil emulsion
	Washing effluent	clarified water	dirty water (waste product)
	Water	clarified water	waste product
	Whey	lactose solution	whey protein concentrate
	Xantan	waste product	concentrated xantan

4. Membrane Materials

The selection of membranes offered by the various suppliers in the business may appear to be confusing since many materials may be used to make membranes, and they are provided under an array of trade names. In reality, relatively few materials are actually used in quantity, and only a few basic membrane types form the bulk of the membranes being sold and used.

Integral membranes

Cellulose acetate (CA) is the "original" membrane and is used for RO, NF, and UF applications. The material has a number of limitations, mostly with respect to pH and temperature. The main advantage of CA is its low price, and the fact that it is hydrophilic, which makes it less prone to fouling. There are many "die hard" membrane users who insist on buying "the same membrane as last time," and who simply stay with CA because it works for them. An inherent weakness of CA is that is can be destroyed by microorganisms.

Various membranes made from polysulfone (PSO) have been used for UF and MF since 1975. PSO's main advantage is its exceptional temperature and pH resistance. PSO is practically the only membrane material used in high quantity for a number of food and dairy applications. As a rule, PSO membranes do not tolerate oil, grease, fat, and polar solvents. However, there is one type of hydrophilic PSO membrane which apparently defies this rule and seems to work well with oil emulsions.

Polyvinylidenedifluoride (PVDF) is a traditional membrane material, but it is not used much because it is difficult to make membranes with good and consistent separation characteristics. Its main advantage is its high resistance to hydrocarbons and oxidizing environments.

Composite membranes

Also called thin-film composite membranes, they appear under various acronyms such as TFC and TFM, and were made to replace cellulose acetate RO membranes. Their main advantage is the combination of relatively high flux and very high salt rejection, as 99.5% NaCl rejection can be achieved with composite RO membranes. They also have good temperature and pH resistance, but do not tolerate oxidizing environments.

Composite membranes are made in two and three-layer designs, the precise composition of which is proprietary. Generally speaking, a thin-film composite membrane consists of a PSO membrane as support for the very thin skin layer, which is polymerized in situ on the PSO UF membrane. The three-layer design has two thin film membranes on top of the PSO support membrane.

Around 1980, FilmTec marketed the two- design which immediately became the industry standard for water desalination, and this type of membrane has dominated the water desalination market ever since. The membrane has been improved over the years but the basic design remains unchanged, and today there are several companies making this type of membrane.

In the mid-1980s Desalination Systems, Inc. (DSI) began making composite membranes with a three-layer design. These membranes had difficulties competing with the two-layer membranes in water desalination, but proved to work better on industrial process streams where it is more stable and less prone to fouling. The three-layer design is available for RO and NF, and it is still the best choice for treating a vast array of difficult process streams. DSI is the only producer of three-layer composite membranes.

Total worldwide consumption of membranes, based on membrane surface area, is approximately as follows.

- Composite RO membranes: 85%
- Composite NF membranes: 3–5%
- Polysulfone UF and MF membranes: 5–7%
- Other membranes: 3–5%

Materials like polyacrylonitrile (PAN), ceramic materials (SiO2), and cellulose (hydrolyzed cellulose acetate) are included in the group of "other membranes" (Wagner, 2001).

During the last two decades, various application of membrane filtration can be found. The most widespread application is in seawater desalinization. Up to 100 desalinization plants are in operation in the world. Capacity has been extended up than 1,000 m^3/h. Other applications are connected with potable water treatment, ultra pure water in chemical and pharmaceutical industry, wastewater treatment of industrial and municipal water (www.sell-inventory.com).

Membrane filtration is a part of membrane technology that Fluid Project Company (2006) successfully applies on the field for potable, ultra pure and waste water treatments. Fluid Project is a Water Treatment Company situated in Skopje, Republic of Macedonia. The company was founded 1980, and the water treatment division was started since 1995. The company is covering the market of water treatment in Macedonia, Bulgaria, Kosovo, and Albania. Among various technologies of water treatment such as: softening, filtration, deferization, demanganization, dearsenization, dechlorination, demineralization, and disinfection, the company is deeply involved in membrane technology application. During their 10 years of experience, more than 20 plants and systems have been constructed.

Case studies:

1. Membrane filtration applied RO is used as part of the final demineralization process into a system that produces ultra pure water model FPRO-4040–800 (Fig. 1). The system is located in the ultra pure supply network of "Clinic Pharmacy" of Skopje. The capacity of treated water is 800 l/h, with average water quality of 0.5 μS/cm^{-1}. The resource of raw water is city distribution system. Pretreatment consists of the following steps: filtration, dechlorination, and deferization. RO unit consists of four membrane elements that are 4″ each, manufactured by Osmonix, and supported elements. Working pressure is up to12 bars.
2. A very similar system was installed at "Remedica" Clinic in Skopje. The capacity of the system (Fig. 2) is 900 l/h. The applied membrane elements are 4″ × 40″ and manufactured by Desal Membranes.
3. The most reliable application in ultra-pure water treatment is at the treatment plant at "Alkaloid" Pharmaceutical Factory, Skopje. It contains a demineralization system and is designed to remove endotoxins and pyrogens according to European Pharmacopeia. Unit model FPRO-8040–3 (Fig. 3). It consists of pretreatment in order to utilize demineralization, provided by ion exchange columns (cation, anion, and mix-bad), UV sterilization, and RO modules. Capacity of the treated water is 4.5 m^3/h, at the pressure up to 14 bars. Model of RO membranes is NW80″ × 40″ manufactured by Osmonix. According to GMP standards, all installation material is manufactured by AISI 316L.

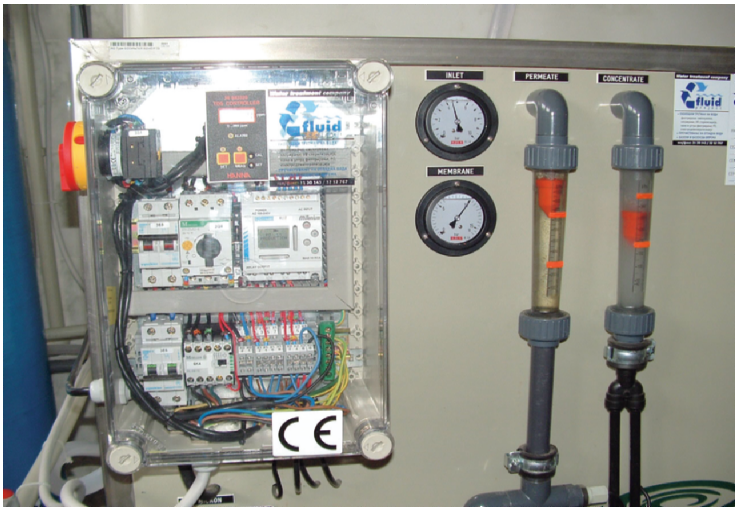

Figure 1. Ultra Pure Water model FPRO-4040–800.

Figure 2. “Remedica” clinic in Skopje.

Figure 3. Unit model FPRO-8040–3.

4. The most common application is in the water bottling industry. A system for partial demineralization is installed at the DOBRA VODA factory near the city of Kratovo, Macedonia. The system model FPRO-8040–2, capacity of 2.5 m^3/h is employed after the filtration and deferization pretreatment (Fig. 4). The number of RO elements is 2, sized 8″ × 40″ each. Working pressure is 12 bars (Fluid Project Company 2006).

Figure 4. Unit model FPRO-8040–2.

5. Conclusion

The membrane separation process is based on the presence of semipermeable membranes. RO is the tightest possible membrane process in liquid/liquid separation. Water is in principle the only material passing through the membrane; essentially all dissolved and suspended material is rejected. NF rejects only ions with more than one negative charge, such as sulfate or phosphate, while passing single charged ions. NF also rejects uncharged, dissolved materials and positively charged ions according to the size and shape of the molecule in question. Finally, the rejection of sodium chloride with NF varies from 0–50% depending on the feed concentration. UF is a process where the HMWC, such as protein, and suspended solids are rejected, while all LMWC pass through the membrane freely. There is consequently no rejection of mono- and disaccharides, salts, amino acids, organics, inorganic acids, or sodium hydroxide. MF is a process where ideally only suspended

solids are rejected, while even proteins pass the membrane freely. Membrane technology has become a dignified separation technology over the past decennia. The main force of membrane technology is the fact that it works without the addition of chemicals, with a relatively low-energy use and well-arranged process conduction. Membrane technology is a generic term for a number of different, very characteristic separation processes. These processes are similar, because in each of them a membrane is used. Membranes are most often used for the creation of processed water from ground-water, surface water, or wastewater. Membranes are now competitive for conventional techniques worldwide as well in the Republic of Macedonia.

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CURRENT STATE OF NANOSTRUCTURED TIO_2-BASED CATALYSTS: PREPARATION METHODS

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Abstract: In this review the current state of preparation methods for nanostructured TiO_2-based catalysts are presented through presentations of experimental results and theoretical discussions published in the literature. Significant numbers of studies have been presented, in order to obtain an overview of this complicated field and to provide a starting point for further investigations. Preliminary experiments of a novel method for TiO_2-based catalysts preparation is shown using ArF pulsed laser-induced chemical vapor deposition with $Ti(OSiMe_3)_4$ as a precursor. The properties and composition of the solid-deposited Ti/O/Si material are revealed.

Keywords: TiO_2 preparation, chemical vapor deposition, photocatalysis, nanostructures, nanomaterials

1. Introduction

Photocatalysis is a well-known process, based on photoinduced phenomena. Irradiation of semiconductor catalyst with UV light carrying enough energy to overcome its band-gap results in creating electron–hole pairs, due to electron promotion from the valence band to the empty conduction band. Excited state conduction band electrons and valence band holes can either recombine and dissipate the input energy as heat, react with electron donors or acceptors adsorbed at the catalyst surface (photocatalysis), be used directly to create

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electricity (photovoltaic phenomena), or valence band holes could be trapped at the semiconductor surface causing high wettability (photo induced superhydrophilicity).

Many semiconductors, due to their electronic structure (filled valence band and empty conduction band) can act as a catalyst for photoinduced processes (TiO_2, ZnO, Fe_2O_3, CdS, etc.) (Hoffman 1995). The most widely used and already with some practical application are nano-sized TiO_2 and TiO_2-based catalysts, due to their chemical stability, nontoxicity and chemical reactivity, as well as low price. The photoinduced phenomena (photocatalysis, photovoltaics, and superhydrophilicity) occurring on the catalyst surface according to the way of action of generated electron–hole pairs, will determine the possible application of nano-sized TiO_2 and TiO_2-based catalysts.

These processes are depicted in Fig. 1, showing the mechanism of these photoinduced processes and at the same time TiO_2 and TiO2-based catalysts, wide applications.

1.1. WHY PRODUCE NANOSTRUCTURED TIO_2-BASED CATALYSTS?

As seen from Fig. 1, one of the photoinduced phenomena on the catalyst surface could be the use of excited electrons from conduction band by some outer circuit for production of electricity. This can result in production of TiO_2-based photovoltaic cells, which produce electricity from sun light (Gratzel 2001).

The most active field of the photoinduced phenomena is photocatalytic reaction, mediated by TiO_2 and TiO_2-based catalysts on their surface:

1. Photocatalytic reactions: solar production of hydrogen from water (Khan 2002), photofixation of nitrogen (Hoshino 2001; Ogawa, 2004), photoreduction of CO_2 (artificial photosynthesis).
2. Degradation of large number of organic and inorganic compounds, viruses, bacteria, cancer cells to CO_2, H_2O, harmless intermediates and inorganic anions (Hoffmann 1995; Zhao 2003; Carp 2004; Anpo 2004).
3. Photoinduced superhydrophilicity across the surface that allows both water and oil to spread accompanied by photocatalytic activity, so the surface contaminants will be either photomineralized or washed away by water (Yu, 2001a, b, 2002). A possible application is self-cleaning windows and self-cleaning ceramics.

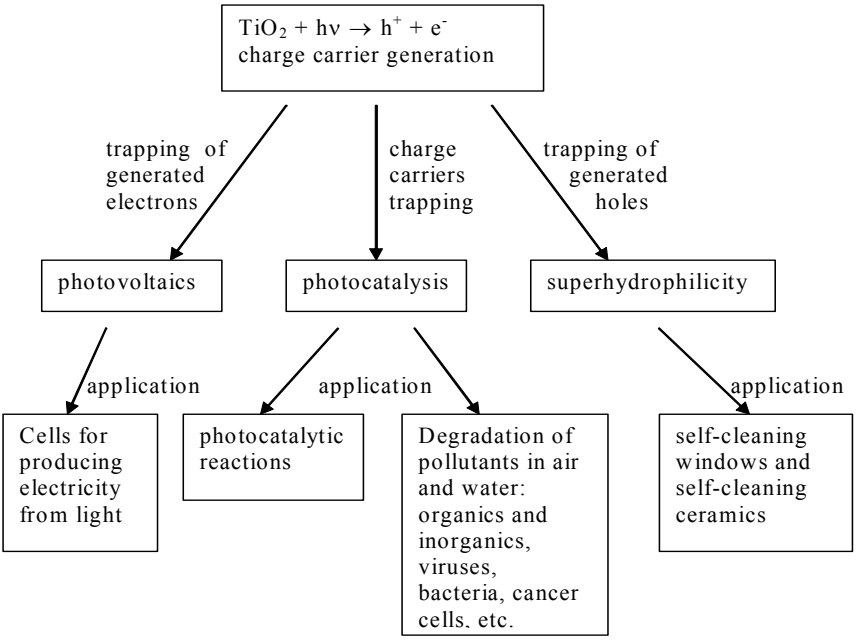

Figure 1. Mechanism of TiO_2 photoinduced processes and their applications.

1.2. TOXICITY OF NANOSTRUCTURED TIO$_2$-BASED CATALYSTS

The explosive growth of nanotechnology has brought opportunities in various fields: engineering, diagnostics, and information technology. On the other hand, questions have arisen about the possible toxicity, and environmental consequences. However, research concerning the biological effect of nanoscaled materials is lacking in comparison with other aspects of nanotechnology development.

According to the published literature, nanoscaled materials may have a higher potential toxicity per given mass than larger particles with the same chemical structure (Oberdorster 2005). Concerning the toxicity of TiO_2-based catalysts (simply substitutionally or interstially doped TiO_2 particles), there are still no experimental studies, except for nanostructured and fine particles of TiO_2 (Oberdorster 2005; Warheit 2006). Oberdorster et al. (2005) have shown that ultrafine particles of TiO_2 (20 nm), when instilled intratracheally into rats and mice, induced a much greater pulmonary-inflammatory neutrophil response in the lung than fine TiO_2 particles (250 nm) at the same mass dose. However,

when the dose was expressed as a particle surface area, for particles with different size and the same chemistry, the authors obtained a neutrophil response that fitted the same dose–response curves. This suggests that it is best to present the inflammatory potential of nanomaterials by expressing the dose as surface area rather the mass when describing effects of inhaled solid particles of different sizes. Warheit et al. (2006) have evaluated the acute lung toxicity in rats of intratracheally instilled pigment grade TiO_2 particles (rutile, 300 nm) and nanoscale TiO_2 (anatase, 10 nm). They noticed transient inflammatory and cell-injury effects that were identical for TiO_2 of both sizes. Toxicity data for TiO_2-based catalysts are needed to establish safe-handling pratices and dosimetry, detecting in biological tissues.

2. Preparation of Nano-Sized TiO_2 and TiO_2-Based Catalysts

Since it was confirmed that the various properties of the photocatalyst could be effectively engineered during its synthesis and processing, a number of different methods has been proposed in order to produce highly reactive and functional catalyst. Concerning TiO_2 catalyst, these methods are directed to produce nano-sized particles with exactly defined physical properties including crystalline structure, surface area, porosity, size and shape, according to their application requirements.

A commercially available TiO_2 catalyst is Degussa P25, with crystal distribution 25% anatase and 75% rutile, particle diameter of 30 nm in 0.1 μm diameter aggregates and a surface area of 50 m^2/g (Hoffmann 1995; Zhao 2003). This catalyst has high photocatalytic activity, and is a standard for photoreactivity in various applications (Hoffmann 1995).

2.1. LIQUID-PHASE METHODS

The most convenient and widely utilized method of TiO_2 synthesis is liquid phase processing of various precursors. Advantages of this method are: possibility of stoichiometry controlling, preparation of composite materials, and production of homogeneous materials (Carp 2004). The most commonly used procedures are as follows:

- **A sol–gel method** is one the most exploited, used mainly for producing thin films and powders. Many authors have used different variants and modifications of this process to produce pure thin films or powders in large homogeneous concentration, and stoichiometry-controlled, with the ability to cover larger areas (Sivakumar 2003; Watson 2003; Campostrini 2003a, b, 2004a, b; Li, Y 2004; Peng 2005; Bu 2006; Xu 2006). Also, this method is widely for the preparation of multicomponent oxides in homogeneous

mixture with different metal ions (Al-Salim 2000; Wang, J 2004; DiPaola 2002; Celik 2006; Kitiyanan 2006; Shifu 2006). The most common precursors are $Ti(OEt)_4$ (Okudera 2003), $Ti(Oi\text{-}Pr)_4$ (Chen, YF 2003; Alapi 2006), $Ti(On\text{–}Bu)_4$ (Xu 2006; Arabatzis 2002; Yu 2005), or $TiCl_4$ (Kuznetsova 2005). Some authors have used the sol–gel method in combination with other methods, for example hydrothermal (Li, L 2005; Zhu 2006) in order to obtain new and efficient photocatalytic materials.

