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LITERATURE STUDY ON RISKS AND RISK ASSESSMENT METHODS RELATED TO NANOBASED PRODUCTS AND THE RECOMMENDED METHODOLOGY FOR ASSESSING RISK OF NANO-FIBRILLAR CELLULOSE PRODUCTS

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CONTENTS

1 INTRODUCTION 5
1.1 INTRODUCTION TO NANOTECHNOLOGIES 5
1.2 INTRODUCTION TO NANO-FIBRILLAR CELLULOSE 6
2 REQUIREMENTS FOR RISK ASSESSMENT OF ENM'S 7
2.1 PHYSIO-CHEMICAL CHARACTERIZATION OF ENM'S 8
2.1.1 Special characteristics of ENMs 8
2.1.2 Methods for nanomaterial characterization 10
2.2 EXPOSURE 12
2.2.1 Tools for measuring exposure 15
2.3 TOXICOKINETICS 16
2.3.1 Absorption 16
2.3.2 Distribution 17
2.3.3 Metabolism 18
2.3.4 Excretion 18
2.4 TOXICITY 18
2.4.1 Immunotoxicity 19
2.4.2 Neurotoxity 19
2.4.3 Mutagenicity and Genotoxicity 19
2.4.4 Carcinogenicity 20
2.4.5 Pulmonary effects 20
2.4.6 Dermal effects 20
2.4.7 Cardiovascular effects 21
2.4.8 Existing diseases and susceptibility to the effects of nanoparticles 21
3 RISK ASSESSMENT METHODS 22
3.1 EXPOSURE ASSESSMENT 24
3.2 HAZARD ASSESSMENT 24
3.3 RISK ASSESSMENT 25
4 ENVIRONMENTAL ASPECTS 25
4.1 NANOMATERIALS IN AIR 26
4.2 NANOMATERIALS IN SOIL 26
4.3 NANOMATERIALS IN WATER 27
4.4 BIODEGRADATION 28
4.5 BIOAVAILABILITY 28
4.6 BIOACCUMULATION 29
4.7 ECOTOXICOLOGY 29
5 LEGISLATION, REGULATION AND RECOMMENDATIONS (OPINIONS) 30

5.1 EUROPEAN UNION 30
5.1.1 Directives considering safety at workplace 31
5.1.2 Integrated Pollution Prevention and Control Directive 31
5.1.3 REACH 32
5.1.4 Waste management directives 32
5.1.5 Other legislation 33
5.2 "NATIONAL SITUATION" – SELECTED COUNTRIES 33
5.2.1 United Kingdom 33
5.2.2 USA 33
5.3 NGO'S 34
5.4 NANOTECHNOLOGY IN MEDIA 35
6 SUMMARY AND RECOMMENDATIONS 37
7 ABBREVIATIONS 39
RFEFERENCES 40

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SUMMARY

This report is a project deliverable D 10.1"Literature study on risks and risk assessment methods related to nanobased products and the recommended methodology for assessing risk of nano-fibrillar cellulose (NFC) products" of the SUNPAP project.

The purpose of this deliverable is to report both the state-of-the-art of risk assessment and results from nano-related studies on heath, safety and environment. The report serves as the basis for the definition of a methodology adapted to the needs of this project. In addition, this report communicates not only to other Modules within SUNPAP project about risk assessment but also to the general public being interested in the safety of nanoparticles.

There are lot of studies assessing risks of different nanomaterials. Nanoparticles can distribute from the site of entry to other sites in the body and it seems that nanomaterials can be found almost everywhere in the body after exposure. Some results suggest that certain nanomaterials might be toxic. Most of the studies focus on inorganic nanomaterials. The characteristics of nano-fibrillar celluloce (NFC) and funktionalised nano-fibrillar celluloce (FNFC) differ from the inorganic nanomaterials and therefore the results and experiences obtained with inorganic nanomaterials can be considered as indicative only. The suggested risk assessment methodologies include in vitro and in vivo tests to give indication from possible cytotoxicity or systemic effects as well as neurotoxicity. Also inhalatory toxicity stydy is included in the methodology.

The responsible partner of this report is Pöyry Management Consulting. For this report, Pöyry has followed the plan outlined in the SUNPAP DoW and made an extensive literature review on nanoparticle risk assessments. Moreover, the report has been complemented and reviewed by other partners in the project (BIOSS, FIOH and VTT).

1 INTRODUCTION

This report is a deliverable D 10.1 "Literature study on risks and risk assessment methods related to nanobased products and the recommended methodology for assessing risk of NFC products" of the SUNPAP project. The purpose of this report is to give information to other SUNPAP partners and public on the state-of-the-art of risk assessment and results from nano-related studies on health, safety and environment. This report serves as the basis for the definition of a risk assessment methodology adapted to the needs of this project.

The first chapter of the report presents a short introduction to the theme. The second and third chapter describes the risk assessment requirements and methods. The fourth chapter concentrates to the environmental aspects of risk assessment and the fifth more broadly legislation, regulation, recommendations and public acceptance related to the risk assessment of nanoparticles. The final chapter summarises the results of the report. The authors of this report are Pöyry Management Consulting (Pöyry), Finnish Institute of Health (FIOH), Technical Research Centre of Finland (VTT) and BioSafe – Special Laboratory Services (BIOSS).

1.1 INTRODUCTION TO NANOTECHNOLOGIES

Nanotechnology, the technology of nanomaterials, is a broad interdisciplinary area of research, development and industrial activity which has been growing rapidly worldwide. It is not a single technology or science; rather, it is a multidisciplinary grouping of physical, chemical, biological, engineering and electronic processes, materials, applications in which the defining characteristic is size /1/.

The nanotechnology industry is rapidly growing with promises of substantial benefits that will have significant economic and scientific impacts, applicable to a whole host of areas ranging from aerospace engineering and nanoelectronics to environmental remediation and medical healthcare /2/. According to Cefic /3/: *"Nanotechnology has the potential to open up new perspectives to economic, environmental and social benefits and is an innovation driver offering significant opportunities for sustainable development, growth and employment in Europe".*

The chemical industry produces already today various nanomaterials that may be used as raw materials for nanotechnology applications including nanostructured materials, nanostructured surfaces as well as nanoparticles /3/. The ability to selectively manage the size of nano-scaled materials allows the chemical industry to develop materials with new properties that offer advantages in macroscopic world. This excitement also has caught the attention of forest product technologists.

It is estimated that thousands of factories are producing or using nanomaterials, but the figures underestimate the true dimension of the occupational exposure, since a lot of

small enterprises are upgrading their production to take advantage of nanomaterial related technologies. Examples of the use of nanomaterials already on the market include sunscreens, plastics, corrosion resistant coatings and lighter and stronger composites for cars. Currently, estimates indicate that there are over 800 consumer products already available containing nanomaterials, the sales of which were valued at $147 billion in 2007 and are expected to soar over the coming years with a predicted value of $3.1 trillion by 2015 /4/. Hence, human exposure is already occurring and is set to increase dramatically in the coming years.

It is claimed that nanotechnology development is likely to require an additional two to ten million workers across the world by 2014 /5/. Many of these jobs are likely to be created in Europe, mainly in start-up companies and in small and medium enterprises. Whether this development may lead to additional hazard and health risks, will depend not only by the nanomaterial characteristics, but by the number of occupationally exposed people and at what levels they are exposed to, i.e., by the likelihood and the nature of exposure /1/. With this many potential workers and consumers exposed to nanomaterials now and in the future, risk assessment is essential.

In relation to risk assessment describing the actual characteristics and properties of nanomaterial in question is essential. To be able to describe nanomaterials it is important to provide some terms for a common understanding. The definitions used in this report follow the definitions suggested by SCENIHR /6/.

The prefix "nano" means a measure of 1×10^{-9} units. In this report, nanoscale refers to a dimension of 100 nm and below. Since the changes in characteristics that are seen on reducing dimension do not occur exactly at the 100 nm size, it is important that some fluctuation is allowed in this definition. An engineered nanomaterial (ENM) is any material that is deliberately created such that it is composed of discrete functional parts, either internally or at the surface, many of which will have one or more dimensions of the order of 100 nm or less.

The purpose of this report is to provide a literature study on both the state-of-the-art of risk assessment and results from nano-related studies on health, safety and environment. The report serves as the basis for the definition of a risk assessment methodology adapted to the needs of the SUNPAP project.

1.2 INTRODUCTION TO NANO-FIBRILLAR CELLULOSE

In the SUPAP project we focus on the risk assessment of nano-fibrillar cellulose (NFC) and functionalized nanocellulose. In different sources the world fibril has been used for relatively long and very thin piece of cellulosic material or thin cellulosic strands that remain attached on the outer surface of fibers, especially in the case of refined chemical pulp fibers /7/. On the other hand, very long and straight crystals of cellulose sometimes have been called "whiskers". In this paper we use the definition fibrillose to describe long

and very thin piece of cellulosic material. In addition to NFC, we study functionalized nanocellulose where other substances are added to nanocellulose in order to alter the existing characteristics or get new characteristics. The composition of the functionalized nanocellulose particles studied in the SUNPAP project will be decided in the near future.

There aren't existing studies on the risk of nanocellulose publicly available. There are some research results about the technical properties of nanocellulose available, but as cellulose is regarded as a non toxic, expected to be biodegradable substance, which has been present in our lives for a long time, no special emphasis has been seen in the risk assessment. Nevertheless, nanocellulose has three properties that are somewhat associated with pathogenicity. Firstly the nano form of cellulose could have more toxicity than larger sized particles. Secondly they are fibres and so might behave like asbestos and other pathogenic fibres which have toxicity associated with their needle-like shape. And thirdly, they are expected to be biopersistent in humans.