- **Thermal methods**. Aqueous (hydrothermal method, (Yin 2003; Lee 2006) or organic media (solvothermal methods, (Yin 2003) solutions of precursor are used at higher temperature (less than 250°C) to produce powders or thin films of the photocatalyst. For sovothermal methods there are usually used organic media as methanol, butanol, toluene, etc. (Carp 2004). As a source for hydrothermal synthesis the most often exploited precursors are $TiOSO_4$ (Inagaki 2001; Hirano 2004; Hidalgo 2005), $H_2TiO(C_2O_4)_2$, (Kolen'ko 2004), titanium butoxide etc. (Zhu 2005).
- **Liquid-phase deposition** method is a novel wet process for preparation of functional titanium-oxide thin films or binary-oxide thin films (Li 2005; Deki 1996; Hu 2006; Imai 2006; Song 2005), deposited directly on the substrate. Usually a starter solution is used, which is a water solution of $[(NH_4)_2TiF_6]$ and H_3BO_3 (Li 2005; Deki 1996), TiF_4 and $TiOSO_4$ with addition of urea (Imai 2006). Compared to other deposition techniques, such as CVD of dry processes, or sol–gel method of wet processes, the LPD method is a synthesis process with lower capital equipments (based on aqueous precursors), lower temperature (30–50°C), and flexibility in the choice of substrate (Li 2005).

Among many advantages of these methods, there are some weaknesses: precursors are expensive, the procedures are quite long, and purity of obtained film is not always a high level.

2.1.1. Gas Phase Methods

Photocatalysts syntheses from the gas phase are based on mainly chemical (Jones 2003; Choy 2003; Pozzo 2006) or physical techniques. One of the most exploited methods is spray pyrolysis deposition (SPD). The SPD technique is mainly used for preparation of mixed oxide films from metal-organic compounds and metal salts as precursors (Ahonen 2001; Veluchamy 2001). Also, there are several sophisticated techniques for preparation of thin films: sputtering by direct current (Mardare 2002; Treichel 2000) sputtering by radio frequency (Okimura 1996), and molecular beam epitaxy (Ong 2001; Herman 2001). TiO_2 films or powders from gas-phase precursors are synthesized in various morphologies: nanorods (Miao 2004), nanotubes (Wang 2002;

Gong 2001), nanoribbons etc. (Yuan 2002). Various morphologies of nano-sized TiO_2 are shown in Fig. 2 a–c.

The different methods for preparation of TiO_2 photocatalyst will result in different crystal structures, sizes and surface morphologies, recombination lifetimes, and different interfacial charge transfer. All these factors together will contribute to the TiO_2 photoreactivity (Hoffmann 1995).

Concerning the possibility of light-illuminated TiO_2 for elimination of hazardous chemical compounds from air, water, and soil, there are opportunites for development of an effective cleaning technology. For that aim, a number of disadvantages of the TiO_2 photocatalyts must be overcome: nonselective oxidation, low reactivity and efficiency, absorption only of UV light.

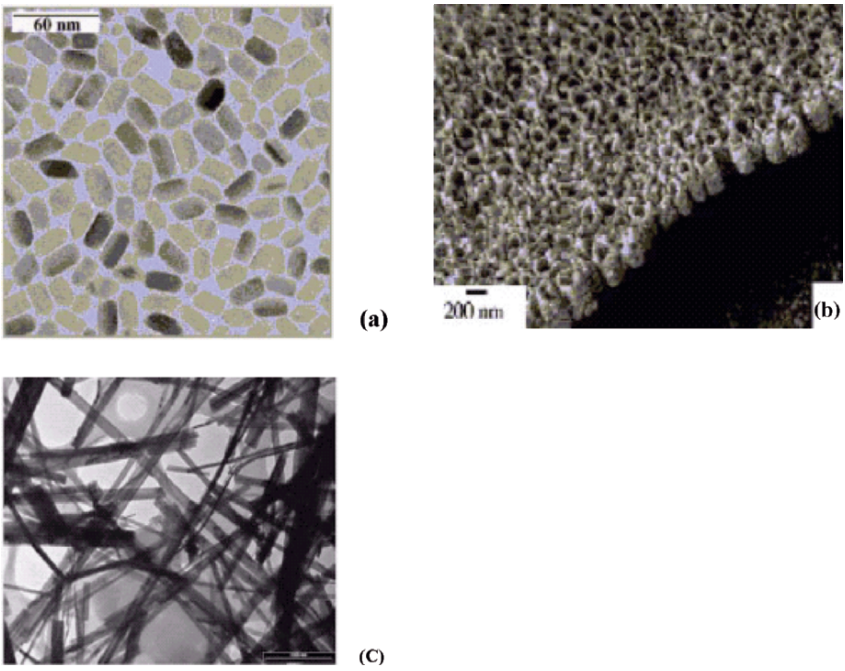

Figure 2. Morphology of nano-sized TiO_2: (a) nanorods (Miao 2004), (b) nanotubes (Gong 2002), and (c) nanoribbons (Yuan 2002).

3. Improving Photocatalytic Characteristics

In relation to the above information, a need has arisen for the development of a photocatalytic system for selective degradation of pollutants and utilization of solar radiation. In that way the effectiveness of the process of

photocatalytic degradation will be ensured. In order to address this problem, extensive research has been performed with main aims to:

1. Develop effective, selective, and highly reactive photocatalytic systems
2. Develop catalytic systems which can operate under visible light ("second-generation TiO_2 photocatalysts" (Anpo 2004))

The progress made in these areas in the last few years will be presented in the next in a other chapters.

3.1. IMPROVING EFFICIENCY OF TiO_2 PHOTOCATALYSTS

In order to improve effectiveness of the photocatalysts, doping of the catalysts with different transition metal ions have been made (Hoffmann 1995; Carp 2004). The effect of improved photocatalytic activity of the doped TiO_2 can be explained by the fast transfer of photo-formed electrons from the bulk TiO_2 to the loaded metal particles, in the way the process of recombination between generated electrons and holes is decreased and the increased efficiency of charge separation (Anpo 2004).

Various results regarding the photocatalytic activity have been obtained, in comparison with that of TiO_2. For example, Fe^{3+} doping of TiO_2 is one of the most investigated process (Wang, J 2004; Arana 2002; Pal 2001; Kwan 2003), and at the same time, one of the most efficient ways to improve the TiO_2 oxidation and reduction capability. According the Carp et al. (2004), doping of colloidal TiO_2 with Fe^{3+} has a controversial influence on the charge-carrier recombination. Some studies suggest that Fe^{3+} behaves as an electron/hole recombination center, while others indicate that doping TiO_2 with Fe^{3+} drastically increases the charge-carrier lifetime, which can be extended to minutes and even hours (in intrinsic TiO_2, the mean lifetime of an electron–hole pair is about 30 ns).

The presence of Fe^{3+} ions in TiO_2 catalysts could shift the adsorption edge of TiO_2 from 380 nm to higher wavelengths and a higher absorbance in the range 400–650 nm compared to bare titania both of which are dependent on the iron content (Carp 2004; Al-Salim 2000; Lee 2006; Wang 2006). Also, this kind of doping decreases the crystallization rate of TiO_2, leading to small particles and enhances the hydrophilic character of catalyst (Kang 2003). Ohno et al. (2006), have introduced a new method of doping Fe^{3+} ions on the surface of S- or N-doped TiO_2, and photocatalytic activity of such TiO_2 photocatalysts for oxidation of 2-propanol are markedly improved under a wide range of incident light wavelengths, including UV and visible light.

Application of Fe-doped TiO_2 photocatalysts with good results include: oxidation of oligocarboxylic acids (Arana 2002); oxidation of o-cresols (Pal

2001), oxidation of 2,4-dichlorophenoxyacetic acid (a herbicide) (Kwan 2003), and even degradation of polyethylene films (Xiong 2005).

In many cases doping of TiO_2 with metal ions have caused detrimental effect on the photoctalytic activity, as observed when TiO_2 is doped with Al^{3+}, Cr^{3+}, Ga^{3+}, and Ln^{3+} (valences lower than that of Ti^{4+}) and Nb^{5+}, Ta^{5+}, Sb^{5+} (valences higher than that of Ti^{4+}) (Carp 2004). Due to the fact that doping ions act as trapping sites, they can influence the lifetime of charge carriers. The inhibitory effect is ascribed to higher rate of the electron–hole recombination as a result of increasing the concentration of conduction electrons (Li, FB 2004). Also, Colmenares et al. (2006) have obtained detrimental effect of TiO_2 doped with Fe and Zr ions on photoxidation of 2-propanol.

Except for the metal ion doped TiO_2, many other methods of catalysts-efficiency enhancement have been studied. One of the most promising methods is metals coating on TiO_2 surface. Different techniques are used (sol–gel; Peng 2005; Moon 1998): photo- (Jin 2004) or chemical-induced deposition (Chen 1999), mechanical mixing (Vorontsov 1999), combination of sol–gel or hydrothermal treatment (Zhu 2006). The enhanced photocatalytic activity is expected due to the same rational as for metal ion doped TiO_2, but it is dependent on the nature of the adsorbed species on the catalyst surface, which should be degraded (Chen 1999; Hu 2003a). For example:

- Pt-loaded TiO_2 is active in the photodecomposition of methanol (Bowker 2003), ethanol (Takeuchi 2003; Vorontsov 2004), tert-butanol, acetone and methyl-butyl-ether (Preis 2004), acetaldehyde (Nakano 2004), EDTA, (Siemon 2002), and ozone (Cho 2004).
- Ag-loaded TiO_2 improves the photodegradation of phenol, (Liu 2004), 4-chlorophenol, (Moonsiri 2004), and some azodyes (Ozkan 2004).
- Au–TiO_2 promotes 4-chlorophenol decomposition (Orlov 2004).
- Pd–Cu/TiO_2, Cu/TiO_2, Pd–Cu–Pt/TiO_2) show a higher activity in the photodecomposition of 2, 4 dinitrophenol, trichloroethylene, and especially formaldehyde (up to five times) in aqueous solutions (Chen 1999).
- Pd, Pt, or Ag results in an increase in molar conversion as compared to bare TiO_2 in photooxidation of 2-propanol (Colmenares 2006).

Physical properties of the TiO_2 catalyst (e.g., crystalline structure, pore size, density of OH groups, surface acidity) could play an important role on photocatalytic efficiency. Modification of these properties could modify the photocatalysts activity (Hoffmann 1995; Anpo 2004; Serpone 1997).

Crystalline structure of the photocatalysts on one hand determines its surface area and the amount of molecules which could be adsorbed and on the other hand influences the amount and velocity of the recombination process between the photoformed electrons and holes. These two affects are opposite

(the catalysts with more crystalline structure will have larger surface area, and at the same time will enhance the process of recombination), thus a balance between surface area and crystalline structure must be found in order to obtain the highest photoactivity (Carp 2004).

Particle size has been found to influence significantly the photocatalysts efficiency (Hoffmann 1995; Zhao 2003; Carp 2004; Anpo 2004). The most attention has been paid to the quantum-sized TiO_2 and TiO_2 doped catalysts, with diameters between 1 and 10 nm, which have shown advantages over the nano-sized TiO_2-based catalysts. The explanation could be found in the following details:

- The characteristics of these small particles are between molecular and bulk semiconductors (Zhao 2003), or the charge carriers appear to behave quantum mechanically (Hoffmann 1995).
- In quantum-sized particles, the wave function of the charge carriers spread over the whole particle, thus there is no need for diffusion of the carriers towards catalyst surface in order to accomplish the reaction (Carp 2004).
- The band edges of these small particles have been shifted, which enhance the redox potential of photogenerated pairs of electrons and holes (Hoffmann 1995; Zhao 2003).
- The effectiveness of the oxidation reactions for surface limited reactions is improved as a result of the high-surface area to volume ratios (Zhao 2003; Anpo 2004). Consequently, photogenerated pairs of electrons and holes can easily diffuse to the surface of the catalysts and form the active sites for redox reactions.

One possibilitiy to develop a highly photoactive TiO_2-based catalytic system is to increase its surface area in a way the adsorption capacity and the selectivity of the catalysts could be influenced. Experimental investigations have been directed toward finding appropriate support for TiO_2 photocatalysts, in a way that is beneficial in that:

Supported TiO_2-based catalysts possess larger surface areas and configurations, which allow efficient UV radiation of all catalysts particles (increase light illuminated and reaction area) (Zhao 2003; Dijkstra 2001).

- Support enables the immobilization of catalysts particles, which is useful for practical reasons, especially in the large-scale continuous processes (Carp 2004).

In general, different materials have been used as a support of TiO_2 catalysts: glass (Lee 2001), silica (Aguado 2002; Hu 2003b; Kim 2006; Bertinchamps 2006), stainless steel (Zhu 2001; Yang 2005; Villacres 2003), Al_2O_3 fiber textile (Bertinchamps 2006; Villacres 2003; Ku 2001), quartz beads (Pozzo 2006; Benoit 2000), fabric (Daoud 2004; Park 2004), paper (Iguchi 2003), activated

carbon (Nozawa 2001; Zhang 2005; Shih 2006), zeolites (Anpo 2004), and borosilicate glass (Hidalgo 2005; Hernandez 2006). For that aim, using previously prepared TiO_2 powder various techniques of immobilization have been investigated. During the procedure of TiO_2 immobilization on some of these support materials, the catalysts active-surface area is decreased. This results in lower photocatalytic activity, as demonstrated in some studies (Carp 2004). The presence of other cations from the support material could enhance the recombination between photoformed electron and hole, which supports the process of deactivation of the catalysts.

Production of highly dispersed TiO_2-based catalysts into the framework of different adsorbent, such as zeolite (Yoneyama 2000; Durgakumari 2002; Reddy 2003), activated carbon (Arana 2003a, b), and silica (Vohra 2003; Tanaka 2002), has also been shown to increase the surface area, and activity of the catalysts (Carp 2004; Anpo, 2004). The idea is to use adsorbents with large-surface area and high-adsorption capacity, which will allow adsorption of the object compounds on the support surface and their diffusion on the interface between the photocatalyst active site and adsorptive inert site (Carp 2004). The experience with such systems (Yoneyama 2000; Aoi 2003), shows that the rate of reaction was enhanced, due to the increased concentration of adsorbed species on the surface. This process is effective even at low-level concentrations of the pollutants (either in water decontamination or indoor applications). Also, deactivation of the catalysts and formation of dangerous intermediates have been significantly decreased. These systems did not provide for the highest photocatalytic activity, and examples for successfully increasing effectiveness of the catalyst as a result of the adsorbent support are:

- In the photodegradation of methylene blue (Tsumura 2002) a high photoactivity and a long-photocatalytic life are observed when TiO_2 is coated with a layer of carbon.
- The higher efficiency in phenol photodegradation is obtained with activated carbon as a support during the synthesis. However, during the calcination step the carbon is removed. The benefit can be explained as a synergetic effect between surface acidity, carbon content, and structural improvement (Carp 2004).
- The presence of SiO_2 is beneficial in the removal of free cyanide (Aguado 2002) and photodegradation of phenol (Tanaka 2002), and dyes (Vohra 2003) in water as a result of the generation of new active sites, due to the interactions between titania and silica and improved mechanical strength, thermal stability, and surface area of the titania.

3.1.1. Second-generation TiO_2 Catalysts

Numerous attempts have been made to design TiO_2-based catalysts, which are able to adsorb not only UV light but also visible light irradiation. It has resulted in development of "second generation TiO_2 catalysts."

Doping of the TiO_2 with a number of metal ions have been investigated by many researchers in order to decrease the band gap or introduce intra-band gap states, which results in more powerful visible absorption. For that aim they have used metal ions such as Ca^{2+}, Sr^{2+} and Ba^{2+} (Al-Salim 2000; Wang 2004), Cr^{6+} (Wang 2004; Wilke 1999), Cr^{3+}, (Zhu 2006; Lee 2006), Co^{3+} (Wang, C 2004), Ni^{2+} (Lee 2006), V^{5+} (Lee 2006), and La^{3+}, Ce^{3+}, Er^{3+}, Pr^{3+}, Gd^{3+}, Nd^{3+}, and Sm^{3+} (Xu 2002). Introducing these metal ions into the TiO_2 lattice, except the shift of photoactivity towards the visible light areas, could alter the surface properties of the catalysts and consequently modifies the adsorption properties (Carp 2004). For example, doping of TiO_2 with La^{3+}, Ce^{3+}, Er^{3+}, Pr^{3+}, Gd^{3+}, Nd^{3+} or Sm^{3+} (Xu 2002) improves NO_2 adsorption. An enhancement of saturated adsorption capacity and adsorption equilibrium constants (compared to bare TiO_2) for 2-mercaptobenzothiazole (Li 2004; La^{3+} doped), a mixture of salicylic acid, t-cinnamic and p-chlorophenoxy acids (Eu^{3+}, Pr^{3+}, Yb^{3+} doped; Ranjit 2001a, b), XRG aqueous solution (Cr^{3+}-doped TiO_2; Zhu 2006) have been reported. Doping of TiO_2 with nitrogen (Belver 2006; Orlov 2006) also has been shown to increase photocatalytic activity of the catalysts under the sunlight-type excitation in the case of photocatalytic degradation of methylcyclohexene. Belver et al. (2006) has found that the samples contain substitutional and interstitial N-containing impurities and a significant number of oxygen vacancies and that the photocatalytic activity is correlated with the density of oxygen vacancies, which above or below decrease the steady state reaction rate.

Anpo (2004), Yamashita and Anpo (2001), and Yamashita et al. (2004) have applied a highly advanced metal-ion implementation method to modify the electronic properties of bulk TiO_2 photocatalysts, by bombarding them with high-energy metal ions, such as V, Cr, Mn, Fe, and Ni. They have discovered that implementation of these ions into TiO_2 lattice induce a large shift in the adsorption band toward the visible light region. On the other hand, implementation of Ar, Mg, or Ti ions exhibited no shift in the adsorption spectra, showing that the shift is caused by some interaction between the metal ions and TiO_2 catalyst (Anpo 2004). They have also found that the shift effectiveness is in following order: V > Cr > Mn > Fe > Ni ions. Figure 2 shows the UV/visible adsorption spectra of TiO_2 and Cr ion-implanted TiO_2 (Anpo 2004) as provides an example of how the implantation of Cr into TiO_2 influence the adsorption spectra of the photocatalyst, and the influence of the implanted Cr amount.

Another approach in the development of visible-light responsive catalysts is the adsorption of various photosensitizing dyes on the catalysts surface. Compounds such as bipyridine (Yang 2002) and phthalocyanine (Carp 2004) are used as a sensitizers. They absorb visible light, which excites them electronically, and makes them capable of donating an electron, improving charge separation between photoformed electrons and holes.

4. Conclusion

As can be seen, a significant number of publications on preparation methods of TiO_2-based catalysts have been published in the past several years. This demonstrates the possibility of such catalysts and their potential applications, especially in restoration of the contaminated environment (water purification and air cleaning). The published results are rather scattered and not always compatible, which make them difficult to compare. However, since many significant questions are not addressed in the literature, especially regarding the practical application of the photocatalysis on a large scale, investigations in this field should continue to develop a photocatalyst system that can selectively degrade pollutants, utilizes solar light irradiation, and is clean, safe, and abundant. Designing of such photocatalyst systems and understanding complex heterogeneous photochemistry in multiphase environment will ensure development of promising technology for wide environmental applications.

5. Experimental Studies

In this review we report on the preparation of nano-sized Ti/O/Si materials (thin film or powder) by laser-induced chemical vapor deposition technique. Laser photolysis experiments were carried out by irradiating a gaseous mixture of $Ti(OSiMe_3)_4$ (3 μL) and O_2 (5 kPa) at 80°C in a Pyrex reactor (140 ml in volume). The reactor was made of two orthogonally positioned tubes, 3.5 cm in diameter, one of which was 13 cm long and furnished with two Ge windows, and the other 10 cm long and furnished with two quartz windows. The reactor contained two ports, one fitted with a rubber septum and the other connecting with a vacuum line. The photolysis was performed by ArF laser (ELI 94 model), operating at 193 nm with a repetition frequency of 10 Hz and pulse energy from 10 to 70 mJ on the area of 1.0 cm^2 (unfocused irradiation). The progress of photolysis was monitored by FTIR spectroscopy (Nicolet Impact 400) using the diagnostic IR bands of Ti-O-Si (929/cm). The volatile products of photolysis were examined directly in the reactor by FTIR spectroscopy and gas chromatography (Shimadzu GC-14A, Porapak P and SE-30 columns, programmed 25–150℃).

The materials formed by the irradiation of the gaseous mixture were deposited on Ta, quartz and glass substrates accommodated in the reactor. Their properties were studied by both FTIR and UV spectroscopies (Shimadzu UV 1601 spectrometer) and electron microscopy techniques. The photocatalytic activity was studied after 1 h annealing at 450℃ by photooxidation of 2-chlorophenol and dichloroacetic acid. This work presents preliminary results. The main aim of the work is preparation of photocatalysts with high photo-activity, and have practical environmental applications.

5.1. RESULTS AND DISCUSSION

When gaseous mixture of $Ti(OSiMe_3)_4$ and O_2 were exposed on ArF laser radiation, it resulted in formation of volatile products and solid deposits. Depending on the fluence of the laser beam, two different photolysis mechanisms were observed.

Low fluence irradiation (up to 13 mJ/cm^2) results in photolytic oxidation of $Ti(OSiMe_3)_4$ and deposition of transparent films. The most abundant-volatile product is trimethylsilanol, along with traces of methane, methanol, carbon dioxide, and hexamethyldisiloxane. The conversion of the initial substances is low, thus prolonged irradiation is required to obtain significant amount of solids (16 min. irradiation with repetition frequency 10 Hz gives the 43% depletion of $Ti(OSiMe_3)_4$).

Under higher-fluence (>13 mJ/cm^2) irradiation, the first pulse induces an explosive reaction with complete depletion of the initial compound. The reaction is followed by bright blue fluorescence in a whole reaction cell, together with formation of white powder, covering the reactor walls. The volatile product from this reaction is mainly CO_2, which suggests the effective oxidation course of the explosive process. In both reaction routes there were no gaseous Ti-containing compounds observed. This reveals that titanium was completely utilized for production of solids.

5.1.1. Properties of the Solid Deposit

The solids deposited during the ArF-laser irradiating of gaseous mixtures of $Ti(OSiMe_3)_4$ and O_2 have shown photoactivity during the experiments of photooxidation of 2-chlorophenol and dichloroacetic acid. Their properties are dependent on the laser fluence. When these mixtures were irradiated with low fluence irradiation (<13 mJ/cm^2), the solids become completely transparent and highly adhesive. The solids deposited under higher irradiation fluence (>13 mJ/cm^2) are powders, white colored and deposited in thin layers all over the reactor. In order to determine the composition of the solids, as well as to

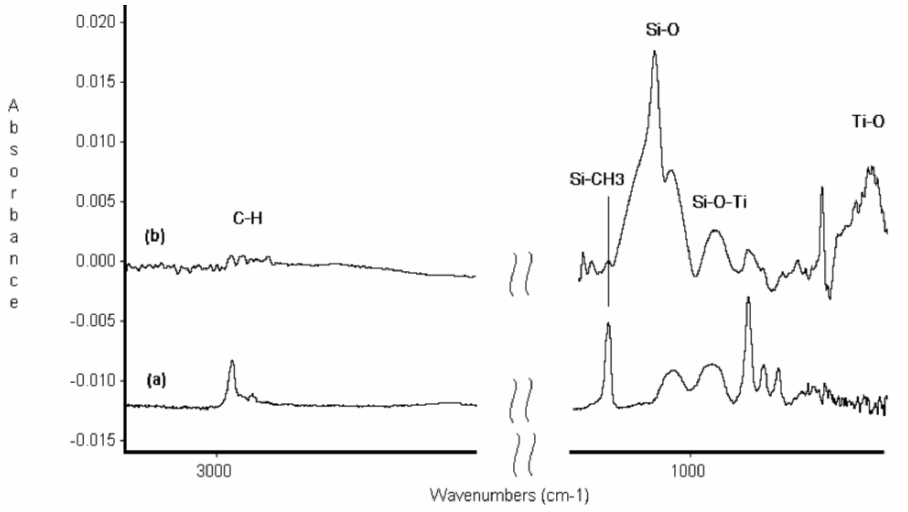

Figure 3. FTIR spectra of the solids deposited under (a) low-fluence irradiation (<13 mJ/cm2); (b) higher-fluence irradiation (>13 mJ/cm2).

compare the solids obtained under two different conditions, FTIR spectra were measured and depicted in Fig. 3.

The spectra in Fig. 3 reveal that both solids contain similar moieties, but their ratio in the solids depends on the conditions under which they were deposited. From these spectra the composition of the solids are presumed to contain C–H, Si–C, Si–O, Si–O–Ti and Ti–O vibrations. Their intensity differs according to the irradiation conditions:

1. Solid deposit prepared by low-fluence photolysis shows intense C–H stretching vibrations, in accordance with intense Si–CH_3. A typical feature of the deposit is low content of Si–O moieties, as demonstrated by weak absorption in the stretching region ~1,100/cm. Ti–O stretching vibration (~480/cm) is almost missing due to the low intensity of this vibration and thinness of the deposited film.
2. The deposit prepared by high-fluence photolysis contains a low amount of hydrogen, as demonstrated by low intensity of vibrations in the C–H stretching region. A very strong and complex absorption band in the Si–O (Si–O–Si) region at ~1,100/cm proves deep oxidation course of the photolysis. Broad bands at 960/cm and 480/cm are assignable to Si–O–Ti and Ti–O vibrations, respectively.

In Figs. 4 and 5, UV spectra of solid films (a) as deposited and (b) after 1h of annealing at 450℃ are depicted. For both as deposited solids under different laser fluence, the absorption maxima are centered at around 200 nm. Annealing induced a shift of absorption maxima:

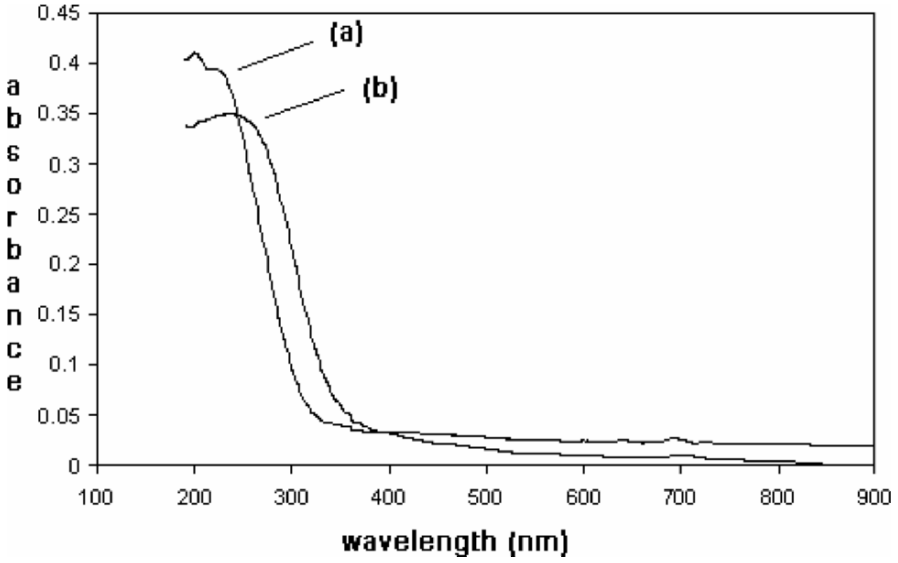

Figure 4. UV spectra of solids deposited under low-fluence irradiation (<13 mJ/cm^2): (a) as deposited, (b) after annealing at 450°C for 1 h.

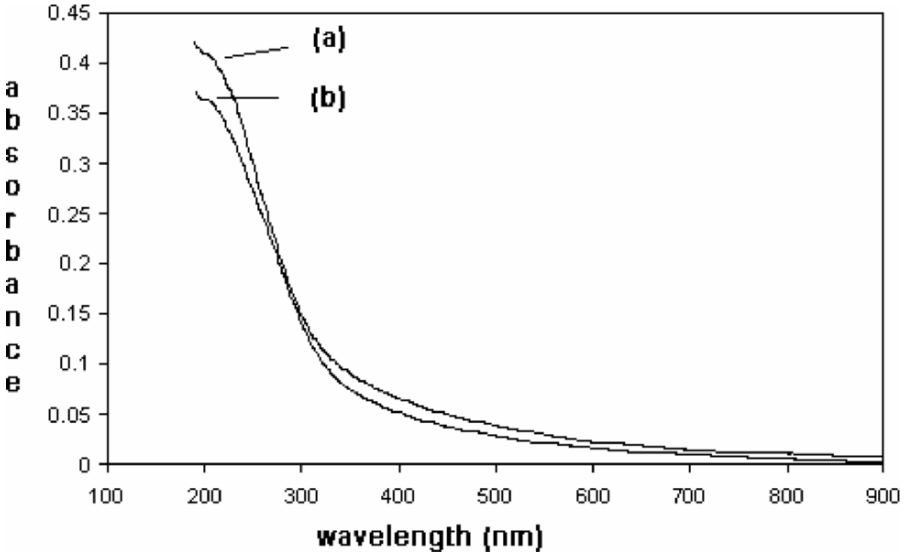

Figure 5. UV spectra of solids deposited under higher-fluence irradiation (>13 mJ/cm2): (a) as deposited, (b) after annealing at 450°C for 1 h.

1. Solid deposits prepared by low-fluence photolysis (Fig. 4), have shown a shift of the absorption maximum toward higher wavelengths (~260 nm). This could be explained by oxidation of the deposit and a structural change of amorphous material to anatase structure, which is known to be formed at this temperature. x-ray and electron-diffraction studies are continuing.
2. The deposit prepared by high-fluence photolysis (Fig. 5), has no significant shift of the absorption maxima after the annealing process. This feature can be explained by formation of a more stable rutile structure of the deposit in the explosive course of photolysis. This behavior is also a subject of further study.

6. Summary

This work presents results of the preliminary experiments, focused on preparation of novel TiO_2-based photocatalysts. The main aim of the work is tailoring the highly photoactive catalysts, which will avoid a disadvantages demonstrated in the literature. The composition of the materials was investigated by FTIR spectroscopy, and the conclusions are that they are Ti/O/Si based, and in some cases with C–H impurity, coming from Si–CH3 bonds. Prepared deposits have shown the photoactivity, as demonstrated by photooxidation of two model compounds. According the UV spectra of the produced solids, the assumption of structural changes in the process of annealing was made. Suggested explanation needs further investigation.

The novel method of ArF pulsed-laser irradiation of the above-mentioned precursor could be quite interesting, because of the possibility to cover a large surface with a thin and, more or less, uniform layer of the photoactive material with only one pulse of UV irradiation.

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EVALUATION OF MEAN DIAMETER VALUES USING SCHERRER EQUATION APPLIED TO ELECTRON DIFFRACTION IMAGES

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Abstract: The aim of this work is to determine mean diameter of polycrystalline samples using the Scherrer equation. These calculations represent a direct connection between the mean diameter, crystal structure and morphology by applying the Bragg angle and shape factor. Two samples are investigated, first a nano-sized particles of MgO and second a CVD (chemical vacuum deposition) obtained polycrystalline aluminum film. First, the shape factor and mean diameter are evaluated using direct measurement of particle morphology from BF–TEM (bright field – transmission electron microscopy) images. The value of mean diameter is determined by assuming a lognormal distribution. Second, the mean diameter values are evaluated using the Scherrer equation applied to SAED (selected area electron diffraction) images. We obtained for MgO nano-sized a mean diameter about 48 nm from the direct measurement, and 70 nm respectively using the Scherrer equation, and for Al film 77 nm and 70 nm, respectively.

Keywords: CVD, BF–TEM, SAED, Scherrer, polycrystalline, nano-sized, lognormal

1. Introduction

Bragg's law states the condition for a sharp-diffraction peak from an infinite crystal with a perfect 3D order. Typically the diffraction peak has a finite width which is associated with imperfection in some of the Bragg parameters. These imperfections can be associated with a beam divergence, a somewhat polychromatic source, or imperfections in the 3D order of the crystals. The latter can be a basis for quantitative measurement of the deviation from the Bragg

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requirement for perfect 3D order which is infinite in all spatial directions. Deviations from the latter requirement have been explained in terms of 3 features: finite crystallite size (Scherrer equation) (Cullity 1978; Fang 2000; Klug 1954), distortions of the first kind, which are random motions of atoms in a crystalline lattice (Debye thermal broadening) or other local randomization of lattice sites which do not disturb the 3D repetition or crystalline motif, and distortions of the second kind, which involve disruption in the long-range order of the crystal, i.e., at long distances the lattice does not repeat perfectly. These usually lead to preferential broadening of high order peaks. The Scherrer equation can be written as:

$$\overline{D} = \frac{57.3 \cdot k \cdot \lambda}{\beta \cos\theta} \tag{1}$$

where $\overline{D}$ = mean diameter, k = shape factor, λ = wavelength, β = the full-width at half maximum, and θ = Bragg angle for studied ring. The factor 57.3 is used for conversion of β from degrees to radians.

The Scherrer equation, (1) above, predicts crystallite thickness if crystals are smaller than 1,000 Å. Since small angular differences are associated with large spatial distances (inverse space), broadening of a diffraction peak is expected to reflect some large-scale features in the crystal. The simplest way to obtain the Scherrer equation is to take the derivative of Bragg's law holding the wavelength constant and allowing the diffraction angle and the Bragg spacing to vary, $2d\sin\theta = \lambda$. Take derivative in d and θ yields $2\Delta d \cos\theta\Delta\theta = \lambda$, since $\Delta\theta$ can be positive or negative, the absolute value must be taken and it reflects the half-width of the peak (really half-width at half-height) so $2\Delta\theta$ is the peak full-width at half-height, β . Δd reflects the crystallite thickness,

$$Thickness = t = \Delta d = \frac{\lambda}{\beta \cos\theta_{\beta}} \tag{2}$$

If a Gaussian function (rather than a triangle function) is used to describe the peak a prefactor of 0.9 occurs so the Scherrer equation is given as: $t = 0.9\lambda/\beta\cos\theta_{\beta}$.