Some risk related studies for nano-scale fibre-like structures like carbon nanotubes have been made. They can be used as comparison, but they can't cover the possible risks related to nanocellulose. There is a definite need for a risk assessment and research concerning the safety of nanocellulose, especially because nanocellulose is likely to be used widely in products where consumer exposure is impending.

2 REQUIREMENTS FOR RISK ASSESSMENT OF ENM'S

Risk assessment (RA) is a scientifically based process where the probability for the occurrence of harmful effects on human or animal health or the environment is evaluated. The traditional RA schema includes four stages; hazard identification, hazard characterization, exposure assessment and risk characterization /8/. Health risk is defined as the combination of the probability of occurrence of harm to health and the severity of that harm. The traditional RA schema is considered an appropriate starting point to address the additional safety concerns that may arise due to the nanocharacteristics of ENMs /9,10,11,12/.

The special characteristics and properties of ENMs, such as their size, surface reactivity and translocation across biological membranes, are issues that probably need special considerations in risk assessment. Interactions of ENMs with the surroundings and effects resulting form it should also be under special consideration. Detailed knowledge of representative ENMs with information about physio-chemical and toxicological properties is still under development. This development is seen essential for the future of risk assessment /13/. We discuss the different stages of RA in the following chapters including characterization, toxicokinetics, exposure and toxicity of ENM's.

There is no established risk assessment method covering all nanomaterials at the moment. As there is very limited public knowledge covering the risks and risk assessment of nano-fibrillar cellulose we have studied risks and risk assessment methods related to other nanomaterials. The results from other studies are adapted in

this report to the appropriate extent. This doesn't change the fact that each nanomaterial should always be tested as their own before any results about the possible risks can be stated.

2.1 PHYSIO-CHEMICAL CHARACTERIZATION OF ENM'S

A wide range of physical and chemical properties is recommended to be provided for the hazard characterisation of manufactured nanomaterials, including elemental composition, purity, density, agglomeration /aggregation, crystal structure, solubility, charge, conductivity, melting point, hardness, magnetic and optical properties, morphology, shape, size and size distribution, surface area and surface characterization: charge, reactivity and layer composition /10,14, 15/. This opinion with some variations is shared by many other researchers in the area of toxicology and ecology of nanomaterials /16,17,18/. An indication of the chemical reactivity of nanomaterial surfaces is also desirable. Where relevant, the description of these characteristics needs to take into account known or anticipated variations over time and under varying conditions /10/. There are various common principles and procedures for approaching particle characterisation that also apply to nanomaterials and which are endorsed by national and international standardisation bodies such as ISO and ASTM.

It is imperative that the sample of particles measured is representative of the substance. The broader the size distribution of the particles, the more significant will be the errors if the sample is not representative. Sufficient sample size must be measured to ensure that the desired limits of accuracy and precision will be achieved. Standard statistical techniques can be used in the determination of representative sample sizes.

To be able to provide representative results, particle size and shape characteristics should be measured in the most relevant dispersed state. Consideration should be given to the possibility that the material characteristics may change during the products life cycle. The most appropriate metrics and the methods of evaluation should be used for the particle and hazard characterisation. E.g. the commonly used mass metric for substances is not necessarily adequate for nanomaterials. More relevant parameters could be number concentration and surface area for the calculation of dose if the dose - response relationship is to be used as a metric for toxic endpoints for nanomaterial effects /10/. Ideally, the characteristics should be measured in conditions that imitate the potential human and environmental exposure.

2.1.1 Special characteristics of ENMs

One of the main reasons why nanoparticles are of interest is the tendency for some of their properties to change as particle size decreases. Properties of nanoparticles like the dynamics of dispersion, the rate of dissolution, the characteristics of nanoparticle aggregates, the surface area and the potential to adsorb substances onto nanoparticle surfaces are all important when assessing the behaviour of and responses to nanoparticles in biological and ecological systems.

Surface area is the total area of the material that is exposed to the environment. Surface area can be external (geometric surface area) as well as internal if the material is porous or is an agglomerate of primary particles. Porous substances of any pore size exhibit higher surface areas than nonporous substances. It is also possible that the characterstics like the total surface area and the effective porosity change in the biological environment as the result of the adsorption of species or the agglomeration of particles themselves.

The principal physical parameters for the characterization of nanomaterials are size, shape, and the morphological structure of the substance /14/. What makes ENMs so different from others is that when the size of the particles decreases, the surface area increases proportional to their size, until the properties of the surface molecules dominate. In practice this means that ENMs have new properties determined by their high surface-to-volume ratios. Many ENMs have changed characteristics compared to their micro/macroscale equivalents due to this fact. ENMs have increased reactivity compared to the non-nanoscale materials since many more molecules are located at the surface. Many types of ENMs act as catalyte, mainly in oxidation reactions. They may also act as nuclei during crystallisation and recrystallisation in material sciences.

In response to their environment, ENMs undergo changes. The high size-surface ratio may create particle interaction. Free ENMs tend to agglomerate or aggregate, resulting in bigger particles which may preserve some of the nanoscale properties, but may loose others.

Case Nano-fibrillar cellulose:
Nano-fibrillar cellulose (NFC) is a cellulose fibril with lateral dimensions of less than 20 nm in diameter.
This nano-fibrillar cellulose is not similar to cellulose whiskers also prepared from cellulose pulp fibres via pulp hydrolysis, because the cellulose whiskers are stiff rod-like particles with a bigger diameter and less aspect ratio as the NFC.
Another well known property of NFC is the very high transparency comparable to those of synthetic polymer foils.
Even more interesting are the rheological properties of NFC suspensions. Intensive dispersion of NFC in water produces a stable gel – even for solid contents as low as 2%/19/.
The degree of the shear thinning behaviour depends mostly on the size dimensions. The viscosity and the linear viscoelastic region are much higher of NFC with 50 nm as NFC with a dimension of 500 nm.
In lab-scale some applications of NFC for food, composite materials and paper have been developed based on the nano-scale properties, which are:
reinforcement of matrix materials/20/
special mechanical properties of NFC films /21/
rheological properties /19/.

2.1.2 Methods for nanomaterial characterization

Microscopy is very powerful and perhaps the most widely used technique on providing information about physico-chemical properties of materials. For nanomaterials electron microscopy is required to have necessary resolution for imaging and it is the only technique that provides reliable information about the shape at this scale. When using electron microscopy it should be kept in mind that the sample size is large enough to be statistically valid representation of full size and shape distribution. This can be very difficult and time consuming and may require image analysis of thousands of particles /22,23,24/.

The two most common techniques within the electron microscopy are scanning electron microscopy (SEM) and transmission electron microscopy (TEM). SEM is primarily used for imaging the surface and topography of materials. TEM can be operated in two modes (image and diffraction). The diffraction mode can reveal whether a material is polycrystalline, single-crystal, or amorphous. The image mode reveals the two dimensional structure of nanomaterials at high spatial resolution, including information e.g. from size, shape, crystalline structure and defects. Because the samples for TEM are much thinner than those typically used for SEM, the spatial resolution in TEM is considerably better /23,24,25/.

One of the most important electron spectroscopy method widely used is energy dispersive spectroscopy (EDS). EDS is based on the fact that electron beam causes ionization of electrons belonging to the inner shells of the atoms that compose the material. Due to these excited electrons, X-rays are emitted with energies that are unique to the ionized atom. Therefore, by measuring the energies or the wavelengths of the X-ray emitted from the sample, the composition of the material can be determined /25/.

There many different surface chemistry analysis techniques that can be useful for the characterization of nanomaterial surface. Among them are FT-IR, X-ray and electron (AES, XPS) spectroscopes, mass spectrometric analysis -TOF-SIMS etc. /26,27/. The techniques for measuring size, size distribution and agglomeration can be divided to light scattering technologies, centrifugal techniques and field-flow fractionation technique.

Dynamic light scattering (DLS) is a versatile set of technique for measuring the sizes and size distributions, nanoparticles in liquids. DLS technique utilizes the ability of particles to scatter laser light. The technique, however, has some limitations. There are some difficulties in estimation of particle size in heterogonous sample with a broad size distribution. Since there is non-linear relation between the intensity of scattered light and the particles' size, this technique is biased toward large particles. The signal coming from large particles present in small amount (often refer as dust particles) may cover the signal from nanoparticles of the small size /28/. Therefore the methods should be used with big care.

One of the biggest advantages of this technique is that the measurements are performed in suspension without any additional sample perturbation. TEM and SEM provide the most direct information on size and shape of the particles. However, one serious disadvantage is that EM is performed on dry sample and there is the risk of changes in particle properties and size during drying and contrasting (performed for SEM) of the sample. Since the biggest concern of toxicology is a tendency of nanoparticle to aggregate, the methods for measuring size of nanoparticle in suspension (in form most relevant for toxicology) are of a special importance. Since the DLS is one of the easily available techniques with the relatively low cost of the apparatus, it has become the most popular method for nanoparticle sizing.

Nanoparticle Tracking Analysis (NTA) is a new technique for direct visualization and size distribution analysis of nanoparticles in suspension. This approach is based on the relationship between the rate of Brownian motion and the particle size /29,30/. This technology has number of advantages. In this technique the particles are tracked on individual basis, therefore it is much less biased by scattering intensity of larger particles that DLS. The other benefits of this technique are a relative ease of use, minimal preparation of the sample, and real-time information. However, it also has some limitations. The lower size limit for detection is around 20 nm and depends on refraction index of the particles. The technique can be used for analyzing particles with the high-aspect ratio (nanotubes). However, since the size estimation is based on assumption that the particles are spherical; the analysis of non-spherical particles should be carried with conscience. Moreover, there is so far no clear information available how long tubes can be analyzed by this method.