The shape factor provides information about the "roundness" of the particle. For a spherical particle the shape factor is 1, for all other particles it is smaller than 1. The formula for this calculation is:

$$shape\ factor = 4 \cdot \pi \cdot \frac{area}{perimeter^2} \tag{3}$$

The mean diameter represents the arithmetic mean of all diameters of a particle (for α = 15°, 30°, 45°, 180°). The value of mean diameter is estimated using the assumption that the mean diameter follows a lognormal distribution (Batlle and Labarta 2002; NIST; Morjan 2002; Dumitrache 2003). The experimental data are fitted using lognormal function, given by:

$$y = Ae^{-\frac{\ln^2(x/xc)}{2w^2}} \tag{4}$$

where A is an arbitrary constant related to particle number, xc represents the distribution maximum, and w – the dispersion of particle diameters. In Figs. 1 and 2 we can observe a good correlation between experimental data and theoretical curve (4).

2. Materials and Methods

The samples are prepared using chemical vaporization. MgO is obtained by burning Mg ribbon in air (Spence 1988). A special copper grid with amorphous carbon thin film is used to capture the smoke. Aluminum film is obtained using CVD. The substrate for the film is a formvar-coated copper grid mounted on a glass slide. The aluminum-prepared grid can be washed using chloroform and ethylic alcohol to remove the formvar substrate.

BF–TEM images (Figs. 1 and 2) are obtained by a Philips CM120 electron microscope with 100 kV acceleration voltages. SAED images (Fig. 5a, b) are taken using 420 mm camera length.

3. Results

Figure 1 presents the BF–TEM images (magnification × 29000) of the Al and MgO samples.

Figure 2 presents the same sample images but processed to increase the contrast of the particles. The processed images are obtained after applying a pseudo filter on the original image. This filter emphasizes the transitions between the bright and dark image points. The transitions from bright to dark are valued positive and those from dark to bright – negative. The image will be normalized in such a way that the zero level becomes a gray value 128. This means, that a gray shade area within the limits –127 and +128 is available. The filter produces the impression of a one-sided lighting effect and a seemingly topographic contrast (pseudo 3D). The employed 3 × 3 matrix are the following:

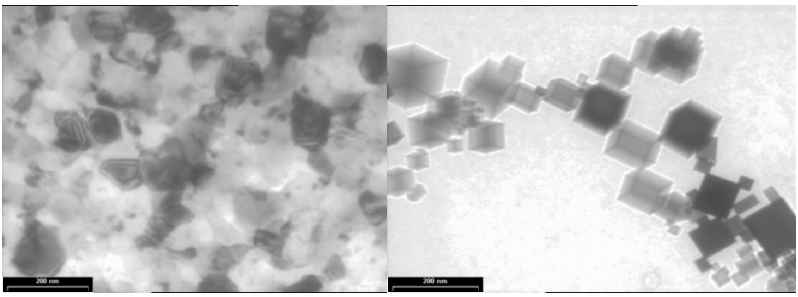

Figure 1. BF-TEM images of aluminum film and MgO nano-sized crystal.

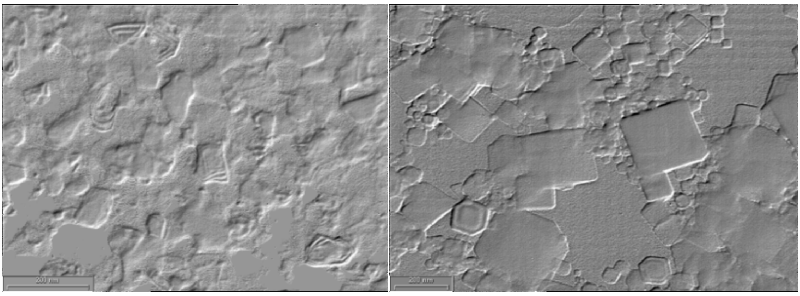

Figure 2. Processed BF-TEM images of aluminum film and MgO nano-sized crystal using a shadow filter.

$$\begin{matrix} 0 & -1 & 0 \\ -1 & 2 & 0 \\ 0 & 0 & 0 \end{matrix}$$

Figure 3 demonstrates the experimental data for the mean diameter statistics. The experimental curves are fitted with lognormal function.

Figure 4 represents SAED images used to evaluate the mean diameter from the Scherrer equation. Figure 5 demonstrates the drawing profiles of radial axis from the SAED images. Initial data are processed in order to remove the background. Results are fitted using 3-multipeaks Gaussian curves.

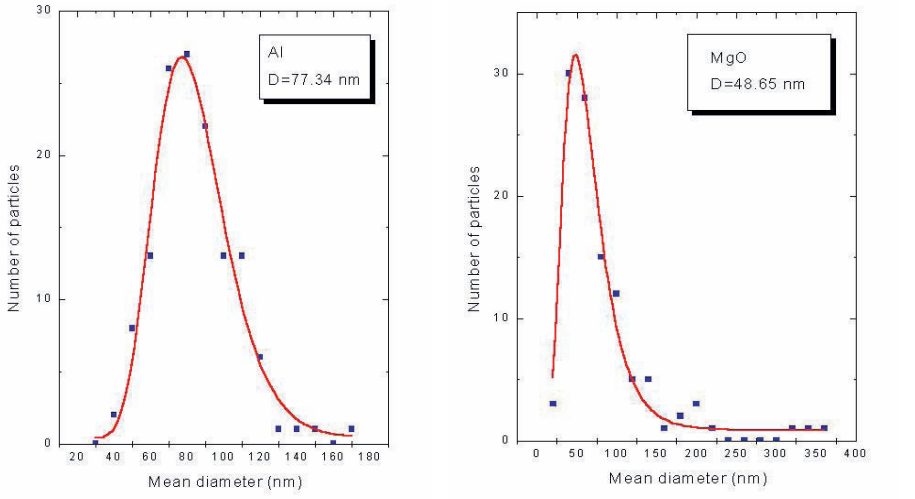

Figure 3. Mean diameter distribution of aluminum film and MgO nano-sized crystal fitted with lognormal function.

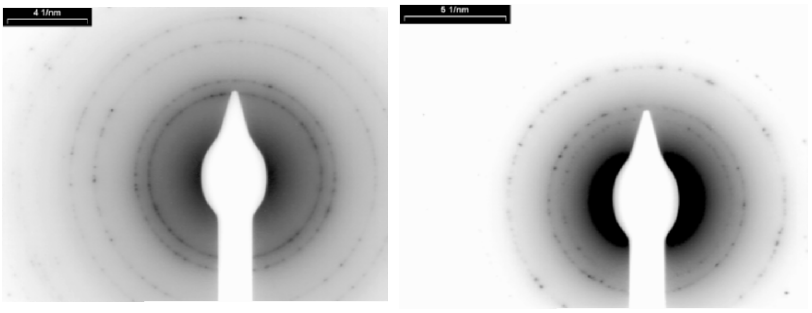

Figure 4. SAED image used for evaluation of the mean diameter. First interior ring in both images represents (111) the reflection used in the Scherrer equation to evaluate the mean diameter.

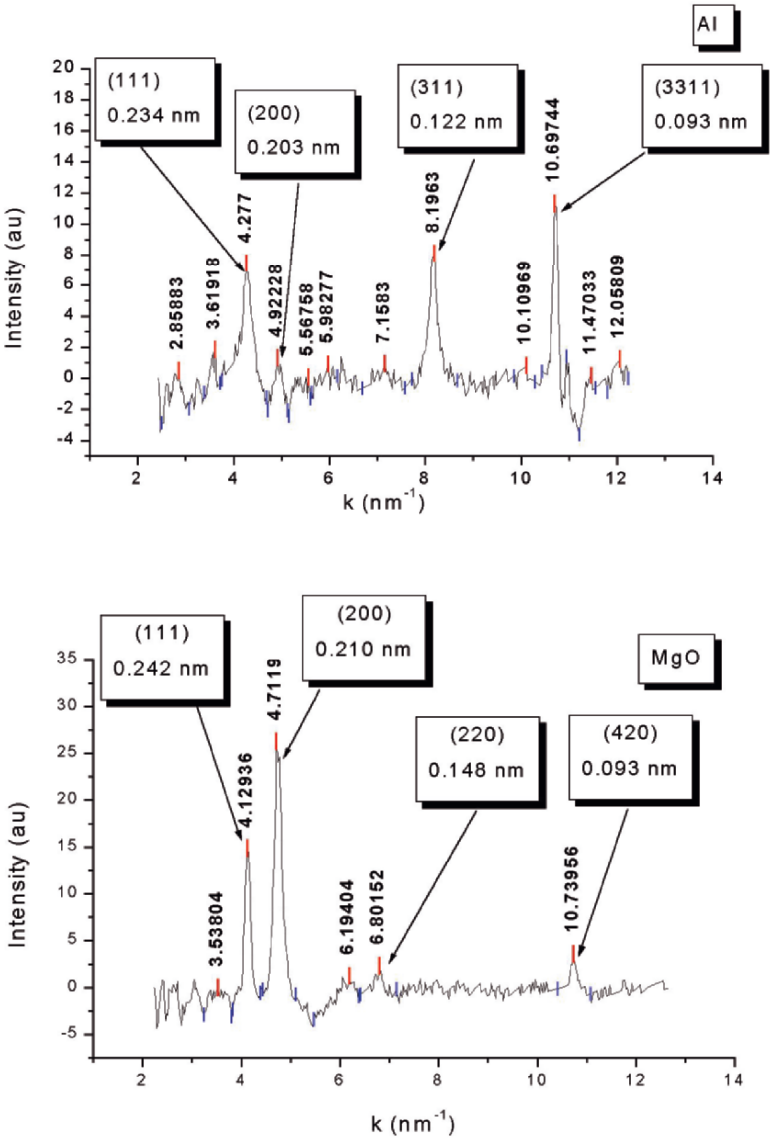

Figure 5. Profiles of diffraction patterns.

Table 1 summarizes mean diameter results obtained by direct measurement or the Scherrer equation.

TABLE 1. Mean diameter comparison.

Samples	Direct nm	Scherrer nm
Al	77.34	70.97
MgO	48.45	69.98

The value determined for MgO using the Scherrer equation is greater than the value determined using direct measurements. This difference can be explained by Debye thermal broadening, which appears in a case of thermal vibration of a lattice site in crystalline structure.

4. Conclusions

This paper shows that the Scherrer equation can be used to estimate the mean diameter in nano-sized particles. In some particular cases, e.g., MgO, this relation must be completed using additional theories which consider the lattice site of crystalline structure. Otherwise, this equation can be used for first evaluation of the mean diameter distribution.

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OCCUPATIONAL RISK ASSESSMENT AND MANAGEMENT: FOCUS ON NANOMATERIALS

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Abstract: A discussion of molecular mechanisms of occupational and industry-related diseases (DNA diagnostics methods) and nanotechnology applications in occupational health are presented.

Keywords: occupational and environmental chemical exposure, risk assessment, reproduction, bronchopulmonary system, DNA diagnostics, occupational diseases, gene polymorphism

1. Introduction

Risk assessment represents fundamental and applied investigations devoted to identifying unfavorable factors, their "dose-effect" relationships, a scientific basis to establish potential health disturbances, and development of models for determining probability of work-related health disturbances. The etiological contribution of work conditions leading to prenatal fetal-health disorders has been studied at our institute (Table 1). Our researches have shown that mothers who lived in industrial-polluted regions gave birth to children with significantly lower Apgar scores, as well as a higher incidence of cerebral circulation disturbances consistent with hypoxia, and prenatal infections (Table 2).

Children in the first year of life and living in industrial-polluted region show a higher incidence of hypertension-hydrocephalic syndrome (HHS) and iron-deficient anemia. Many of these children were low in statural-weight values and

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TABLE 1. Qualitative occupational risk assessment in mothers and newborns living in urban areas.

Mother's working condition before pregnancy (Guide R2.2.2006-05)	Mother (according to indices of pregnancy course)		New-born	
	Relative risk*	Etiological fraction %	Relative risk*	Etiological fraction %
Harmful 3(1)	1.5	33.3	1.7	41.2
Harmful 3(2)	1.7	41.2	2.7	63.0
Harmful 3(3)	2.0	50.0	3	73.7

* $P < 0.05$

TABLE 2. Morbidity rate of newborns and association with living site (per 100 newborns).

Indices	West p±m	Southeast p±m	Odds ratio OR	Conf. int. 95% CI
Low Apgar score (6 pts and lower)	5,3 ± 1,2	15,6 ± 2,6*	3,35	2,01 ÷ 5,72
Chronic pre-natal hypoxia	12,4 ± 1,8	20,3 ± 2,9*	1,81	1,08 ÷ 3,02
Hypotrophy	8,3 ± 1,5	11,5 ± 2,3	–	–
Cerebral circulation disturbances due to hypoxia	9,0 ± 1,5	15,1 ± 2,6*	1,56	1,31 ÷ 2,99
Prenatal infections	7,7 ± 1,5	14,1 ± 2,5*	1,95	1,06 ÷ 3,61

* $P < 0.05$

had longer or more complicated respiratory catarrh than children from clean regions.

Fluorine levels were twofold higher in the blood of newborn children in industrial areas compared to the control group (Table 3). Fluorine exposure, reduced barrier functions in the placenta. Risk to the fetus and newborn increased with increasing doses of fluorine. Twenty-five percent of the circulating fluorine-ions passed through the placenta from the mother's blood to the funic blood of the fetus. After birth, the newborn is further exposed to fluorine and related compounds from the mother's milk during breast-feeding. Research has shown that children receive up to 50% of the fluorine circulating in mother's blood. The incidence of abnormalities was higher in female newborns than in males.

Evidence from these investigations indicates that the effects on the reproductive system occur not only at the molecular level but at the cellular, organ, and system levels. Biomarkers associated with toxicity include changes in

TABLE 3. Concentration of Soluble Fluorides in Placenta and New-born's blood.

Placenta, mg/kg			Blood, mg/l		
Main group	Controls	Statistic significance	Main group	Controls	Statistic significance
1,76 + 0,07	0,78 + 0,04	P < 0,001 OR = 2,3	0,16 + 0,001	0,08 + 0,001	P < 0,001 OR = 2,0

albumin levels, hormone concentrations, as well as damage to cell membranes and the endothelium. Studies at the organ level involved investigations into the function of the liver, kidneys, gonads, and placenta; the latter being formed and which functions only during pregnancy. Understanding the pathogenic mechanisms, including neurophysiological and psychometric contributions in the early stages of fetal development will promote improvement of the mother and child's health and safety. Occupational health-risk assessment in reproductive health is closely related to advances in the nanotechnology industry. Prophylaxis, medical treatment, and rehabilitation are based not only on regulations, but also on the application of new preventive technologies and drugs involving nanomaterials.

Advances in molecular biology and their application in occupational health are based upon development of novel biotechnology, genomic, proteomic, and nanotechnology developments. This, in turn, opens possibilities for new approaches to risk assessment for occupational and work-related diseases, and will dictate the necessity of defining standard biotest systems at the molecular, cellular, tissue, and organ levels. Currently, more than 30 technical, scientific, and medical organizations in Russia are conducting projects dealing with nanobiotechnology and nanomedicine. Some topics under study are as follows:

- Systems medicine addresses delivery (directional transport), passive directional transport (lightened penetration of natural barriers), and specific delivery ("recognition" of pathological materials) are being studied. In the near term, practical applications can be reached in use of phospholipids agents, liposomes, and fullerenes as containers for drug delivery, including vaccines. Long-term projects are directed to specific delivery systems, on the basis of antibodies or aptamers, which are able to connect selectively with pathological changes in cells.
- Studies of nanoparticles as medicines are being examined. Applications of various modified fullerenes' for treatment of influenza, neoplasms, tuberculosis are being explored, as well as the application of nanoparticles to help deliver vaccines.

- Medical nanorobots facilitate the correction of molecular and cellular processes in organisms. For example, glucose level and insulin production can be monitored in diabetes patients with nanorobots. Creation of artificial blood cells by molecular simulation is possible.
- Self-productive genomes. Minimal artificial biosystems for self-replication using existing technological-based oligonucleotide constructions synthesis are being developed.
- Biocompatible nanomaterials. Special properties of nanomaterials can be used for growing artificial organs and materials. Scientific-practical results in the field of nanomaterials use for recovery of enamel properties, technology of surfaces processing by method of nanospattering with the object of giving them antibacterial properties, creation of new volumetric nanostructural material implants are studied.

In our opinion the introduction of microtechnology in occupational health will be extremely useful in the field of biomonitoring for chemical exposure. The sensitivity of these systems will allow us to revise standards for many occupational and environmental pollutants. Results of conducted genomic and proteomic research in the RAMS Institute of Occupational Health has revealed important genes regulating biotransformation and has been used to study pathology of allergic dermatosis and lead intoxication. Nanotechnologies with application to DNA-test-systems of gene polymorphisms for glutathione-transferase M1, alpha-1 proteinases inhibitor, NO-synthase, delta- amino-levulinic acid, cytokines, etc. are used for these purposes. Test-system data are based on the estimation of nucleotide replacement (point mutations, different disorders of nucleotide chain or deletions) and identification of protein substances with amino acid substitutes. Recent studies established the role of particular phenotyopes and genotypes on individual susceptibility, as well as resistance to hazardous materials (Beckman and Cedorgen, 1985; Izmerov et al. 2002; Kuzmina, 2002, Spitxyn et al. 1992, 2000).