3-D tracking for sizing of the nanoparticles has been reported by Xu et. al. /31/. However, this approach required confocal microscope and not easy to perform. Since Nanosight technology is rather easy to use and affordable, it is receiving attention as a tool in toxicological research /32,33,34,35/.

Centrifugal techniques can be applied for the separation of particles of different size. However, the error can arise due to particle shapes and the aggregation of the particles in the medium used for centrifugation. These techniques have rather low size resolution. Size-exclusion chromatography (SEC) is a technique based of chromatographic separation of the particles based on their size. It is easily available technique. There are some limitations, such as risk of non-size interaction in column. The upper limit for the particle size is around 0.2 μm.

Field-flow fractionation (FFF) is similar to chromatography, but uses a thin flow channel through which a solvent transports the sample. FFF has high resolution for particle size range between 1 nm and several μm. The advantage of this method is that the further analysis of sub-fraction is possible using for instance UV-Vis detector, DLS, mass spectroscopy (ICP-MS) etc. The main limitation is, similar to centrifugation and chromatography, the possible errors due to particle shape. This basically means that the application of these methods for nanofibrillated cellulose can be very challenging.

The zeta potential, also called the electro kinetic potential, is defined as the value of the electrical potential at the "shear plane" of the particle. The most common method to define it is the separation of the particles by electrophoresis (the migration of particles in an electric field) followed my detection of the particles by, for instance, DLS technique. The measured electrophoretic velocity is mathematically converted to the zeta potential. The zeta potential is well recognized methods for examining stability of colloid systems. The particles showing high z-potential value tend to disperse well and form stable suspension. Measuring of z-potential is a well established technique with ready available instrumentation. Therefore it, together with DLS, has become most used method for the physicochemical characterization of nanoparticles and is often offered as a set of characterisation methods. In the toxicological research z-potential may be very useful parameter in optimization of the sample preparation since it indicated the dispersion potential of the sample. Z-potential has been used for characterization of nanofibrilles/nanowhiskers of various sources /36,37/.

Case Nano-fibrillar cellulose:
Z-potential has been used for characterization of nanofibrilles/nanowhiskers.
In centrifugation, chromatography and field-flow fractionation the main limitation is the possible errors due to particle shape. This basically means that the application of these methods for nanofibrillated cellulose can be very challenging.
Nanocellulose can be analysed at the nanoscale by electron microscopy. Limiting factor with high-resolution EM is that the samples need to endure high vacuums requiring e.g. dried samples.

2.2 EXPOSURE

The exposure to nanomaterials is likely to vary throughout the product life-cycle. To be able to fully understand all the possible sources and modes of exposure, a product or material should be examined throughout its entire life-cycle from manufacturing to recycling and finally to post disposal stages (Figure 1). This kind of assessment can provide information about what environmental routes are likely and what biological routes are important. Primary considerations in exposure assessment are the likelihood, magnitude and route(s) of exposure.

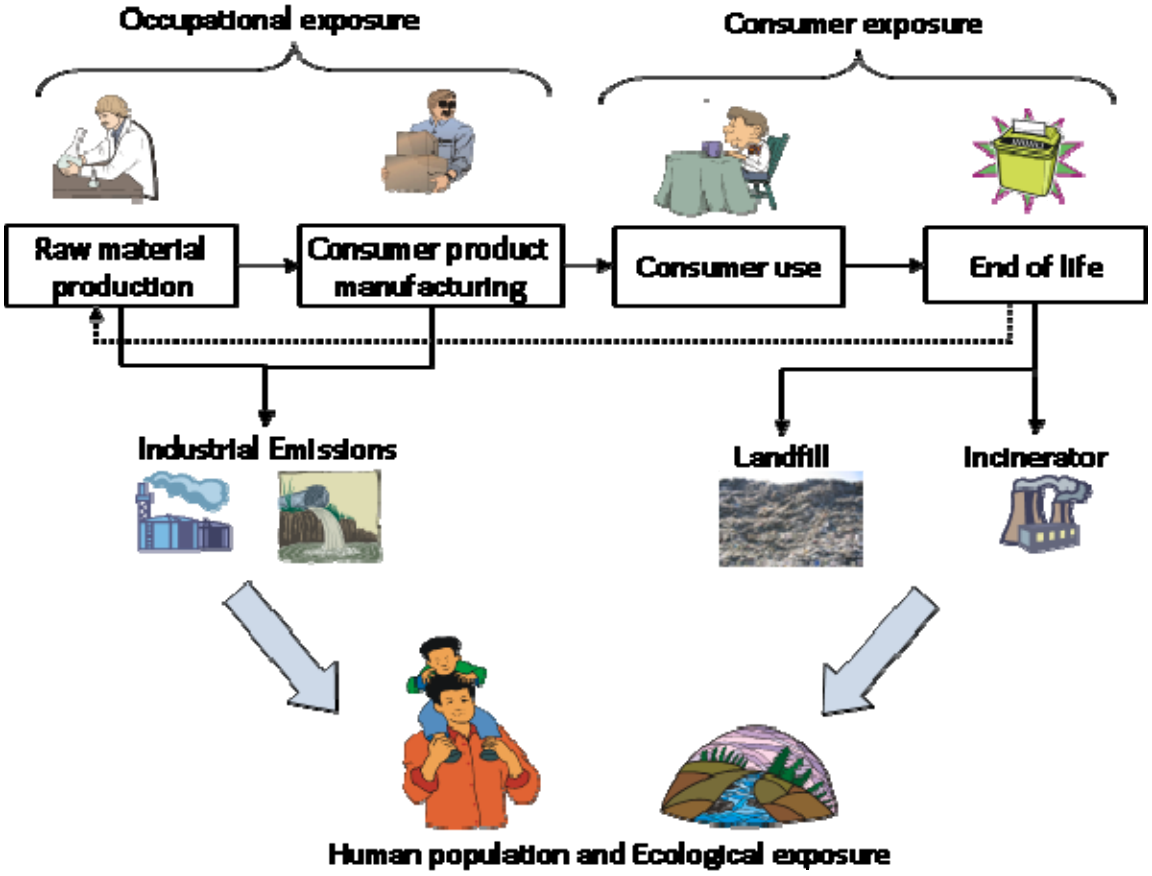

Figure 1. Life cycle analysis and potential exposures Source: Pöyry

Occupational exposure is more likely to occur earlier and have higher quantities per person compared to the general population or consumers. The exposure may occur through inhalation, ingestion, skin absorption and/or exposure of the internal systems of the body (Figure 2). The most studied route in occupational exposure is inhalation /38,39/. However, the exposure studies by the other routes are becoming increasingly important. In different processes different particle sizes with different particle morphology are produced. The amount of nanomaterials depends on the combination of the processes and the materials used; where as the toxicity of the mixture depends on the material.

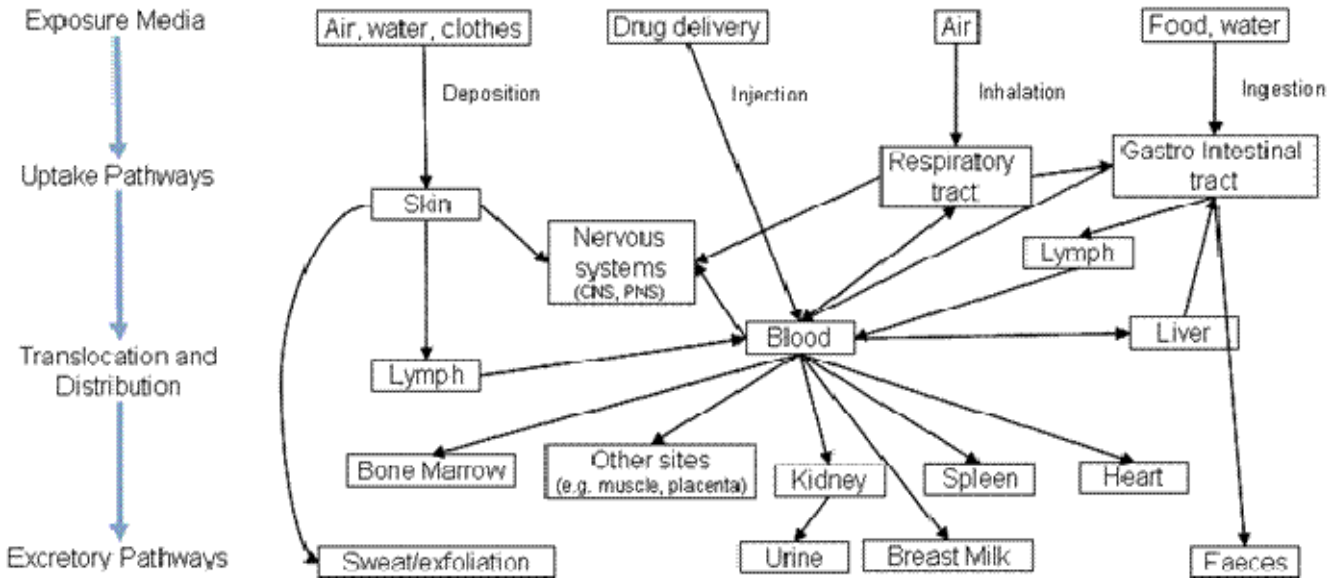

Figure 2. Routes of exposure. Adapted from Mlčoch 2009 /40/.

Surveys have indicated that nanotechnology-related industry workers have the potential to be exposed to nanoaerosols and the degree of skin contamination could not be negligible /41, 42/. Unfortunately there are so little data available that it cannot be used to estimate the exposure of workers in any categories.

Case Nano-fibrillar cellulose:
Occupational exposure to nanocelluloce is possible both on the raw material production site as well as in the consumer product manufacturing phase. It is possible that the occupational exposure route is through air to both to the respiratory tract as well as to skin. It is possible that nanocellulose is handled as dry matter which could introduce exposure to nanocelluloce dust. On the other hand, nanocellulose can be used in foams or in liquids. In this case exposure could be e.g. small droplets of liquid containing nanocelluloce to skin.
It is likely that nanocellulose is used widely in consumer products. As the possible products are still unknown, it is difficult to assess the possible exposure routes. It might be that no or very little nanocelluloce is actually detached from the product during normal use.
The end of life of products containing nanocellulose can be directed to recycling, incineration or to landfills. Both recycling and incineration requires more studies regarding possible exposure routes. It is likely that nanocellulose acts similarly to cellulose in incineration and as cellulose is biodegradable, it is likely that nanocellulose is biodegradable as well.
During the manufacturing of raw material or consumer products, it is possible that some nanocellulose is released with other pollutants. The possible routes of pollution should be studied in more detail e.g. some nanocellulose could be emitted from effluent treatment plants.

2.2.1 Tools for measuring exposure

In order to measure exposure to ENMs, it is necessary to take into account the background exposure to ambient nanoparticles. At present there are limited data from occupational and environmental monitoring of manufactured nanoparticles, for an assessment of their contribution to the overall exposure to be made.

Industrial environment is a complex structure, which contains several gas and particulate pollutant sources. The emissions are dependent on processes, material transport and other activities. The variation in emission sources and in air exchange, make the discrimination of process nanoparticles from background particles very difficult. However, this is essential to do when considering the airway exposure of workers to nanoparticles.

Exposure to all aerosol particles can be measured in real time with several different particle counters and aerosol monitors. At the current level of knowledge, exposure assessment to nanoparticles follows the methods used in exposure assessment to ultrafine particles (particles which diameter is less than 100 nm) /43/. The particle concentration, in the size range of 3 nm to 3 µm, can be measured with condensation particle counter (CPC) which are available both portable (for example TSI Portacount) and stationary. Size-sectional particle analysis can be made with scanning mobility particle sizer (SMPS) (in the size range of 10 nm to 1 µm) or electronical low pressure impactor (ELPI) (in the size range of 10 nm to 7 µm). If the measurement range starts from around 200 nm, the aerosol particle size-sectional analysis can be done with optical instruments which are available on portable size (for example Fluke 983 Particle counter, Grimm Dust Monitors).

With present-day aerosol instrumentation and knowledge, the discrimination of nanosized process particles from background particles in real-time is challenging. Aerosol instruments measure collective properties of the aerosol, like surface-area, mass concentration or concentration of size-fraction. This is usually insufficient to trace the particle origin and off-line chemical or microscopic analysis is needed. In real-time monitoring, one way to identify the origin of the particles is to measure several particle physical quantities, which are also characteristic properties of process particles. For example Rosted et al. /44/ shows an example of effective density measurements by using combined differential mobility analyzer and electronical low pressure impactor. They measure mobility classified particle aerodynamic diameter from which they are able to quantify effective density of the aerosol. With this method, it might be possible to identify dense nanoparticles from background aerosol particles. Another combination could be mobility classified optical diameter which gives information about refractive properties of the particles as a function of mobility size. However, these methods are not tested in laboratory conditions with background aerosol or in real industrial environments. The instruments resolution limit functionality of these methods when background aerosol particle properties are close to process particles or the background particle concentration is high compared to process particle concentration.

Case Nano-fibrillar cellulose: Atmospheric concentrations of NFC can be estimated by analyzing electron microscopy

collection samples. When the collection efficiency of nanoparticles is known, and the particles are non-volatile, the concentration can be estimated. Accuracy of the method depends on sampling location, filter burden and sample volume

2.3 TOXICOKINETICS

Risk is dependent on both the exposure and the hazards, and it is driven by the uptake of nanoparticles by different routes. Nanoparticles can distribute from the site of entry to other sites in the body. Studies have shown that nanoparticles can accumulate in areas with increased permeability and cross barriers like the blood brain barrier /45,46,47,48/. Toxicokinetics is the science dealing with absorption, distribution, metabolism and excretion of substances in the body. The whole series of events occurring after an exposure determines the internal exposure of organs at risk to potential toxic substances.

For nanotechnology studies metallic colloidal gold nanoparticles are widely used as a model. They have several good characteristics like 1) they can be synthesised in different forms 2) are commercially available in various size ranges and 3) can be detected at low concentrations. Besides, human cells can take up gold nanoparticles without cytotoxic effects /49/.

Case Nano-fibrillar cellulose:
There are no toxicokinetic studies available for Nanofibrillous cellulose.

2.3.1 Absorption

As described before (Figure 2), nanoparticles normally have three possible portals of entry, the lungs, skin and gut. In addition intravenous injection is studied for medical purposes. The uptake of nanoparticles is also possible after absorption at the nasal epithelium and transported by the olfactory nerve.

In the lungs, the site and the extension of exposure will depend on the thermodynamic and aerodynamic diameters of the particles. The aerodynamic diameter is important to be able to determine which compartments of the respiratory system would be exposed, the upper respiratory tract, the airways, or the alveoli /50/. Migration of the nanoparticles from the surface of the lungs across the epithelium to the interstitium is of fundamental importance. This susceptibility to translocate to the interstitium may differentiate nanoparticles from larger particles.

There are some evidence that particle formulations are susceptible to transdermal transport, especially if the skin is flexed. Nevertheless, the ability of nanoparticles penetrate through the healthy skin is questioned in the literature. There is some evidence that small particles can enter the dermis /51/. Nanoparticle charge has reported to be the determining factor in skin penetration /52/.

In general, both nano- and microparticles are ingested every day. It is estimated that 10^{12}-10^{14} particles, are ingested per person per day in the Western world from products like food or toothpaste /53/. Little is known about the behaviour and fate of ENMs in the gastrointestinal tract. It is possible that they react with e.g. acids or enzymes or loose their free form due to transformations or reactions with other components of food. Thus, nanomaterials may disappear totally or partially. There are several potential routes for nanoparticles to be taken up from the gut, including uptake by intestinal wall.

Absorbtion of particles through intestinal wall depends on their physico-chemical structure, e.g. size, surface charge, lipophilicity or hydrophilicity /54/. Studies with rats and humans suggest that TiO_2 particles can absorb from the gastrointestinal tract to the blood and accumulate in the liver and spleen /55/. Smaller particles are generally absorbed more easily and faster than larger particles /56,57,58,59/. Absorption of ENMs is 15-250 times greater than that of microparticles /60/.

2.3.2 Distribution

Kreyling et. al /47/ have demonstrated the translocation of nanoparticles from the upper respiratory tract into the brains. Studies with rats suggest that nanoparticles have high efficiency of deposition in the nose, followed by some migration to the olfactory neurons, entering the olfactory bulb of the brain /61/. The uptake of different kind of inhaled nanoparticles in the secondary target organs (liver, spleen, kidneys, heart and brain) is shown by various different studies /62,63,64,65,66,67,68/. When rats were exposed by inhalation for 5 days to nanoparticles, gold was only detected in the lung and olfactory pulp, after 15 days exposure, gold could also be detected in heart, lived, pancreas, spleen, kidney and testes /69/. The available studies suggest that both the blood brain barrier (BBB) and the blood testis barrier (BTB) can be penetrated by nanoparticles.

There is some information available about the long term biokinetics of ENMs. According to Semmler et.al /70/ and Semmler-Behnke et. al. /68/ after a short-term inhalation about 1-5% of the ENMs crossed the air-blood-barrier and accumulated in liver, spleen, kidneys, heart, brain, bone and remaining carcass. Nanomaterial concentrations remained constant over six month’s period. Studies with nanoparticles for drug delivery purposes have found that for example coating with polyethanol glucol diminish the uptake into the tissue for any particle present in the blood, thus potentially prolonging the circulation of nanoparticles /6/.

An important characteristic of ENMs is interaction with proteins which may enhance membrane crossing and cellular penetration /6/. It is estimated that interaction with ENMs may affect the structure of a protein resulting in functional disorder /71/. The distribution patterns after oral exposure has been studied for silver and gold nanoparticles and polystyrene ENMs. In rat studies the highest NP levels for silver were in the stomach /72/. Silver was found also in kidney and liver, lungs, testes, brain and blood /72/. Mice test with gold nanoparticles suggested that smaller particles had larger distribution to organs /73/. The smaller particles were found in kidney, liver, spleen, lungs and brain, while the bigger ones remained mainly inside the gastrointestinal tract.

One study reported about that about 7 % of up taken polystyrene were found in liver, spleen, blood and bone marrow /58/.

When rats were injected with solutions containing various sized nanoparticles, the distribution was found to be size dependent. The smallest particles showing the most widespread distribution, including blood, heart, lungs, liver spleen, kidney, thymus, brain and reproductive organs /74/. The largest particles were present in liver and spleen. Studies with ultrafine particles indicate that liver is the major organ of their uptake /75/.

The distribution of nanoparticles to the placenta and foetus has been studied for C_{60} fullerenes and for gold nanoparticles. C_{60} fullerenes seem to be able to pass through the placental barrier and end up to the embryo /76/. On the other hand, gold nanoparticles injected did not seem to penetrate the placental barrier /77/, but after intratracheal or intravenous administration to pregnant rats small fractions of gold nanoparticles were found in the placenta and foetuses /78/.

2.3.3 Metabolism

The metabolism of ENMs depends e.g. on their surface chemical composition. It is possible to design some polymeric ENMs to be biodegradable. On the other hand slow dissolution is important for metal and metal oxide ENMs. Metabolism of soluble substances is different from the substances in the form of persistent solid particles. However, it is not clear what the metabolic fate of any persistent nanoparticles is. It is possible that some nanoparticles such as carbon nanotubes may be excreted directly or undergo transformation. In addition carbon based nanoparticles are likely to have carboxyl and hydroxyl groups on their surfaces which may allow metabolism /9/.