One of the most promising trends in molecular medicine is proteomics. Understanding structural and functional properties of proteins, advanced in part by development of new proteolytic processes is allowing a better understanding of the biological functions of proteins, as well as their disorders in disease. The proteinases inhibitors are regulated by several genes, and, depending upon their level of synthesis influence leukocytic elastase, collagenase, and cathepsins. Information gained in recent years has made it possible to form the current concepts of the physiological and pathogenetic role of proteolysis, and to determine the importance of evaluating the activity of proteinases and their inhibitors for understanding inflammatory diseases. For example, we now understand the role of proteinase alpha-1 inhibitor deficiency in early onset of obstructive bronchitis and emphysema, as well as the important role of the

proteinases-inhibitor systems in development of occupational diseases of the respiratory organs (Barnes 1999; Faber et al. 1990; Kuzmina 2001; Yarovaya et al. 1999). However, despite numerous studies of the "proteolysis-antiproteolysis" system, the findings are still somewhat ambiguous. Epidemiological data indicate that 90% of all the ZZ genetic variants are not identified in identified in routine examination of proteinase alpha-1 inhibitor in blood (Hutchinson 1990). In this case alternate methods of genotyping of the alpha-1 proteinase inhibitor are available including, isoelectrofocussing and determination of the proteinase alpha-1 inhibitor gene mutations by means of the polymerase-chain reaction to be followed by restrictase cleavage (Frants et al. 1978; Lucotte and Sesboue, 1999).

In the presence of the Z or S mutation of the alpha-1-antitrypsin gene, deficiency of protein alpha-1-antitrypsin is diagnosed, which is a predisposing factor for the development of respiratory diseases (bronchitis, emphysema, pneumoconiosis, bronchial asthma). Studies of the "proteolysis-antiproteolysis" system were carried out in 111 patients suffering from occupational respiratory diseases: 48 patients with dust bronchitis, 24 with pneumoconiosis – silicosis, 22 with toxic-dust bronchitis and 17 with diffused toxic-dust pneumosclerosis, and in 30 with non-occupational bronchopulmonary pathology (NOGBPD). The findings were compared with a control group consisting of 40 apparently healthy people having had neither occupational exposure to industrial factors, nor chronic or acute respiratory diseases. The comparison group was composed of 30 patients with various non-occupational bronchopulmonary diseases. During the routine clinical examination of the patients, special attention was paid to their respiratory organs. All the subjects underwent X-ray studies of the chest organs (roentgenoscopy, roentgenography in the direct and oblique projections, magnified roentgenograms, tomograms), external-respiration function (lung-vital capacity, FRC1, FRC1/LVC), and analysis of the peripheral blood and immunological indices. In the group 5% of the patients with occupational-pulmonary diseases from industrial aerosol exposure had the gene variant compared to 10% with nonoccupational bronchopulmonary diseases. A lower incidence of the gene variants in patients with occupational pathology may be explained by elimination of the people with a similar genetic defect in the beginning of occupational activity due to development of respiratory diseases with severe clinical course.

Comparing the findings of genetic polymorphism of α1-PI with clinical manifestations showed that the presence of the homozygous-deficient variant (ZZ) of the α1-PI gene is characterized by formation of rapidly progressing, chronic occupational bronchitis even in individuals with comparatively short lengths of service (5–7 years). Comparing the results of actual levels of α1-PI with genotype revealed a discrepancy between genetic polymorphism and the content of α1-proteinase inhibitor in serum. Thus, 57% of the patients with

hyposecretory genotypes of MZ had a normal level of the inhibitor in serum. Apparently, this discrepancy was connected with the fact that heterozygous carriers simultaneously synthesize both the normal and defective proteins.

In the systemic responses to exposure to industrial factors and in detection of the biomarkers of individual susceptibility, it is important to study polymorphism of genes which control xenobiotics detoxification. The functionally defective allelic variants of these genes following exposure to hazardous chemicals may cause development of pathological processes in the body. Gene variants of cytochrome P-450 are interesting to study including glutathione S-transferase (GST). GST polymorphism may promote development of occupational diseases of the bronchopulmonary system. Glutathione-mediated detoxification plays a key role in providing cellular resistance to lipid peroxidation, free radicals, alkylation of proteins, and preventing DNA breakdown. GST synthesis is controlled by genes in different chromosomes. The molecular structure of these genes is well studied. GSTM1 exists in three allelic variants, two of which, GSTM1 and GSTM1B, encode the proteins, that differ in their enzymatic activity while GSTM10, due to a prolonged deletion, produces neither RNA nor protein at all (Seidegard et al. 1998).

The PCR-analysis method was used to study the prevalence of homozygous carriers of the GSTM1 deletion. Existence of homozygotes of the normal allele, genotype GSTM1 +/+, was determined by its presence on electrophoregrams of the amplification fragment sized 213 bp. Lack of (0/0) was indicative of the homozygous variant. Comparing the results of distribution of the wild-type and variant homozygotes in groups of patients with occupational-respiratory diseases (pneumoconiosis and occupational chronic bronchitis revealed, no statistically significant differences were observed when compared to the control group. The prevalence of the zero variant of GSTM1 in the patients with occupational pathology (OGBPP group) is considerably higher (Table 4) than that in the control sample, ($\chi^2 = 4.41$; $p < 0.05$).

In comparing the sample of the patients with NOGBPP with the control group, attention is drawn to the lack of differences between them in the prevalence of genotypes GSTM1 0/0 ($\chi^2 = 1.89$; $p < 0.1$). The proportion of the deletion obtained in the control (0.468) was comparable with the literature concerning the Russian populations, 42%, (Baranov et al. 1999) as well as corresponding to the distribution of this mutation in European populations, from 39 to 62% (Cotton et al. 2000). Hence, the findings favor greater susceptibility of the carriers of the GSTM1 0/0 to the risk of developing bronchopulmonary diseases induced by exposure to industrial aerosols (in particular, quartz-containing dust).

TABLE 4. Distribution of prevalence of genotypes of the GSTM1 gene in patients with occupational-respiratory diseases and in the control group.

Groups (n)	Prevalence of genotypes				Incidence of GSTM1 0/0
	GSTM1 +/+		GSTM1 0/0		
	abs.	%	abs.	%	
OGBPP (77 people)	30	39.0	47	61.0	0.6104*
NOGBPP (29 people)	11	37.9	18	62.1	0.6207
Control group (297 people)	158	53.2	139	46.8	0.4680

*$p < 0.05$.

The glutathione system is an important antioxidant system preventing formation and accumulation in the body of active oxygen forms. We have attempted to assess the condition in patients, depending upon the GSTM1 genotype (Table 5). There is a clear-cut relation between a more severe course of bronchopulmonary pathology in patients (hypoxemia and respiratory insufficiency of 2° and 3°) and the zero allele of the GSTM1 gene. The percentage of the subjects in the group of carriers of the GSTM1 null genotype was significantly higher ($\chi^2 = 7.0$, $p < 0.01$), compared to the group of patients

TABLE 5. Characteristics of the patients depending on the GSTM1 genotype.

Diagnosis	Genotype GSTM1 +/+ (n = 42)		Genotype GSTM1 0/0 (n = 64)	
	abs.	%	abs.	%
Hypoxemia 2, 3 degree	12*	28.6	35*	54.7
Respiratory insufficiency 2, 3 degree	24	57.1	46	71.9
Tuberculosis	2	4.8	10	15.6
Developmental defects of the bronchopulmonary system	7	16.7	16	25.0
Diseases of the upper respiratory pathways	23	54.8	25	39.1
Pulmonary emphysema	31	73.8	53	82.8

*$p < 0.01$

with the genotype GSTM1 +/+. Attention is drawn to the fact that the group with the zero allele GSTM1 gene has a higher share of the subjects with a history of bronchopulmonary-system defects and suffer from lung tuberculosis. Also, those individuals that were more susceptible to diseases of the upper respiratory pathways were those with the normal allele GSTM1 gene. This may be a protective mechanism in people with the genotype GSTM1.

An association has also been found between the incidence of chromosomal aberrations, mutagenic activity, and the presence of the GSTM1 0/0 genotype (Sram 1998). In such people, if they smoke, the mutagenic and carcinogenic risks are especially pronounced.

In conclusion, the advantages achieved using nanotechnology in molecular genetics open new horizons for studying occupational medicine. Nanotechnological sensors (biochips), which are able to reveal DNA molecules or amino acids and their substratums, will allow great sensitivity and specificity in studies of occupational diseases. The application of bionanotechnology methods in occupational health will contribute to improvement of methodical approaches and help decrease economic expenses in the workplace.

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APPROACHES IN ENVIRONMENTAL ECOTOXICOLOGY

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Abstract: Ecotoxicology represents an outgrowth of the link between toxicology, ecology, and chemistry. In this review, different methodical approaches commonly used in the ecotoxicological studies are identified, and their application to aquatic and soil systems critically evaluated. An obvious priority is a need for process-based testing rather than single-organism tests. Novel developments in the detection of ecotoxicological effects based in part on nanotechnological principles are discussed.

Keywords: ecotoxicology, methodical approaches, single organisms tests, process based tests, links to nanotechnology

1. Introduction

The science of ecotoxicology represents an outgrowth of the link between toxicology, ecology, and chemistry. The term itself was coined by TRUHAUT in 1969 when the scope of investigation into the harmful effects of chemical substances on humans and other organisms was expanded to include entire ecosystems (Butler 1984). Ecotoxicology is devoted to investigating possible effects of different "foreign chemical substances," i.e., xenobiotics, on single organisms, communities, and/or ecosystems. In this sense, ecotoxicology differs from ecological chemistry which deals with the fate of chemicals in ecosystems, including their possible abiotic impacts (Korte 1987). Also, in this context, xenobiotics, including pollutants, represent chemical substances which are released into the environment primarily, but not exclusively, as a result of

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human activities, and which have adverse or damaging effects on living organisms and their ecologically important functions. Thus, the appearance of chemicals in an ecosystem represents a precondition for dealing with ecotoxicology on either a small or large scale.

Another realm of toxicology, specifically nanotoxicology, has appeared recently. Nanotoxicology is the study of toxicity of *nanomaterials*. Because of their small size and large-surface area, these materials have unique properties compared with their larger counterparts. The nanomaterials, even when they are made of inert elements like gold, become very active at the *nanometer* range. This may pose a threat to the environment and to human beings. Currently, little

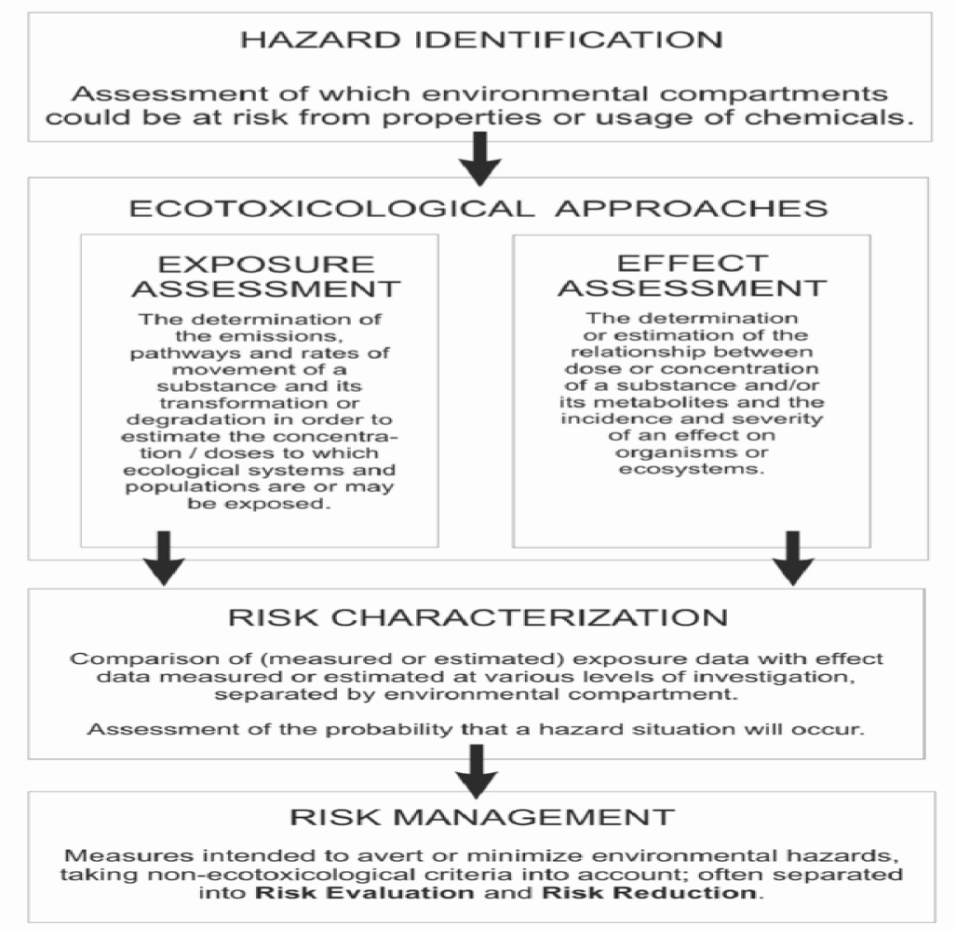

Figure 1. Basic steps in the management of a chemical hazard. (Modified from EPA 1992.)

is known about nanotoxicity due to lack of knowledge about their properties, and appropriate testing principles (http//en.wikipedia.org/wiki/Nanotoxicity).

1.1. BASIC APPROACHES IN ECOTOXICOLOGY

Within hazard management, ecotoxicological issues fall into categories: exposure and effect assessments. The individual steps starting from the hazard identification and up to the risk management are schematically shown in Fig. 1.

Ecotoxicological effects may appear and be observed in three environmental media, i.e., air, water, and soil, and in structures and functions of biota that inhabit these media. Many tiered procedures and tests have been developed in order to estimate ecotoxicological effects at different levels. They include: (a) laboratory tests under artificial conditions including cellular and molecular systems; (b) semi-field tests using, e.g., intact soil cores or water columns and; (c) field tests that include monitoring of diverse natural populations, and/or soil and water sampling. Basic characteristics of individual approaches in ecotoxicology are shown in Fig. 2:

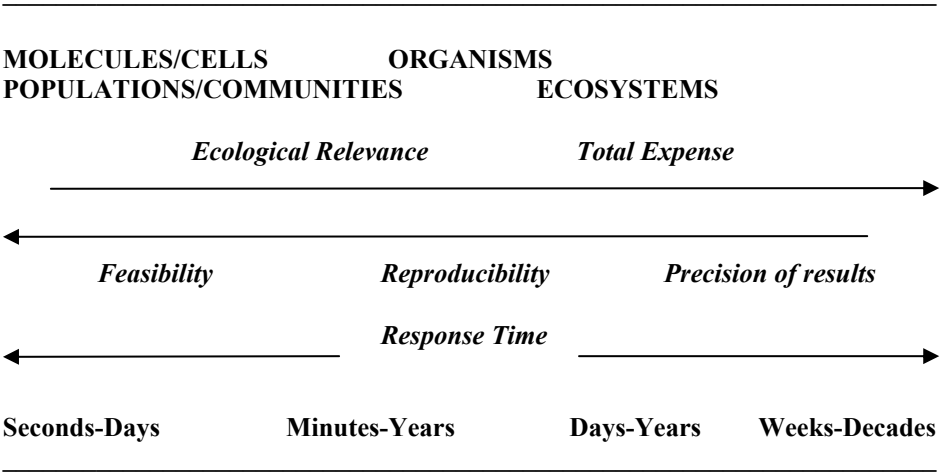

Figure 2. Relationship between biological levels, ecosystem relevance, methodological aspects, and response time in ecotoxicological studies.

Historically, single-species or multispecies tests for risk assessment have been used in monitoring and extrapolation to field conditions and ecosystems. Either a stepwise procedure or direct extrapolation (Fig. 3) has been applied in ecotoxicological assessment of aquatic environments.