2.3.4 Excretion

Some studies of excretion have been made for ENMs, but the data available is limited. After intravenous administration in mice, gold nanoparticles were partially filtrated into the preurine /77/. In another study intravenous administration in mice resulted nano gold composite in both urine and faeces /79/. For polymethyl methacrylate nanoparticles administered orally to rats, 95% of the total amount absorbed was eliminated after 2 days and after 8 days less than 0,5 % of the dose remained /80/.

After intravenous administeration in rats, TiO_2 nanoparticles were retained in the liver for 28 days, decreased in spleen and a returned to control levels in 14 days in the lung and kidney /81/. A study made with quantum dots resulted to kidney clearance of particles with a diameter less than 5.5nm while larger particles were less cleared and accumulated in secondary target organs /82/.

2.4 TOXICITY

Toxicity is an essential part of risk assessment and hazard characterisation. At this stage most information about the toxicological characteristics of nanoparticles has been derived from inhalation toxicity studies. Some important data, like the effects of

repeated doses of nanoparticles, are not yet available. Most of the studies are made using insoluble metals and metal oxides.

Case Nano-fibrillar cellulose:
No nanocellulose toxicity studies are known to be published. It is possible that nanocellulose acts like other fibre-like nanoscale structures like carbon nanotubes. Fibers such as asbestos cause fibrosis and cancer that could be due to the direct effects of fibers on cells or as a result of oxidative stress from the fibers or the inflammatory response. Carbon nanotubes are known to cause cytotoxicity and there are evidences that carbon nanotubes may cause oxidative stress and DNA damage /83,84/.

2.4.1 Immunotoxicity

Fine particles, both model particles and ambient air particles, are known having adjuvant activity under which may worsen responses to allergens /85,86,87,88,89/. In these studies smaller particles were found to cause the stronger adjuvant effects. In addition, to evoking pulmonary inflammation in healthy subjects, the airway exposure to engineered nanomaterials may also influence the development and severity of other allergic pulmonary diseases. Oxidative stress and/or production of proinflammatory cytokines can trigger immune effects /22,90,91,92/.

2.4.2 Neurotoxity

Nanoparticles could gain access to brain basically by two different mechanisms, the trans-synaptic transport, and uptake from the blood through the blood-brain barrier /93/. The possible impact of nanoparticles on neuronal tissue has not yet been investigated thoroughly. Nanoparticles may induce the production of reactive oxygen species and oxidative stress in vitro. There are some indications that nanoparticles may migrate to the brain and have some effects on the brain although whether these effects results in disease remains unknown /10/.

A number of pathologies have been associated with increases in the permeability of the blood brain barrier to nanoparticles in experimental animals, increasing their susceptibility to diseases /91/. In addition, the nanoparticle surface charge has been shown to alter blood-brain integrity suggesting that such factors should be considered when studying brain toxicity /94/.

2.4.3 Mutagenicity and Genotoxicity

The chemical composition and surface reactivity are known to play major modifying roles. Especially particles with an insoluble or poorly soluble core onto can carry various adsorbed mutagens from the environment into and throughout the human body/10/. DNA adduct formation has been linked to specific combustion-generated nanoparticles /95/. The possible genotoxic and mutagenic effects of particle-associated organics or metals will depend on their bioavailability /96/.

The production of reactive oxygen species has been regarded as a major mechanism through which genotoxicity is induced by particles. Increased levels of reactive oxygen species can be generated by particles themselves, upon particle-cell contact or as a consequence of particle deriving inflammation /97,98,99/. It is suggested that nanoparticles can penetrate the mitochondria and nucleus causing uncoupling of respiration and increased oxidative stress or interference with the genomic replication or repair /100,101/. It's also likely that some ENMs are genotoxic including iron/platinum, cobalt/chromium, ZnO, SiO_2, TiO_2, carbon black, carbon single walled nanotubes and carbon multi walled nanotubes /102,103,84/. For TiO_2 and carbon black it has been found that the nanosized particles induced DNA damage, while larger particles did not /104,105,106/. Similar results are reported for cobalt nanoparticles /107/.

2.4.4 Carcinogenicity

The overall ability of any particle burden to cause chronic inflammation and fibrosis, and therefore potentially to be carcinogenic, will depend on the surface area and reactivity /108, 10/. This has important implications for engineered nanoparticles which have very high surface areas per unit mass with the potential to have a reactive surface.

Studies of the carcinogenicity of particles are difficult to interpret when considering dose responses, the ability to extrapolate between species and the appropriate metrics /10/. It is likely that inhaled, non-toxic, nanoparticles can induce lung tumours by mechanisms similar to those found with fine particles. The mechanisms include DNA damage and increased cell proliferation associated with a persistent inflammation in the lung. Weather the metric driving to response is dose, mass, surface area, volume or particle size data is still unclear. Surface area has the strongest support from toxicological evidence. Because nanoparticles have higher surface area, they have a stronger theoretical potency to induce lung tumours /96/.

2.4.5 Pulmonary effects

Pulmonary toxicity studies suggest that nanoparticles generate enhanced inflammatory responses compared to larger-sized particles or identical chemical composition at equivalent mass concentrations /109,110/. The high size-specific deposition rate of nanoparticles inhaled as single nanoparticles contribute to the effects of toxicity /91/. Pulmonary effects caused by lung particle overload were studied for TiO_2 nanoparticles. Chronic inhalation studies show that less than one-tenth the inhaled mass concentrations of the aggregated nanoparticles, compared with the fine particles, produced equivalent numbers of lung tumours in rats /111,112/. In addition, studies with carbon black, nickel and TiO_2 particles in rats have demonstrated enhanced lung inflammatory potency of the nanoparticles compared to fine-sized particles of the similar composition /113,114,115/.

2.4.6 Dermal effects

Particles with a size of 50-500 nm are widely used in cosmetics products, e.g. to improve the homogeneity of the product, or to act as UV filters against sun radiation. The concentration of nanoparticles in cosmetics products usually under 3% /91/. The

discussion on dermal effects has concentrated on the question on whether these particles are able to penetrate into or through the skin. In some studies focusing on the sunscreens containing nanosized TiO_2, nanoparticles were not found to enter the skin /116,117/. On the other hand, there is some evidence that some small particles can enter the dermis /51/. It seems that nanosized particles are more likely to enter more deeply into the skin than larger ones. This suggests that the penetration of the skin is size dependent. Different types of particles are found in the deeper layers of the skin and at present too little is known to predict the behaviour of a particle in the skin /118/. Materials, which can dissolve or leach or break into smaller parts, can possibly penetrate skin.

It's known that fine fibres can cause strong skin irritation e.g. glass fibres and rockwool fibres which are widely used as insulation material can induce dermatitis through the mechanical irritation /118/. The reason why these fibres are such strong irritant has not been examined in detail.

2.4.7 Cardiovascular effects

Nanoparticles have been studied as agents for molecular imaging or drug delivery. These studies have lead to some understanding of particle properties that can affect penetration in tissue without affecting tissue function. A size dependent nanoparticles penetration in aorta vessel wall was noted after local delivery of polystyrene nanoparticles /119/. Cationic nanoparticles like gold and polystyrene have been shown to cause haemolysis and blood clotting, while these usually anionic particles are quite non-toxic /91/.

Animal studies with combustion nanoparticles show that high exposures cause cardiovascular effects. Toxicological studies have demonstrated that combustion and model nanoparticles can gain access to the blood following inhalation or instillation and can enhance experimental thrombosis /120/. Diesel particles instilled into hamster lungs also enhance thrombosis /115,121,122,123/. High exposures to diesel exhaustion particles by inhalation caused altered heart rate in hypertensive rats /124/. In contrast to previous studies, a study found that inhaled nanoparticles didn't pass directly from the lungs into the systemic circulation /125/. Another study found that tiny fractions, at most, of nanoparticles have access from the peripheral lungs to systemic circulation and extrapulmonary organs /126/.

2.4.8 Existing diseases and susceptibility to the effects of nanoparticles

There are likely to be individuals vulnerable to unfavourable effects of nanoparticles on the basis of existing diseases /10/. Individuals with diseases like asthma or chronic obstructive pulmonary disease may experience exacerbation of their disease, when exposure to nanoparticles is elevated. The effects will be related to the oxidative and pro-inflammatory effects of nanoparticles, inflamed lungs being more permeable such that nanoparticles may cross the epithelium more readily in these individuals. Diabetic patients are also at potential risk as they have endothelial dysfunction, similar to the

patients with cardiovascular disease. It is also possible that the uptake of nanoparticles may be enhanced in smokers /10/.

3 RISK ASSESSMENT METHODS

According to European commission /127,128/ "Extensive guidance on information requirements and chemicals safety assessment" (IR-CSA TGD) is available from ECHA /129/. The principles and approaches to risk assessment of substances as elaborated in the IR-CSA TGD are considered to be applicable to the risk assessment of substances at nanoscale. However, the guidance does not yet address specific properties of substances at nanoscale and it will need further adjustments to be able to fully assess "the information related to substances at the nanoscale/nanoform, to assess their behaviour and effects on humans and the environment, and to develop relevant exposure scenarios and risk management measures" /129/.