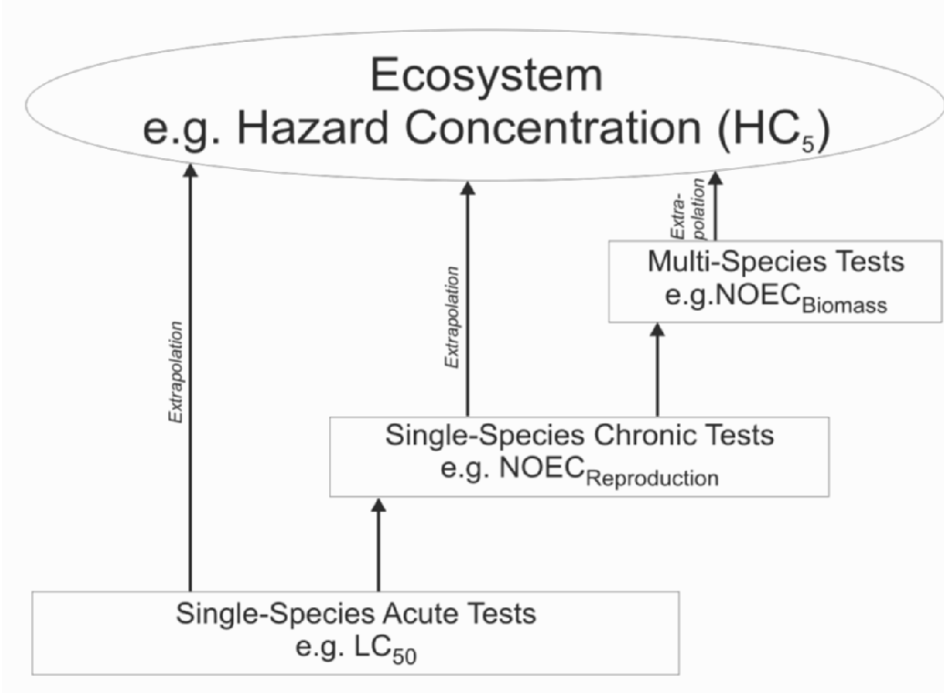

Figure 3. Extrapolation steps in the hazard and risk assessment.

The explanation for the parameters and abbreviations used in Fig. 3, and for some other terms frequently used in the evaluation of ecotoxicological tests, are given in Table 1.

For results obtained in the individual tests, the respective effects can be rated according to their importance, and relationship to control values. Römbke and Moltmann (1996) ranked these tests based upon severity (Table 2).

Recently, Van Wijngaarden et al. (2005) discussed ecological threshold values, i.e., $NOEC_{eco}$, and $LOEC_{eco}$, in a review focusing on the ecological impact of neurotoxic insecticides. The authors considered the $NOEC_{eco}$ to be the highest concentration tested at which little or no effects on the structure and functioning of the studied (model) ecosystem are observed. The $LOEC_{eco}$ was defined as the lowest test concentration at which significant treatment-related effects occur. Also, considering different test organisms as belonging to (a) microcrustaceans, (b) macrocrustaceans, (c) insects, (d) fish, (e) rotifers, (f) other macroinvertebrates, or (g) algae & macrophytes, as well as (h) community metabolism, the authors determined structural endpoints (categories

TABLE 1. Terms and parameters frequently used in ecotoxicological tests.

Term/Parameter	Objectives
Mortality	Effects causing death and/or permanent immobilization of organisms exposed for a short-term to chemicals. Expressed as an LC_{50} or EC_{50} (Lethal Concentration and Effect Concentration, respectively, for 50% of the test organisms).
Growth	Changes in either total biomass or body size (weight) of the test organisms after a long-term exposure and usually expressed as a NOEC (No Observed Effect Concentration), e.g., $NOEC_{Biomass}$
Reproduction	Depending on the test organisms, numbers of eggs or juvenile organisms (including juvenile development), and expressed usually as NOEC.
Morphology	Damage to the external appearance of the test organisms after either short-or long-term exposure to chemicals; eventually expressed as NOEC.
Physiology, Metabolism	Alterations, e.g., in respiration activity, production of enzymes and/or secondary metabolites; eventually expressed as NOEC after a short- or long-term exposure to chemicals.
Behavior	Mainly motility alterations after a short- or long-term exposure to chemicals.
Teratogenicity	Embryonic defects after exposure to chemicals.
Carcinogenecity	Unregulated cell growth and tumor formation tissues after a long-term exposure.
Mutagenicity	Induction of genomic changes after short- or long-term exposure to chemicals.

TABLE 2. Rating of effects obtained in ecotoxicological tests as related to unaffected controls. (Modified from Römbke and Moltmann 1996).

Rating	Mortality or lowered beneficence in %	
	Laboratory tests	Semi-field and field tests
Not harmful	<50	<25
Slightly harmful	50–79	25–49
Moderately harmful	80–99	50–75
Seriously harmful	>99	>75

"a" to "f"), and functional responses (category "h") for about 18 individual compounds. The structural endpoints considered densities (numbers) and/or biomass of populations while functional endpoints considered oxygen balance, water chemistry, and decomposition of particulate matter. Effects reported on these endpoints can be classified into five classes:

Class 1: "Effect not demonstrated" (no effects observed or no clear causal relationship between treatment and controls).

Class 2: "Slight effect" (effects on individual samplings only, short term effects, effects shortly after treatment, and/or quantitatively restricted).

Class 3: "Pronounced short-term effect" (clear response in several species but recovery within 8 weeks).

Class 4: "Pronounced effect in short-term study" (clear effects observed but the study is too short to demonstrate recovery within 8 weeks).

Class 5: "Pronounced long-term effect" (clear effects on many species, on various subsequent samplings, and recovery is longer than 8 weeks).

Toxicity data on the most sensitive standard test organism (*Daphnia magna)* were used to calculate toxic units (TU_{mso}). The use of TU_{mso} has been shown to be an adequate reference for estimating field responses due to direct toxic effects. If standard species are not representative of the sensitive taxonomic groups then the choice of TU_{mso} will be less successful. A field effect concentration (e.g., FEC 5%, FEC 95%,) can also calculated on the most sensitive endpoints. Basically, the authors recognize the necessity of a more specific formula that may be used to protect the ecosysytem and be considered as "unacceptable damage" to the ecosystem.

Davoren et al. (2005) pointed out the importance of aquatic sediments that are critical to the health of an aquatic ecosystem. The authors used a test-battery approach for the ecotoxicological evaluation of estuarine sediments, i.e., porewater and elutriate extracts, with the aim to assess the overall sensitivity and applicability of each test. Three bacterial bioassays were employed in their study: (a) Microtox Solid Phase Test with lyophilized *Vibrio fisheri* bacteria; (b) Toxi-ChromoPad bacterial bioassay, as based on inhibition of ß-galactosidase in a mutant strain of *Escherichia coli*; (c) MetPAD bioassay based on a similar principle as (b). In addition, an algal-toxicity test, using axenic cultures of a marine diatom *Skeletonema costatum*, and a standardized crustacean bioassay with *Artemia salina*, an herbivorous invertebrate, were employed. Toxicity data was used to calculate statistically significant effect concentrations, i.e., EC_{10} and EC_{50} values. Microtox and algal bioassays appeared to be more sensitive than bacterial enzyme assays and the invertebrate lethality test. For the first time, the authors recognized the necessity of including salinity controls in the experimental design, as the degree of salinity and geophysical properties of the sediment significantly affect the results of bioassays.

Recently, Jager et al. (2006) criticized standard summary statistics approaches in ecotoxicological risk assessment such as NOEC, LC_{50}, and EC_x values

because they change with exposure duration in a manner that may vary considerably with the test species and the toxicant. As an alternative, process-based models can be used. These models allow for toxicity measures that are independent of exposure time, and are better suited for educated extrapolation (e.g., from individual to population, and from continuous to pulse exposure). Reducing animal testing is also important for ethical reasons, and it can reduce costs for chemical risk-assessment. Thus, radical changes in current test protocols should be made to allow better use of process-based modeling of ecotoxicological effects.

In this respect, we recently reported on the effect of numerous organic chemicals, not on individual test species, but on the size of biomass (ATP contents), and metabolic activities (dehydrogenase, and respiratory activity) in a mixed population of groundwater microorganisms enriched in a complex nutrient medium (Filip and Demnerova, 2006). Since groundwater represents a capital resource of drinking water in many countries, there is a growing concern of chemical contamination of groundwater aquifers. To be effective in transformation or degradation of chemicals, microorganisms indigenous to natural groundwater aquifers should be capable of resisting toxic effects of chemicals. By estimating minimum effect concentrations (MECs) of the individual chemicals on the above sum parameters after 1 day and 42 days of exposure (MEC 1 day; MEC 42 days), we found a high degree of toxicity at low concentrations for some anilines, chlorophenols, and nitrated aromatic hydrocarbons (Table 3).

TABLE 3. Minimum effect concentration of some organic chemicals on amounts and activities of groundwater microorganisms (values in ppm). (From Filip and Demnerova 2006.)

Chemical compound	Water solubility	MEC 1 day	MEC 42 days
N-methylaniline	30,000	300[a]	<30
2,4,5-trichorophenole	2,000	0.3[a]	>3
Pentachlorophenole	2,000	0.3[b]	3
Nitrobenzene	1,900	10[b]	10

[a] = value obtained in ATP test; [b] = value obtained in dehydrogenase test

1.1.1. Ecotoxicological Approaches in the Evaluation of Soil Quality

Soil is the foundation of the entire biosphere and a part of terrestrial ecosystems with a highest population density of different organisms (Filip 1973; Kovda 1975). Due to their physiological and biochemical activities, soil organisms are critical determinants not only of soil production capacity (fertility) but they have also an environmental safe-guarding activity as prominent agents in the degradation of chemical pollutants that could either directly or through a soil

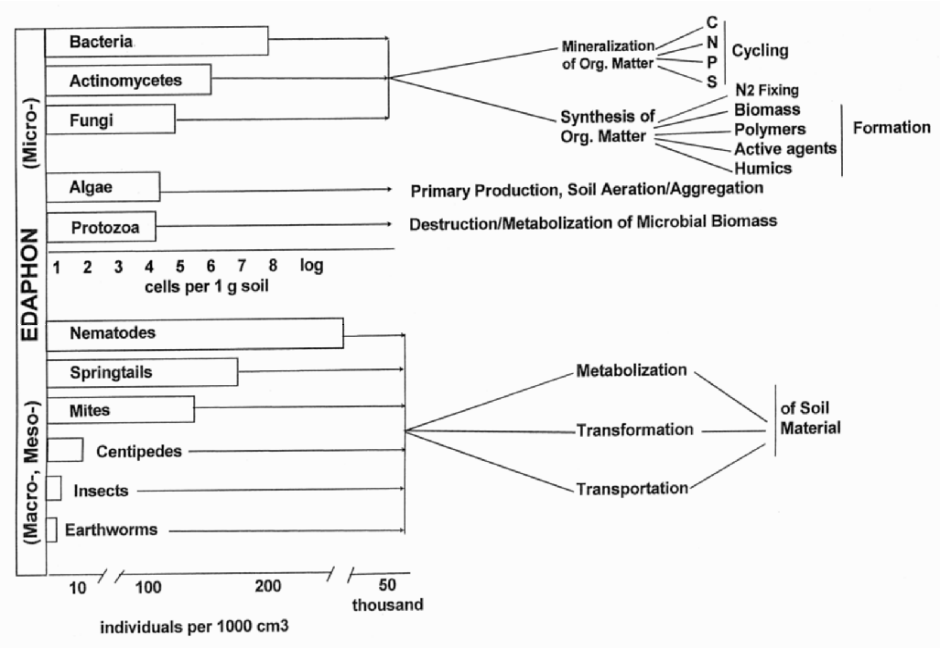

Figure 4. Soil organisms, their approximate counts and ecologically important activities. (From Filip 2002.)

passage endanger the quality of water and air. There is no doubt that firm linkages exist between different communities of soil organisms, their biochemical activities, and ecologically important soil processes, such as mineralization and transformation of plant residues and other organic materials (Fig. 4).

In a review, Filip (1995) reported the effects of chemical contaminants on size and composition of soil microbial populations as well as populations of micro- and meso-fauna, turnover of carbonaceous substrata, turnover of nitrogenous substrata, influences on enzyme activities, degradation of plant residues, and resistance of soil microorganisms against a heavy metal soil contamination. The author also emphasized the importance of site-specific non-biotic factors that strongly influence the effectiveness of chemical contaminants on soil organisms and their metabolic activities. The behavior of chemicals in soil and their interactions with microorganisms are reviewed in Fig. 5.

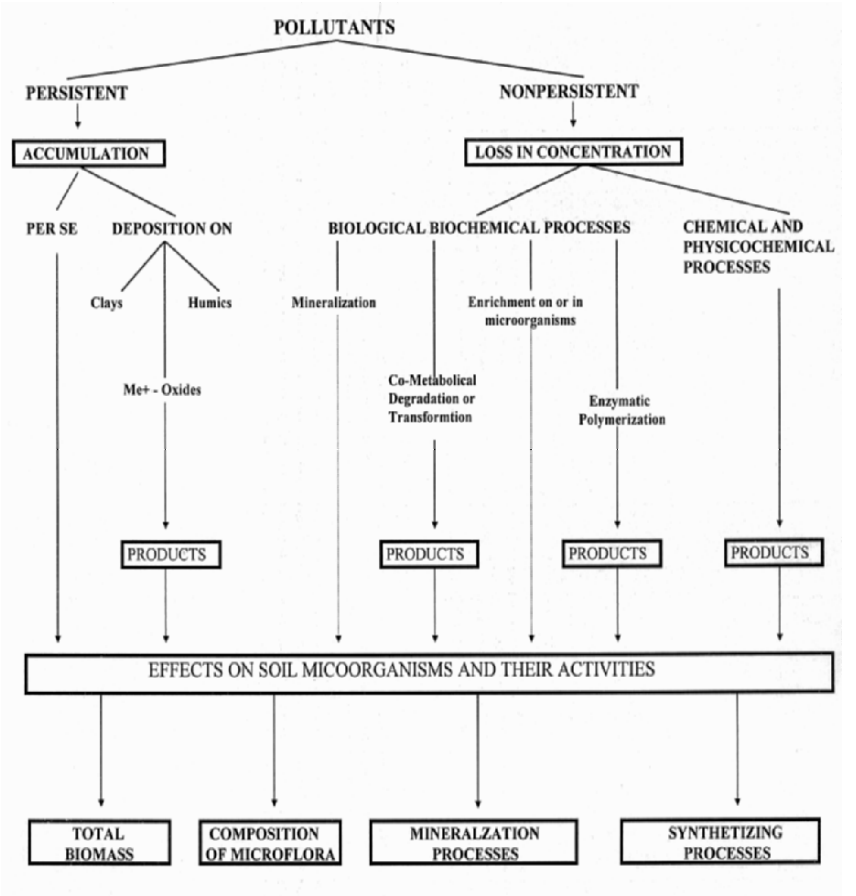

Figure 5. Behavior of chemical pollutants in soil and their interactions with microorganisms. (From Filip 1998.)

Paces (1998), predicted a role for soil as an interface between biogeochemistry, environmental science, economics and politics. In Central European countries the loss of natural soils due to different anthropogenic activities amounts to about $1m^2/s$ (Nestroy 2003). Considering the necessity of developing a biologically based testing protocol of soil quality, a multinational approach consisting of more than 20 ecologically related biological parameters were selected and performed in order to identify the best methods for determining chemical contamination in soil (Filip 2002). The individual parameters employed in the international testing ring are depicted in Fig. 6.

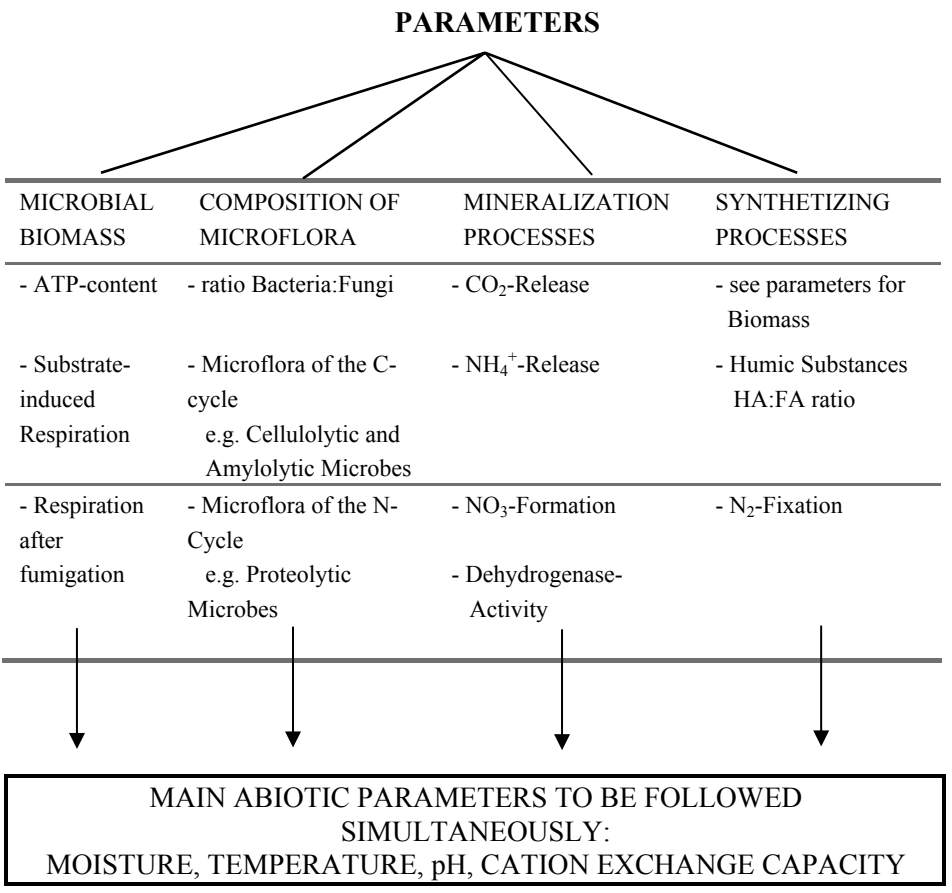

Figure 6. Selected parameters to evaluate effects of chemical pollutants on ecologically important soil characteristics. (From Filip 2002.)