The EU Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) has produced two opinions in relation to risk assessment of nanomaterials /130,131/. In the first opinion SCENHIR concluded that the "existing toxicological and ecotoxicological methods… may need to be supplemented by additional tests, or replaced by modified tests, as it cannot be assumed that current scientific knowledge has elucidated all the potential adverse effects of nanoparticles" /130/. Specifically "attention needs to be given to the mode of delivery of the nanoparticle to the test system to ensure that it reflects the relevant exposure scenarios" /130/. SCENIHR /130/ also has expressed the view that "the use of mass concentration data alone to express dose is insufficient, and that the number concentration and/or surface area would need to be used as well". Equipment to be able to measure exposure to free nanoparticles routinely is not yet available. Especially the equipment for measuring environmental exposure are not yet developed enough to assess the environmental fate adequately. According to SCEHIHR current risk assessment procedures may require modifications /131/.This could lead to additional requirements of test methods to demonstrate potential new hazards.

Because of the uncertainties related to nanomaterials Control Banding (CB) as a risk assessment and management method has been tested for nanomaterials /132/. CB is a technique that determines a control measure based on a range of hazards like skin/eye irritant, very toxic, carcinogenic, etc. and exposures in a three step scale /133/. The method is based on two basic ideas 1) there are limited number of control approaches and 2) many problems have been met and solved before /133/. CB uses the solutions previously used and uses them to other tasks with similar exposure situations; this focuses resources on exposure controls and describes how strictly a risk needs to be managed. CB is a qualitative risk approach and it's intended to help small companies by providing an easy-to-understand, practical approach to controlling hazardous exposures at work /133/.
Derived No-Effect Levels (DNELs) and Derived Minimum Effect Levels (DMELs) are used in assessing e.g. the level of exposure accepted or the possible risks of a substance to humans. When considering EU chemical regulation DNELs are of more importance because EU REACH legislation requires DNELs from manufacturers and importers of

chemicals as a part of the Chemical Safety Assessment (CSA) for any chemicals used in quantities of 10 tonnes or more per year /134/. In REACH DNEL is defined as the level of exposure above which humans should not be exposed /134/.

In REACH DNELs are used in the risk characterisation part of the CSA as a benchmark to determine adequate control for specified exposure scenarios. Risk to humans can be considered to be adequately controlled if the exposure levels estimated do not exceed the appropriate DNEL. According to REACH, DNELs should reflect the likely exposure routes, duration and frequency /134/. If more than one exposure route is likely to exist, DNEL should be established for each route of exposure and for the exposure from all routes combined.

The used DNEL methodology in REACH is intended to harmonise the approach to occupational health risk assessment /134/. DNELs can be compared with the Predicted No-Effect Concentration (PNEC) used in assessing environmental risks required in REACH. This is important, as manufacturers must assess not only human health risks, but environmental and physical safety risks as well.

Predicted No-Effect Concentrations (PNECs) and Predicted Minimum-Effect Concentrations (PMECs) are used similarly like DNELs and DMELs but in relation to environment. Again, REACH requires the use of PNECs in risk characterisation. PNEC is defined in the REACH legislation as "the concentration of the substance below which adverse effects in the environmental sphere of concern are not expected to occur" /135/. PNECs are used in characterising the environmental risk of a substance.

The risk characterisation of REACH consist "a comparison of the exposure of each human population known to be or likely to be exposed with the appropriate DNEL, a comparison of the predicted environmental concentrations in each environmental sphere with the PNECs and an assessment of the likelihood and severity of an event occurring due to the physicochemical properties of the substance" /135/. In REACH it's considered that the risk is adequately controlled if the estimated exposure levels do not exceed the appropriate DNEL or PNEC levels.

According to Pronk et. al. /136/ in the derivation of DNELs and PNECs for nanomaterials, there are uncertainties whether the procedure for deriving DNELs and PNECs for non-nanomaterials is applicable for nanomaterials. Principally, the procedure is about converting measures of dose-response in toxicity studies into DNELs and PNECs by application of assessment factors. These factors address uncertainties in the extrapolation of experimental data for the real exposure situation.

For non-nanomaterials, there are standard default assessment factors have been established, based on common regulatory practice and comprehensive toxicological database analysis. Since these factors are not available for nanomaterials at the moment, it's uncertain if it's possible to apply the standard default assessment factors of non-nanomaterials also to nanomaterials.

Case Nano-fibrillar cellulose:
No estimates for DNELs/DMELs or PNECs/PNELs for nanocellulose are known to be published.
The most extensive study published covering DNELs and PNECs for nanoparticle is presented in nanosilver case study conducted by Pronk et. al. /136/. In the same study it has been stated that the methodology for the derivation of DNELs and PNECs in REACH is not necessarily applicable for nanomaterials /136/.

3.1 EXPOSURE ASSESSMENT

Exposure assessment of NFC and FNFC is challenging as it's likely that both NFC and FNFC are used widely in different consumer products. Nevertheless, in the SUNPAP project, the goal is to provide realistic exposure conditions by characterization of the behavior of NFC and FNFC in aerosols and liquid media used *in vitro* trials. This is done by standard physical and optical methods (e.g. electron microscopy, dynamic light scattering, SMPS, ELPI, DPM). The results of the exposure models for *in vitro* and *in vivo* studies are described more closely in the SUNPAP project deliverable "D9.1 Description of *in vitro* and *in vivo* exposure model".

3.2 HAZARD ASSESSMENT

Cellulose as such is considered as a safe natural material, and the safety assessment of nanofibrillarcellulose (NFC) or functionalized nanofibrillarcellulose (FNFC) depends on the specific novel characteristics possibly introduced by the small particle size, the eventual functionalization, and the accompanying bioactivities. Inorganic nanoparticles and nano-/microfibers are not particularly suitable as risk assessment models for NFC, because of the different chemical composition, size range, shape and physicochemical properties. Therefore the experiences obtained with inorganic nanomaterials can be considered as indicative only.

The crucial safety aspects of NFC depend on its ability to interact with cells either directly or indirectly (for example, via formation of reactive oxygen species). The in vitro cytotoxicity and immunotoxicity tests, together with the physical characterization of the materials, will give indication whether the NFC fibres will be able to cause cellular damage and whether their systemic effects will be likely. Also a nematode model based on a well known test organism (*Caenorhabditis elegans*) that can be used to investigate both potential systemic effects and neurotoxicity will be employed for the safety assessment.

While the exposure of the consumers is likely to happen via the oral route, the inhalatory exposure is of special importance considering the occupational safety. Therefore, an inhalatory toxicity study with experimental animals is included in the test program. The testing scheme is outlined in Figure 3. It should be noted that the in vitro assays and nematode tests are relatively high throughput assays, suitable for the testing of several materials, while the inhalatory studies are technically demanding.

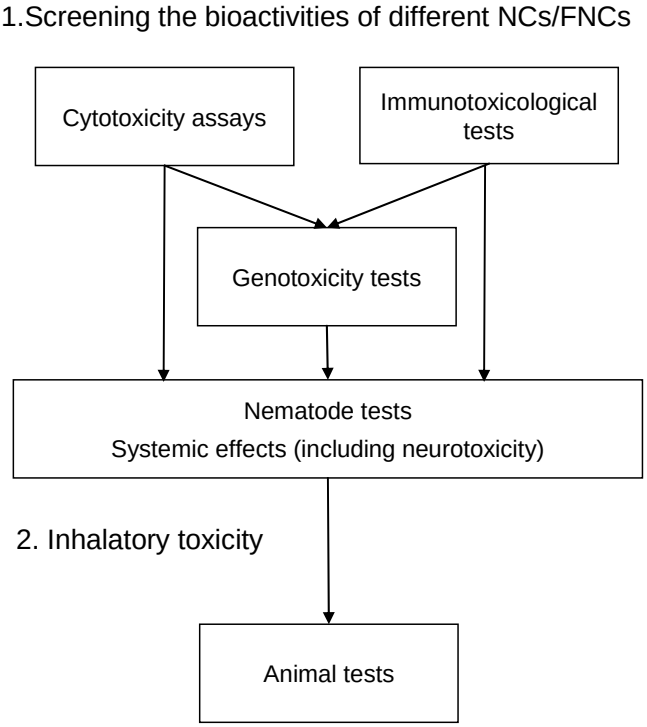

Figure 3. Hazard assessment testing scheme

3.3 RISK ASSESSMENT

The final risk assessment is a combination of results from exposure and hazard assessment. The final methodology will be developed during the project. As a result a verbal risk profile for each studied material is formed. Risk assessment reports and draft chemical safety reports will be generated for the evaluation of the applicability of the methods used in exposure and hazard assessment. Control banding approach will be used as a part of risk management approach of the NFC and FNFC. This means classification of the chosen nanocellulose fibers into appropriate hazard categories based on the toxicity data available. In addition DNELs and PNECs for selected substances are formed and tested as one approach of assessing risk.

4 ENVIRONMENTAL ASPECTS

As more products containing nanomaterials are developed, there is greater potential for environmental exposure. Potential nanomaterial release sources include direct and/or indirect releases to the environment from the manufacturing, processing and use of nanomaterials. The fundamental properties concerning the environmental fate of nanomaterials are not well understood, as there are just a few available studies on it /9/.

Case Nano-fibrillar cellulose:
Potential nanocellulose release sources to environment include manufacturing, processing and use of products containing nano-fibrillar cellulose. They have not been closely assessed as the technology for manufacturing and processing nanocellulose is still under development and the products where nanocellulose could be used are not defined.
During the different production and usage phases it is possible that nanocellulose end up in air, water and through water to soil.

4.1 NANOMATERIALS IN AIR

In addition to the dimensional and chemical characteristics, several processes and factors influence the fate of airborne particles. To be able to understand the potential atmospheric behaviour of particles, processes like diffusion, agglomeration, wet and dry deposition and gravitational settling are of utmost importance. These processes are relatively well understood for ultrafine particles and the results may be applicable to nanomaterials /137/. With respect to the length of time particles remain airborne, particles with aerodynamic diameters in the nanoscale range (<100 nm) may follow the laws of gaseous diffusion when released to air.