In Table 4, the relative sensitivities, as estimated for the most reliable test parameters, are shown. Nitrogen fixing bacteria, the enzymatic activity (dehydrogenase), and the soil respiration (CO_2 release) appeared capable of establishing soil pollution. Figure 7 shows examples of the sensitivity of a few parameters to an artificially induced contamination of a podzol soil by Pb $(NO_3)_2$, as estimated in the Russian part of the project.

TABLE 4. Relative sensitivity of the selected microbiological and biochemical parameters for the assessment of effects of chemicals on soil as obtained from repeated (2 years) analyses of 49 soil sites in four European countries. (From Filip 2002.)

Parameter	Relative Sensitivity
Microbial biomass	+/++
Composition of microbial communities (colony-forming units, and most probable counts, respectively)	
Copiotrophic bacteria	+/++
Oligotrophic bacteria	++
Actinomycetes	++
Microscopic fungi	++
Proteolytic sporeforming bacteria	–/+
Cellulose decomposing bacteria	+/++
Nitrogen fixing bacteria	++++
Pseudomonads	–/+
Biochemical process-linked activities	
Respiration (CO_2 release)	+++
Ammonification (NH_4 release)	++
Nitrification	++/+++
Denitrification	++/+++
Dehydrogenase activity	+++/++++
Humification activity	++

Sensitivity (relative to control soil): – = no effect; + = low effect; ++++ = very strong effect

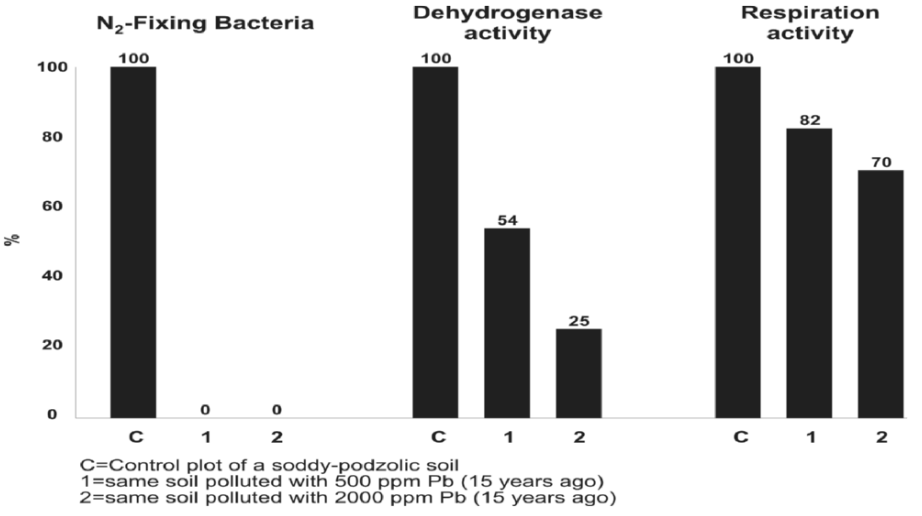

Figure 7. Relative sensitivity of some microbiological and biochemical indicators in soil contaminated with $Pb(NO_3)_2$. (From Filip 1998.)

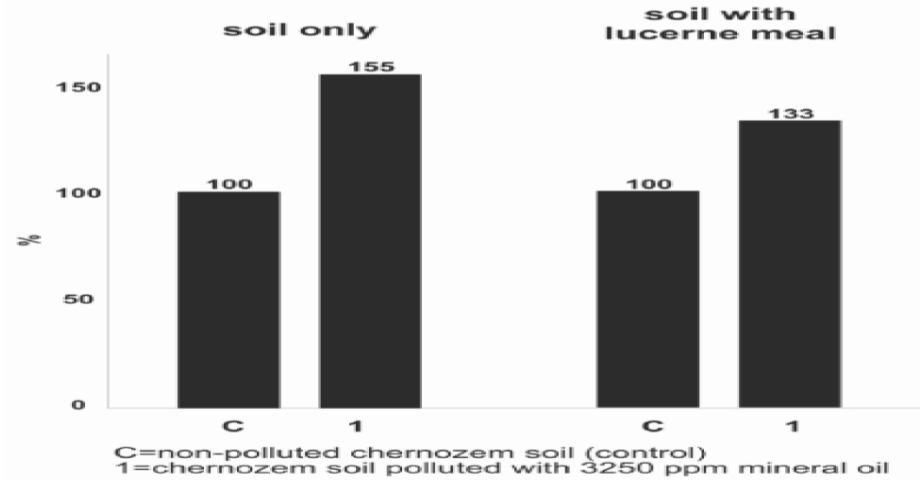

Figure 8. Relative effects of a long-term soil contamination by mineral oil on the respiration activity of soil samples, without or with organic substrate (lucerne meal), added. (C = control soil; 1 = soil with 3250 ppm mineral oil). (From Filip 1998.)

If soil becomes contaminated with multi-structured organic chemicals, an increased activity, instead of inhibition has been observed. As shown in Fig. 8, such a phenomenon was observed in samples of a Slovak chernozem-type soil from a site located near to oil refinery.

In order to obtain ecotoxicological data on the effects of chemical contaminants in soil, more simple and rapid tests are required. Kördel (2000) and Pfeifer et al. (2000) listed aquatic tests with soil eluates based on a luminescence and/or growth of *V. fisheri* bacteria, growth of daphnids and algae, and mutagenity tests with soil eluates. Earlier, Römbke and Moltmann (1996) reported on tests in higher vertebrates (birds, mammals), soil saphrophagous invertebrates, plants, and honey bees for examining terrestrial media, including soil. According to Filip (2002), some of these test strategies may have an advantage based on simplicity, and ease in standardization. However, such methodical approaches oversimplify both the biological and non-biotic structural complexity and heterogeneity of soil, and disregard soil ecological functions. Therefore, the usefulness of these methods are restricted to toxicity testing of individual chemicals, and only if the respective test organism possess the expected sensitivity and is representative of the soil environment. Certainly, with several millions species of invertebrates living in soil and thousands of bacterial species in every gram of soil (Pankhurst, 1997; Torsvik et al. 1997), it is impossible to select a single representative test species. In addition, it remains questionable whether a substantial correlation exists between soil-ecological functions and the degree of biodiversity in soil organisms (Wardle and Giller,

1996). This criticism is also valid for soil testing using higher plants, as discussed in brief by Kalsch and Römbke (2000), and in more detail by Riepert et al. (2000). Indeed, higher plant should be employed if the capacity of soil as a natural site of plant to growth is to be evaluated. Contrary to this, there are firm linkages between complex microbial communities, their biochemical activities, and ecologically important soil processes, such as mineralization and transformation of plant residues and other organic materials (Fig. 4). In this respect, Wilke et al. (2000) reported on the usefulness of methodical approaches, such as estimation of soil microbial biomass, respiration activity, and aerobic as well as anaerobic processes involved in the cycling of essential nutrients such as carbon, nitrogen, phosphor, and sulfur. Recently, and following international discussions, a comprehensive approach to characterize soil by a microbiological approach has been proposed by Andren et al. (2004). The main categories and investigative methods involved in this ecologically oriented soil evaluation system are shown in Table 5.

Similar to our methodical approach shown in Fig. 6, Filzek et al. (2004) underlined the necessity of estimating nonbiotic pedological factors that affect soil responses to chemical contaminants. For example, soil collected along a transect from a primary Cd/Pb/Zn smelting works, disturbances occurred from soil humus up to a 1.5 km distance from the smelter. In an opposite effect, we were able to measure a significant increase of humic acid and fulvic acid contents in long-term anthropogenic affected (chemically fertilized) arable soils (Filip and Kubat 2004).

In spite of the ecologically based approaches summarized in Table 5, some authors still employ tests with single organisms to assess ecotoxicological effects of different chemicals in soil. Robidoux et al. (2004) used soil mesocosms with the earthworms, *Lumbricus terrestris* and *Eisenia andrei* to assess toxicity of explosives in soil from an antitank firing range. The results indicated that the survival of earthworms was reduced up to 100% following 28 days of soil exposure. Bundy et al. (2004) sampled earthworms across an environmental gradient of metal contamination.. Both indigenous (*Lumbricus rubellus, Lumbricus terrestris*) and introduced species (*Eisenia andrei*) were analyzed for small-molecule metabolites as possible biomarkers by ^{1}H NMR spectroscopy. The spectral data revealed that biochemical changes were induced across the metal-contamination gradient. Native worms of *L. rubellus* from the most polluted site were associated with an increase in the relative concentration of maltose and histidine but the concentrations were significantly reduced by metal contamination in *L. terrestris*. These results show that contradictory results may be obtained in tests based on the use of a single-test species. Fountain and Hopkin (2004) examined the effects of metal contamination (Cd, Cu, Pb, Zn) on the population of Collembola (springtails) in five soil sites. The

highest number of species was found at the most contaminated site, although the Collembola population also had a comparatively low-evenness value with just two species dominating. Coja et al. (2006) found a collembolan, *Folsomia candida*

TABLE 5. List of methodical approaches and tests to characterize soil ecological capital using natural microbial characteristics. (From Andren et al. 2004.)

Methodical approach	Tests: normalized, calculated, upon research
Microbial biomass	*Normalized tests:*
	ISO 14240-1 (substrate-induced respiration)
	ISO 14240-2 (fumigation–extraction)
	Calculated test:
	Microbial quotient
	Tests upon research:
	Fumigation–incubation
	Specific group biomass (e.g., ergosterol determination for fungi)
	Physiological method (initial mineralization rate of glucose added)
	ATP method (adenosine triphosphate concentration in soil)
Microbial activity	*Normalized tests:*
	ISO 15685 (nitrification – NH_4 oxidation)
	ISO 14238 (N mineralization and nitrification–incubation method)
	ISO 14239 (C mineralization = respiration–incubation method)
	ISO 16072 (basal respiration of heterotrophs)
	ISO 17155 (respiration curves)
	Calculated tests:
	Respiratory quotient
	Fungal/bacterial respiratory ratio
	Tests upon research:
	Denitrification (e.g., N_2O production – anaerobic conditions)
	N fixation (e.g., C_2H_4 production – incubation – nitrogenase activity)
	Mycorrhizae (percentage of root colonized in a test plant)
Enzymatic activity	*Normalized tests:*
	ISO 23753-1 (dehydrogenase activity – TTC method)
	ISO 23753-2 (dehydrogenase activity – ITC method)
	Tests upon research:
	Enzymatic activity tests (other than dehydrogenase: phosphatase, sulphatase, peptidase, urease, esterase, cellulase, amylase, xylanase, laccase, peroxydase, maltase, saccharase, cellobiase)
	Enzyme index
Root pathogens	*Tests upon research:*
	Detection of root pathogens
Microbial diversity	*Tests upon research:*
	Community level physiological profiles (e.g., BIOLOG-system)
	Nucleic acid analysis (e.g., soil DNA extraction and PCR, RPFT, DGGE)
	Dilution plating and culturing methods
	Ester-linked fatty acids estimation (e.g., PFLA, FAME, MIDI, SLB – chemical microbial signatures)

and a carabid, *Poecilus cupreus* quite useful in testing lethal and/or sublethal effects of benzoxazolinone, some degradation products and related pesticides. Rather than a single-organism test, Garcia-Gil et al. (2004) used microbiological and enzymatic biomarkers for soil monitoring. Sometimes, test results may differ from a single-soil sample. For example, Tobor-Kaplon et al. (2006) showed contaminated soil (Zn, Cd) had the lowest stability to additional pollution with Pb and heat stress with regard to respiration. However, bacterial growth rates were affected in an opposite way than respiration. Some controversial results also occurred using methods for evaluating human impacts on soil microorganisms (Joergensen and Emmerling 2006). In general, however, the authors confirm the opinion we expressed earlier (Filip 1973, 1998, 2002); that total microbial biomass, structure of microbial community, and different activity rates, represent the most useful parameters for assessing soil-ecological functions, and thus, they should be primarily considered in soil-ecotoxicological examination and systematic long-term soil monitoring.

Nonetheless, some shifts in methodical approaches can be expected, especially due to novel genetic parameters. Muyzer (2000) claimed that up to 99% of all microorganisms in nature cannot be isolated in culture and, therefore, favored genetic fingerprinting of microbial communities as a key perspective. Genetic techniques can provide a profile of the community diversity based upon unique nucleic-acid species. There are direct methods, whereby nucleic-acids are extracted and directly analyzed, such as a low-molecular-weight (LMW) RNA profiling, or indirect methods, whereby the molecular marker are first amplified, such as in denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), single stranded-conformation polymorphism (SSCP), randomly amplified polymorphic DNA (RAPD), DNA amplification fingerprinting (DAF), bismenzimide–polyethyleneglycol electrophoresis (Bb–PEG), restriction fragment length polymorphism (RFLP), amplified ribosomal DNA restriction analysis (ARDRA), and terminal or fluorescent RFLP (T/Flu–RFLP). According to the authors, these methods are rapid, relatively easy to perform and allow for simultaneous analysis of multiple samples, which makes it possible to compare genetic diversity of microbial communities from different habitats, or to study the behavior of individual communities over time. It is not certain, how beneficial these alternative approaches will be. According to Soulas and Lors (2000), no unbiased calibration techniques are available to control the efficiency of DNA-extraction procedures and there is no evidence that PCR amplification contributes definitely to the profiling of microbial communities. According to Zvyagintsev et al. (2002), data obtained by molecular genetic methods are more ambiguous that those obtained by culture techniques. The majority of soil clones have a low degree of homology with the known

nucleotide sequences of bacterial taxa, and thus, there are designated as new phylogenic groups. On the other hand, common soil inhabitants often cannot be detected. Furthermore, one cannot obtain information on physiological peculiarities and ecological functions of soil bacteria. The authors recommend further research to improve existing and developing novel-cultural techniques for broad-scale soil monitoring.

2. Ecotoxicity Measurement for Polychlorinated Biphenyls (PCBs) and Intermediates in their Degradation

As was mentioned before, different systems for ecotoxicity measurement were evaluated during last 15 years. Many of them are based on measurement of viability of different organisms and their ability to survive in the presence of different toxicants. We have studied metabolism of PCBs in bacteria and plants (Wilken 1995; Kucerová 1999). These organisms are involved in transformation of toxicants in nature and the fate of many xenobiotics in the environment. Bacterial strains and several plant species that are able to transform PCBs. were studied. Toxicity of identified bacterial and plant products was studied using plant, microbial, and mammalian cell systems (Kucerová 2000).

2.1. MEASUREMENT OF TOXICITY

CBs were produced from mixtures of different congeners varying in their degree of chlorination. The main products of bacterial degradation of PCB are CBs which can be further degraded by other bacteria present in contaminated areas. Analysis of the products of bacterial and plant metabolism has shown that hydroxychlorobiphenyls are the first step in PCB transformation, which is similar to that which occurs in mammalian cells. Monochlorobiphenyls, which are usually not present in commercial mixtures can be formed in the environment by microbial dechlorination or degradation of more highly chlorinated congeners. We used monochlorobiphenyls and their products identified in bacterial and/or plant cells as models for the toxicity measurements (see Fig. 1).

From these results it was concluded that hydroxychlorobiphenyls, as primary products of PCB metabolism in plants, are the most toxic of the three groups of compounds tested. A similar response was previously obtained in mammalian cells (keratinocytes), which also are highly sensitive to hydroxychlorobiphenyls. Comparing both systems for the ecotoxicity measurement, luminescent bacteria are more susceptible to lower concentrations of tested compounds.

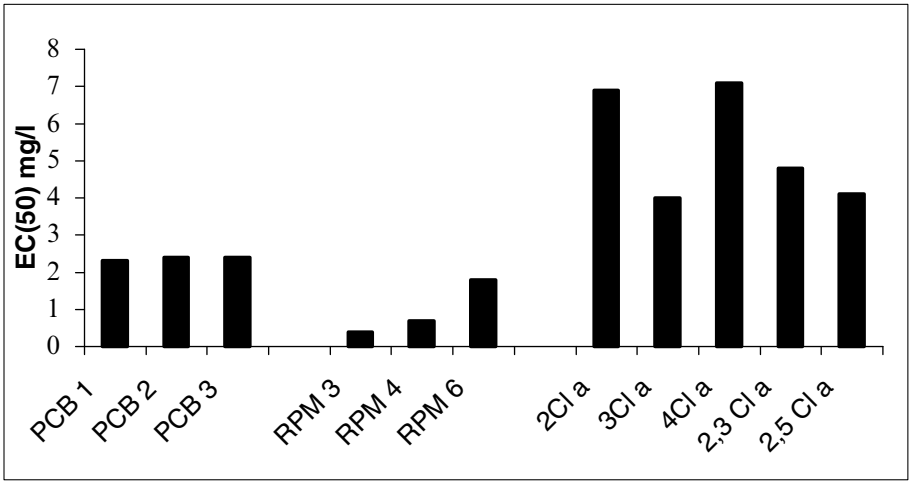

Figure 9. Toxicity of monochlorobiphenyls and products of bacterial and plant metabolism measured by luminescent bacteria *Vibrio fischeri.*

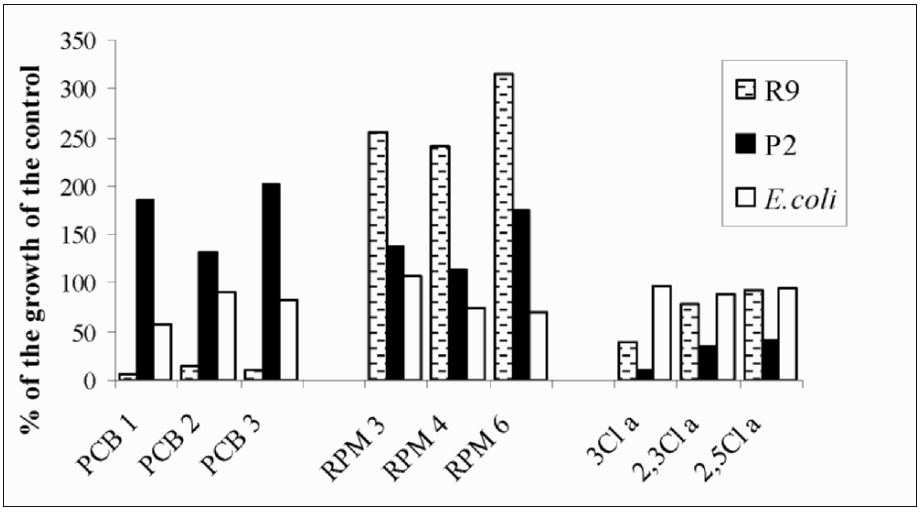

Figure 10. Evaluation of specific growth rates of three bacterial strains incubated with PCBs, chlorobenzoic acids, or hydroxychlorobiphenyls at a concentration of 10 mg/l.

Toxicity of the same compounds was tested using an independent method, namely the Bioscreen measurement. Using this method, the growth of three different bacterial strains incubated in medium with and without toxicants was followed over 2 days. The Bioscreen test showed a different response to the selected organisms. *Pseudomonas* sp. R9, which is not able to degrade PCBs, exhibited the strongest response to individual congeners of PCBs, while hydroxychlorobiphenyls and CBs were less toxic. The opposite effect was

observed with *Pseudomonas* sp. P2, which can degrade products of PCB (CBs) but not parent PCBs. *E. coli* exhibited a similar response to all three groups of tested compounds (Fig. 10).

Test results with seeds of *Lactusa sativa* are shown in Table 6. Monochlorbiphenyls were not toxic to the plant system, but CBs and hydroxychlorobiphenyls were more toxic than PCBs, probably due to better solubility in water.

TABLE 6. Toxicity of products of bacterial and plant metabolism measured by seeds of *Lactuca sativa.*

I (%)	PCB 1	PCB 2	PCB 3	RPM 1	RPM 3	RPM 4	3 CIa	2,3 Cla	2,5 Cla
3 mg/l	19.1	16.5	7.8	4.7	2.0	15.7	55.5	44.5	53.7
50 mg/l	25.8	27.3	7.5	86.9	81.8	93.6	79.1	93.7	99.6

3. Measurement of Genotoxicity by Ames Test

Due to the toxicity of the tested substances for model microorganisms it was necessary to establish a concentration which did not kill the bacterium. The lowest concentration showing a mutagenic effect was chosen and a concentration gradient was prepared. Genotoxic effects were observed with CBs. PCBs and hydroxychlorobiphenyls showed less genotoxicity than CBs, as only TA 100 cells showed evidence of weak genotoxicity.

3.1. CONSIDERATIONS ON "NANOMATERIALS & THE ENVIRONMENT"

The Nanoforum, a pan-European nanotechnology information network founded by the European Commission (EC) and the Institute for Environment and Sustainability of the EC Joint Research Center, Ispra, Italy, organized a workshop on "Nano and the Environment" in Brussels in March 2006 to discuss ways in which nanotechnology could be used for the benefit of the environment. The program was divided into three topics: (a) monitoring, (b) pollution and remediation and, (c) resource saving.

Rickerby (2006) reported the development of solid-state sensors, based on nanocrystaline SnO_2 films, which can reversibly and selectively detect CO and NO_2, at or bellow the existing legal limits, and which can be linked with a global positioning system (GPS). Other candidates for sensor detection included methane, ozone, and benzene gases. Over 1 million sensors are expected to be deployed in fixed locations over the coming years for an air-quality early-warning service. In an EU-funded project, automated water analyser computer

supported system (AWACSS) was developed. Requiring only a pre-filtration step to remove particulate matter, the device can analyze water by means of an integrated optical chip, which uses immunoassays systems to detect organic pollutants such as pesticides, antibiotics, natural toxins, carcinogens, and different industrial waste chemicals (Proll and Gauglitz 2006). The chips can be reused up to 500 times before the surface chemistry must be reapplied. Systems developed by another EU-funded project are based on indium, zinc, and tungsten oxides deposited in the form of thin films, nanowires, and nanocombs, which allows cost-effective monitoring of different environment-linked odors and early detection of smoulder fires (Vomiero 2006). Colvin (2006) believes that the interface between "dry" site of inorganic nanostructures and "wet" side biological systems offers enormous opportunities for medicine, environmental technologies, as well as development of new types of nanomaterials. Current safety studies have examined nanomaterials in water-soluble systems and their effects on natural processes. While water-suspendable nanocrystals (C_{60}) appear cytotoxic to various cell lines, the closely related fully hydroxylated $C_{60}(OH)_{24}$ is nontoxic, producing no cellular response. Similarly, single-walled carbon nanotubes are nontoxic to cells in culture.

The report from the workshop also contained information on magnetic iron-oxide nanoparticles for removing arsenic from groundwater. The system relies on the increased ability of iron-oxide nanoparticles to bind arsenic irreversibly (up to tenfold higher than micro-sized particles) and the supramagnetic nature of nanoparticles, which allows them to be separated from water by the application of magnetic field. In laboratory tests, more than 99% of the arsenic in water was bound by iron particles that are no more than 12 nm in diameter.

It was estimated that 60% of environmental nanoparticles are due to road transport, and 27% originate from combustion processes. These small particles can cause health-related problems by penetrating the lung epithelum, entering the vascular system, and migrating to different organs. The surface chemistry of nanoparticles and their ability to form aggregates are also key considerations in determining their toxicity in cell tissues. Furthermore, different by-products of nanomaterials production also may need to be tested for their possible toxic effects. A close cooperation of scientists, regulators, and consumers is required to recognize positive and negative health and environmental related impacts of nanomaterials.

From an ecotoxicological perspective, future efforts should be focused on the development of molecular based nanosensors (biochips) that will allow detection of early changes in microbial communities related to community activities that influence biogeochemical cycles of elements, matter and energy in both aquatic and terrestrial ecosystems. Only in this way, and not by

monitoring the fate of selected test organisms, can environmental stressors be identified, evaluated, and hopefully eliminated.

4. Conclusions

Various methods are in use for ecotoxicolocical testing of natural and anthropogenic affected environments. As shown by several examples, process-based testing, although often more complicated, is preferred over those based on the use of a single test species. Future development should be concentrated on molecular based nanosensors (biochips) that will allow detection of early changes in microbial communities in relation to community activities influencing the key processes of biogeochemical cycles of elements, matter and energy in both aquatic and terrestrial ecosystems. In this way, environmental stressors can be identified, evaluated, and hopefully eliminated early enough to avoid serious disturbances to natural ecosystems.

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PARTICLE EXPOSURE THROUGH THE INDOOR AIR ENVIRONMENT

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Abstract: Sources, exposure and measuring techniques for indoor particulate matters (PM) are overviewed. To evaluate indoor air quality in two subway stations in Budapest, concentrations of PM_{10}, $PM_{2.5}$ and total suspended particulates (TSP) were measured during a 5-day period in a preliminary study. The following results were found: PM_{10} pollution was 2–3 times higher in the metro station than in matched sampling in the street. The levels of $PM_{2.5}$ were much less. PM pollution level was not influenced by the depth of the platforms.

Keywords: indoor environment, particulate matter, exposure, PM on subway

1. Introduction

It is well known that air quality has a significant impact on human health. We can drink bottled water if piped water contains too much chlorine. We can select higher-quality food, so-called bio-products currently on the market, if we are afraid of chemical residuals. However, we cannot choose the air we breathe. We spend a large part of our life indoors, at home, or other public environments, such as schools or restaurants. Having clean indoor air is very important for the health of the population as a whole and it becomes particularly important for infants, children and the elderly or people predisposed to disease, particularly respiratory or cardiovascular diseases.

The public health significance of indoor air pollutants, including particulate matter (PM), is studied worldwide and scientific evidence shows a significant impact on the health of the population (http://www.acidrain.org/pages/publications/factsheets.asp). Environmental tobacco smoke (ETS), combustion products, volatile organic compounds and biological pollutants are all responsible for, or increasing respiratory diseases and in some cases, cardiovascular diseases

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(Gamble, 1998). When exposures are sufficient to cause acute health effects in a population (as may occur in certain accidental catastrophes) causal relationships are easier to demonstrate. However, low-level exposure environmental or occupational exposure is difficult to investigate. In principle, there are two basic approaches commonly used to address indoor air issues. The most obvious is to reduce indoor exposure to known air pollutants for which health impacts have been established or are strongly suspected. The second is to promote investigations aimed at a better understanding the exposure – health effect relationship in order to support policy development and implementation (WHO/Euro, 2006). Regarding PM, we have to reduce sources of emissions or take measures, such as optimizing building design and ventilation, to keep indoor concentrations of pollutants as low as possible. There is also a need to conduct investigations on the source, hazard, exposure and effect of different types of airborne particles and informing the populations at risk on behaviours that minimizes exposure. This strategy is already followed by many national and international organizations such as WHO and EU.

2. Sources of Indoor PM

The major indoor source of fine-particle exposure, besides smoking, is cooking, particularly from frying and broiling. For ultra-fine particles, gas-burners, gas ovens, and electric toasters are also important point sources (Ott and Siegmann 2006). Other sources of indoors air polluatants are derived from the day-to-day activities of humans and dosmestic animals, as well as handling of organic materials like firewood. Biological particles that have settled indoors, along with other particulate matter, may become resuspended into the ambient air through normal household activities and other mechanical disturbances. The relative importance of these sources depends on the environment and lifestyle of the occupants. Particles present in the indoor environment may also be bound to surfaces, attached to dust accumulated in the building, or present in internal parts of the building structure or their operating system, such as air-conditioning units, and ducts (Morawska and Salthammer 2003).

Besides active indoor sources, particles generated by outdoor sources can penetrate from outdoor into indoor air either through open windows or doors, or through cracks, gas or holes in the building envelope. Particle deposition on indoor surfaces is related to particle size and surface characteristics, with rough surfaces resulting in higher deposition than smooth ones. The depositing particles contribute to the surface accumulation, and thus the process of deposition can also be described in terms of an increase in deposited materials on the surfaces. The deposition of house dust has been the subject of many studies.

There is a significant difference in the role of outdoor air, as a source of indoor particles, compared to the role of indoor sources. Indoor sources, while

affecting outdoor characteristics to varying degrees, have a direct effect only in houses in which they are present. Since the characteristics of the sources and pattern of their usage differ from house to house, the resulting particle-concentration levels and other characteristics will differ from house to house as well. Outdoor air, however, provides the same background levels for all houses in the area. Although the fraction of outdoor particles penetrating a building differs due to differences in air-exchange rates or the filtering systems, the time variation of this background tends to remain the same.

The indoor/outdoor (I/O) relationship for mechanically ventilated buildings is even more complex than that for naturally ventilated buildings. The mechanically ventilated buildings investigated, in most cases, have been non-industrial workplaces or public buildings, such as offices, hospitals, restaurants, schools, shopping centers or public transport buildings (Poupard et al. 2005). A common characteristic of indoor areas in all such buildings is that the mechanical ventilation and filtration systems affect the characteristics of PM entering the building in terms of concentration and size distribution.

3. Exposure to Indoor PM

Human movement has frequently been shown to result in an increase in particle-mass concentration. Activities such as walking, cleaning or dressing can significantly increase the concentration of PM in the air. It has been demonstrated that even light activities could be a significant source of PM. However, such physical activities do not contribute to PM in the air, which are basically non-resuspendable under conditions present in residential environments.

In addition, the impact of cigarette smoking on particle concentrations has been investigated in terms of the increase in particle concentrations in the houses of smokers compared to the houses of non-smokers based upon various averaging periods, number of cigarettes smoked and indoor/outdoor ratios for houses with and without smokers. Increased concentrations of $PM_{2.5,}$ as a result of cigarette smoking, have been found in many places. Moreover like smoking, the effect of cooking on indoor particle mass concentration levels has been investigated and expressed in a number of ways (Saraga et al. 2006).

For the assessment of human exposure to indoor pollutants, the analysis of settled house dust and adsorbed organic, inorganic, and biologically active compounds is of increasing scientific and medical interest. Particulates pass into the body through oral and dermal intake. The main mechanism for intake of airborne particles by the human body is through inhalation of particulates and deposition in the respiratory tract (Morawska et al. 2005). Large-sized particulates mainly deposit in the upper part of the respiratory tract due to impaction, interception, gravitational sedimentation as well as turbulent dispersion (Oberdorster et al. 2005). Very fine particles, such as those generated

through combustion processes, have a high probability of deposition in deeper parts of the respiratory tract, due to their high diffusivities. An understanding of the mechanisms of particle deposition in the human respiratory tract and the ability to quantify the deposition in individual parts of the respiratory tract is of fundamental importance for dose assessment from inhalation of particles, which can then be used for risk assessment (Gwinn and Vallyathan 2006). Over the last three decades, a large number of studies have been conducted to investigate particle deposition in the human respiratory tract (Donaldson et al. 1998, Oberdorster et al. 2005).

4. Measurement Techniques for Indoor PM

Electronmicroscopy is the most common technique used for particle analysis at both the morphological and chemical levels. Scanning electron microscopy (SEM) can provide information on particle surface structures or 3D interpretations. The added advantage of SEM is the capacity to determine the elemental composition of airborne particles at both the individual and bulk-particle levels that yield specific information about the elemental features of a specimen. Characterization of indoor PM, by combining scanning and transmission electron-microscopy, yields additional information about the size, morphology, and the chemical and phase composition of individual particles (Hoflich et al. 2005). It should be noted, however, that no single electron-microscope technique will provide a total elemental characterization of a specimen and a synergistic approach is generally required, which includes several other micro-analytical techniques.

5. Preliminary Results on Indoor Air-Quality Assessment on Underground Platforms in Budapest

As people spend about 10% of their time per day with transportation, and a large number of people are exposed to traffic-related pollution in big cities every day (http://www.levego.hu/caag.htm). Nowadays the underground transport mode has an important role in Budapest as 23% of the inhabitants make use of the metro. This represents 863,140 persons travelling by underground lines on average in 1 day last year. The main sources of respirable tunnel dust in the underground rail system are particles from abrasive forces acting on rails and wheels from traction and braking. These are likely to contain iron and particles shed from humans and their clothes. The results of a study by Hurley et al. (2003) showed that dust in the London Underground differs from outdoor particles and accordingly risks from outdoor particles are misdirecting for estimating its health effects. Tunnel dust is coarser, being generated by interaction

of brakes, wheels and rails rather than by combustion, with higher-mass concentrations and lower-particle numbers. It contains about 90% iron, 1–2% quartz and the remains of other metals. One of the main aims of their work was to characterize the physical quality and composition of the dust and to make measurement that would allow evaluation of the exposure levels of the London underground workers.

The goal of our investigation was to establish the passenger's exposure to PM on two metro platforms being at different depths (deep and subsurface) of stations in Budapest. Manual sampling was carried out using a high-volume sampler (TSP) and a Harvard impactor (PM_{10} and $PM_{2.5}$) during 24 h on 5 consecutive days. Gravimetry analysis was performed. Energy-dispersive x-ray SEM was used for the characterization of particles morphology as well as for the elemental composition.

It was found that the differences for $PM_{2.5}$ between the metro stations and the above-ground sampling location were less than for PM_{10}. Five-day means of the PM_{10} were 200 ug/m^3 on the subsurface and deep metro stations. The outdoor (ambient-air) concentrations were between 55 and 95 ug/m^3. Five-day means of the $PM_{2.5}$ were 60 and 80 ug/m^3 on the subsurface and deep metro stations, respectively. The ambient air concentrations were under 50 ug/m^3. Hourly TSP concentrations exceeded the national standard (200 ug/m^3) for ambient-air.

Morphology and element composition was analysed by SEM. Regarding the composition of particulates the major components of PM_{10} fractions sampled in metro stations were Fe (30%), O (23%) and C (29%). $PM_{2.5}$ fractions contained less Fe (10%) and more C (50%) than PM_{10} particles. These outcomes in some ways are similar to the composition of tunnel dust received in the London underground. Morphology of particles with iron content was found in amorphous and needle forms as well. Mutagenicity assay of TSP samples taken on metro platforms showed moderate mutagenic activity with responses varying from 1,47 (1,19) to 4,42 (3,31) revertants per metric cube depending on depth of the station.

Our preliminary results indicate absences of elevated risk to the traveling public from exposure to PM in Budapest metro and have firmed that the dust metro is mainly from abrasion comprised of iron. Since concentrations of PM found in the subway indicate meaningful presence of these contaminants, further control measures should be considered.

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