The rate of diffusion is inversely proportional to particle diameter, while the rate of gravitational settling is proportional to particle diameter /138/. Deposited nanoparticles are typically not easily resuspended in the air or re-aerosolized /139,138/. Because particle size is a critical property of nanomaterials, it's important that the particle size is maintained during the use of nanomaterials. In the future materials may be more easily dispersed, because e.g. carbon nanotubes which don't form clumps are developed /140/.

Many nanosized particles are found to be photoactive, but their tendency to photodegradation in the atmosphere has not been studied /139/. Nanomaterials are also known to readily adsorb a variety of materials /137/, and many act as catalysts. However, no studies are currently available that examine the interaction of nanosized adsorbants and chemicals sorbed to them, and how this interaction might influence their respective atmospheric chemistries.

4.2 NANOMATERIALS IN SOIL

The fate of nanomaterials in soil is uncertain. Due to their high surface areas they could be sorbed to soil and be immobile. At the same time, nanomaterials are able to fit into smaller spaces between soil particles, and might therefore travel farther than larger particles. The fate of a nanomarial will be dependent on the size, chemistry, applied particle surface treatment and the conditions under which the material is applied. It has been demonstrated that there are differences in mobility of different insoluble nanosized materials in a porous medium /141,142/.

Additionally, the types and properties of the soil and environment can affect nanomaterial mobility. For example, the mobility of mineral colloids in soils and

sediments is strongly affected by charge /137/. Transformation of nanomaterials on soil surfaces is possible because of the surface photoreactions. Studies of nanomaterials in field situations are complicated because humic substances, which are common in the field, are known to photosensitize a variety of organic photoreactions on soil. In addition there are naturally occurring nanomaterials present in the field conditions.

4.3 NANOMATERIALS IN WATER

Solubility or dispersability, interactions, bilociald and abiotic processes control the fate of nanomaterials in natural water environments. Waterborne nanoparticles generally settle more slowly than larger particles of the same material. Because of their high surface are relation to mass, nanoparticles have the potential to sorb to soil particles /90/. If these soil particles are subject to sedimentation, the sorbed nanoparticles can be removed from the water column. Some nanoparticles might even be subject to biotic and abiotic degradation thus being removed from the water column.

These reactions may alter the physical and chemical properties of nanomaterials and so alter their behaviour in aquatic environments. Certain organic and metallic nanomaterials may possibly be transformed under anaerobic conditions. From past studies, it is known that several types of organic compounds are generally susceptible to reduction under such conditions /143/. Complexation by natural organic materials such as humic colloids can facilitate reactions that transform metals in anaerobic sediments /143/.

In contrast to processes that remove nanoparticles from the water column, some dispersed insoluble nanoparticles can be stabilized in aquatic environments. The stability of the particles and suspensions is sensitive to pH, ionic strength and presence of natural organic matter /144,145/. In addition, the intrinsic properties and characteristics of the materials, including their specific chemistry, will influence their fate and behaviour. Sea surface microlayers could possibly sorb nanoparticles and transport them in aquatic environments over long distances /143/. This kind of interactions could delay nanoparticle removal from the water column.

Treating drinking water, waste- and groundwater uses increasingly heterogeneous photoreactions on metal oxide surfaces. Nanomaterials like titanium dioxide and zinc oxide have been shown to be effective catalyst in reduction of halogenated chemicals and oxidation of different pollutants. The fate of nanoparticles in water and wastewater are being studied /143/.

The fate of nanosized particles in wastewater treatment plants is not well characterized. Wastewater may be subjected to many different types of treatment, like physical, chemical and biological processes, depending on the characteristics of the wastewater and the type of the treatment plant etc. Nanosized particles are most likely to be affected by sorption processes and chemical reactions. On going research in this area includes production of microbial granules that are supposed to remove nanoparticles from simulated wastewater /143/. It is possible that those nanomaterials which escape primary treatment may be removed from wastewater after biological treatment. The rate of gravitational settling of nanomaterials in water is dependent on particle diameter.

However, settling of nanomaterials could be enhanced by entrapment in large sludge flocs, removal of which is typically objective of secondary clarifiers.

4.4 BIODEGRADATION

The most used nanomaterials currently are inorganic chemicals like ceramics or metals, which are not expected to be biodegradable. However, biodegradation of nanomaterials composed of organic molecules may result in their breakdown as typically seen in biodegradation of organic molecules, or may result in changes in the physical structure of surface characteristics of the material. The biodegradation of nanomaterials have begun to be investigated. C_{60} and C_{70} fullerenes have been found to be taken up by wood decay fungi after 12 weeks /146/. This suggests that fullerens were metabolized. For other nanomaterials biodegradability may be integral to the material's design and function. This might be the case for some biodegradable polymers being investigated for use in drug transport /147,148/.

Biodegradability in waste treatment and the environment may be influenced by a variety of factors. In some studies C_{60} fullerenes have suggested toxicity to bacteria under aerobic and anaerobic conditions/149,150/. More studies are needed to find whether fullerenes may be toxic to microorganisms in water. It's also possible that the photoreactions and other abiotic processes alter the bioavailability and biodegradation rates of nanomaterials. As a summary, not enough is known to enable any estimation on the biodegradation of nanomaterials in the environment.

Case Nano-fibrillar cellulose:
As cellulose is biodegradable, it is likely that nanocellulose acts similarly. Nevertheless, it is possible that biodegradation results in changes in the physical structure or surface characteristics of the material. There are no existing publicly available studies regarding the biodegradation of nanocellulose.

4.5 BIOAVAILABILITY

Because the uptake, distribution, clearance and elimination of ENMs may differ from those chemical substances for which the ecotoxicity tests were developed, it is uncertain whether these tests are sufficient for the testing or if new tests are needed. Several mechanisms are available to enable organisms to take up ENMs. One example is the ability of micro-organisms to carry out pinocytosis and/or phagocytosis /10/. There is some knowledge for the upper limits of particle size for these processes, but the uptake of nanoparticles is not very well understood in relation to these processes.

Ranges are reasonably well defined for some taxa although it is important to note that there are both active and passive mechanisms for ENMs uptake. It's possible that environmental fate processes are too slow to be able to remove nanomaterials before they can be taken up be an organism. Processes like gravitational settling are slower for small particles like nanoparticles. This kind of effect would lead to an increased potential

for inhalation exposure to terrestrial organisms and for increased exposure of aquatic organisms.

As there is practically no information on how ENMs behave in different environmental conditions, and as it is unclear what the main exposure and uptake routes may be for different species, it is unreasonable to produce any guidance for appropriate standard test taxa or procedures to assess the effects of nanoparticles on different environmental conditions.

Case Nano-fibrillar cellulose: There are no publicly available studies concerning the bioavailability of NFC

4.6 BIOACCUMULATION

The standard way to estimate bioaccumulation is to measure Kow (log of partition coefficient octanol-water) or Koc (organic carbon partition, soil, sediment, suspended organic matter and sludge) or the bioconcentration factor (BCF) which is the ratio between the concentration in the organism and the concentration in water in steady-state or equilibrium situation. Because this test is not designed for ENMs the food chain transfer of nanoparticles should be assessed in the light of the fact that the conventional BCF (bioconcentration factor) assumptions as presently proposed may not hold for nanoparticles. It is suggested that the simplest way to estimate the potential of a substance to bioaccumulate in environmental species is through experimental measurement of BCF /10/. As bacteria and many aquatic multi-cellular organisms can uptake nanosized materials there is a possibility that nanomaterials accumulate in the food chain /151,10/.

Case Nano-fibrillar cellulose: There are no publicly available studies concerning the bioaccumulation of NFC

4.7 ECOTOXICOLOGY

Very few published studies focus on the effects of nanomaterials on the biota or on the mechanisms of any potential ecotoxicity. A range of metal oxide and silver nanoparticles have been developed as antibacterial substances. The effects of these nanoparticles on non-targeted microorganisms in the environment are of concern. The effects of fullerens have been studied for soil microorganisms, suggesting reduction in growth and respiration/144/. It's suggested that silver nanoparticles can accumulate in Escherichia coli bacteria causing death /152/. In addition, TiO_2–coating on hollow glass beads have been shown to inhibit the photosynthetic activity of bacteria and diatoms, suggesting potential useful applications in preventing excessive algal growth /153/. The antimicrobial properties of some nanoparticles have been used in biocides /154/. The widespread use of such biocides would result in to a release of nanoparticles. This may lead to imbalances within the microbial populations and needs to be addressed appropriately.

Aluminium nanoparticles of size 13 nm have been found to inhibit the root growth of five different plant species, while larger particles had no effect /155/. It was suggested by the authors that the effects were due to the presence of free hydroxyl groups on the particle surfaces /155/. On the other hand it was suggested that the toxicity effects may have resulted from increased solubility of nanoscale aluminium /156/. Harmful effects like oxidative stess and some behavioural and reproductive effects on crustacean and fish exposed to C_{60}, TiO_2 and carbon black have been reported /47,157,158,159,160/.

Case Nano-fibrillar cellulose: There are no publicly available studies concerning the ecotoxicology of NFC

5 LEGISLATION, REGULATION AND RECOMMENDATIONS (OPINIONS)

Both of the potential benefits and hazards of nanomaterials have been debated in recent years /161/. Data on risks potentially associated to nanoparticles have worried dome scientists about their potential impacts on the health and safety of both humans and environment /162/. During the last few years, legislative policies have been proposed and discussed by different institutes like European Commission, Royal Society and U.S. Environmental Protection Agency /140,143,162/. In addition several non-governmental organisations like Greenpeace, Friends of the Earth and Natural Resource Defence Council are also concerned about the safety of nanomaterials /163,164,165,166/.

The different views on how to regulate nanomaterials in the future vary substantially. For example Davies /167/ supports the establishment of a separate, specific regulation on nanomaterials but recognizes political obstacles which make passing new legislation unlikely. On the other hand Reynolds /168/ highlight the ineffectiveness of a generalised prohibition and brings forward a voluntary approach. However, voluntary programs cannot ensure that all companies actually take actions to ensure safety /169/.

In Europe, the European Commission has adopted an "incremental approach" which adapts existing laws to the regulation of nanotechnology in Europe. This approach uses existing legislative structures like product legislation and dangerous substances legislation to the maximum, revisits them, and when appropriate only, alters them in order to deal with nanomaterials. The possibility of founding separate regulation for nanomaterials is considered unfeasible because of the difficulty of establishing links between different pieces of legislation /161/.

5.1 EUROPEAN UNION

Different laws apply in different stages of the products life cycle. In the manufacturing of the ENM the EU directives considering safety at workplace (e.g. directives 31/1989, 24/1998) and Integrated Pollution Prevention and Control Directive (IPPC) are the first relevant pieces of legislation. In addition, the European Union regulation for the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) has to be

taken into consideration in the manufacturing, import, sales and in some cases also development of ENMs.

Depending on the products life cycle, there can be more relevant regulation but at least most of the cases some Waste management Directives (e.g. directives 12/2006, 686/1991, 439/1975 and 53/2000) have to be considered in disposal. In addition there is product group specific legislation which might be applicable depending on the product where ENMs are used.

There has been some criticism regarding the legislation in EU. In the absence of nanospecific provisions in Community law it is criticised that the current legislation doesn't cover the risks related to nanomaterials. It's stated that better implementation of current law alone can't bring necessary level of protection but new nanospecific laws has to be implemented based on developed risk assessment methods. The current situation is seen not to fulfil the REACH principle "no data, no market" and is not adequate to protect human health and the environment /170/.

5.1.1 Directives considering safety at workplace

There is no direct reference to nanoparticles in the Safety at Workplace Directives or in the communitarian and national legislation on the protection of workers' health at workplaces /161,171/. As there are limited information about the exposure of ENMs in workplaces and as such it is impossible to evaluate the safety at workplace. It is possible to define Occupational Exposure Limits (OELs) for workers, but with nanoparticles some problems arise. The establishment of OELs is usually based on a complete risk analysis procedure which is not presently possible for ENMs. In addition ENMs are not necessarily detected by existing instruments and the most optimal parameter to determine the toxicity of nanoparticles is still undefined. At the moment there are some concerns for applying OELs defined for related bulk substances e.g. in the case of nanotubes the respective OELs is graphite dust. This approach is seen not fully representative and should not be used since the properties differ substantially between bulk and nano forms /172/.

5.1.2 Integrated Pollution Prevention and Control Directive

Directive 2008/1/EC on the Integrated Pollution Prevention and Control (IPPC) is the main environmental regulative tool in EU. It establishes an authorising regime for activities with a high pollution potential. The performance of processes must ensure a high level of protection, proper pollution preventive measures as well as comply with the notion of Best Available Techniques (BAT). The relevance of the IPPC to manufacturing plants of nano-engineered substances and materials depends on whether they are encompassed by the list of industrial activities with high pollution potential. All industrial pulp producing facilities and paper and cardboard mills with a production capacity exceeding 20 tonnes per day are listed.

It is likely that some other environmental legislation covers parts of the life cycle of nanomaterials. There are directives controlling the release of materials to water, air or

soil. If nanomaterials are likely to be released during the manufacturing or use of products containing nanomaterials, different environmental directives have to be considered.

5.1.3 REACH

REACH, the European Union regulation for the Registration, Evaluation, Authorization and Restriction of Chemicals is a key instrument of EU legislation considering safety of chemicals. REACH provides legislation applying to the manufacturing of chemicals, placing them on the market and use of substances on their own, in preparations or in articles and to the placing on the market of preparations. REACH also complements current product regulations /173/.

REACH does not cover explicitly nanomaterials. However, as REACH applies to substances on their own, in preparations or in articles, it covers areas in which nanomaterials are being used. Furthermore, REACH provides the input necessary for a proper implementation of regulation in other areas /173/. When substances that are already on the market as bulk are produced or imported at the nanoscale without modifications, they will, for registration purposes, not be considered as different from the "bulk material" /173/. If nano properties or uses differ between the nano and the bulk the following info is required: "the information about the properties and uses, safety assessment for the nano form, any different or additional classification with regard to hazardous properties of the nano form, and any risk management measure and operational conditions required" /173/. Even if the properties or uses of the nano form differ from the bulk, it is possible to use safety assessment methods of the bulk form which are not necessarily applicable for nanomaterials.

At the moment produced or imported substances require a registration over the threshold of 1 ton/year, including impurities. For many nanoparticles this limit would not be reached. Furthermore, the usually low concentration of nanoparticles in the final article is likely to exclude any imported nanoengineered articles from the REACH legislation, since no registration is required when the concentrations of a substance is lower than 0.1% w/w.

5.1.4 Waste management directives

Wastes containing nanoparticles are likely produced in different phases of the life cycle of the product. Nanomaterials are covered by waste management regulations in a non-specific way, because there are no specific references to ENMs in existing laws. In general, nanomaterials follow the products life-cycle where they have been used and their fate depends on how these products are treated in their disposal. In some cases special regulation applies e.g. in situation where nanomaterials would be used in paints. It is likely that nanoparticles used in consumer products end up in municipal solid waste. Wastes are typically disposed of in landfills or incinerated. There is practically no information about the fate of ENMs in a landfill. Release depends on the nanoparticles mobility as well as on the degradability of the host material where nanoparticles are

attached. When nanoparticles are incinerated, their thermal properties determine their fate.

In some cases certain nano-waste might fall within the scope directive on management of hazardous waste (681/1991) which would introduce more severe obligations during waste management. The little amount of toxical data of ENMs makes it hard to define if nanoparticles meet the criteria of hazardousness.

5.1.5 Other legislation

Other existing regulation which might become relevant includes classification and labelling of chemicals substances according of their dangerous properties or banning or restricting the use of chemicals. These directives become relevant only if the ENMs are found to be highly dangerous. If nanomaterials are used in e.g. personal protective equipment, medical devices, cosmetic products or food specific product legislation applies in the EU.

5.2 "NATIONAL SITUATION" – SELECTED COUNTRIES

5.2.1 United Kingdom

United Kingdom has been one of the countries which have raised concerns about the safety of nanomaterials. In 2004 the United Kingdom's Royal Society, said that given the early warning signs of nanotoxicity, nano-ingredients shouldn't be allowed in products until they pass safety testing by independent authorities /140/. Since then, several studies and scientists have identified inadequateness of safety assessments concerning nanomaterials /174,175,176/.

In the UK government called for food manufacturers and others to provide any information on nanotechnologies they are working on in 2006. The reporting programme is part of the UK government's bid to assess the risks nanotechnology poses to the public. At the same time the UK government also launched a review of its nanotechnology policy over concerns about the health and environmental risks. The review is being led by the UK Council for Science and Technology (CST).

In 2007 British Standards Institution developed guidance for labelling, specifying and handling of nanomaterials. It notes that "given the lack of current knowledge about the toxicity of nanomaterials and the concern that current safety data sheets do not adequately reflect the hazardous nature of nanomaterials, it is recommended that all nanomaterials are considered potentially hazardous unless sufficient information to the contrary is obtained" /177/.

5.2.2 USA

Regulation of ENMs in USA is controlled by the U.S. Environmental Protection Agency (EPA) with the Toxic Substances Control Act (TSCA). There have been resent (2008-2009) regulatory efforts concerning nanoscale materials under this act. These include

e.g. issuing new use rules for two siloxane-based nanoparticles, intimating that new testing and data collection rules will be implemented for certain nanoscale materials, and proposing and/or requiring acute toxicity rat inhalation testing regimes in certain instances /178/.

In principle nanoscale substances are not considered as "new" just because their size. If they have a distinct molecular identity that is not shared with any other chemical on TSCA's existing chemical substance inventory they are considered "new". Nevertheless, carbon nanotubes are considered new chemical substances under TSCA. Silica and alumina nanoparticles are considered as significant new uses of existing chemicals which requires premanufacturing notice (PMN) and approval. Submitting PMN can result to a negotiation between EPA and the manufacturer. In this negotiation EPA can state that the use of under specific conditions would not cause an unreasonable risk, but that the use in other conditions might /178/. It is possible that EPA develops a consent order based on finding of potential unreasonable risk or substantial exposure. Risk based consent orders typically include requirements regarding toxicity or environmental fate. E.g. in the case of silica and alumina nanomaterials a 90-day inhalation toxicity study on rats with a post-exposure observation period of up to 3 months is required from the manufacturer.

EPA has clearly taken a case-by-case approach to the regulation of nanoscale materials, rather than the implementation of new generalized approach argued for by many environmental, health and safety advocates /178/. Nevertheless, EPA is taking the risks of nanomaterials in to serious concern and is requiring animal inhalation studies to be undertaken to be able to demonstrate whether these materials present human health concerns or not.

5.3 NGO'S

Several non governmental organisations (NGO's) have concerns about the safety of nanomaterials. E.g. Greenpeace, Friends of the Earth and Natural Resource Defence Council has raised issues relating to nanotechnology. Most of the organisations see the safety assessment of nanomaterials insufficient at the moment. They focus on different aspects of nanomaterials, but most of them urge public discussion and labelling of products where nanomaterials are used. If these issues are not taken in to consideration, it is likely that the reputation of nanomaterials in the market will suffer.

Greenpeace has environmental and socio-political concerns regarding manufactured nanomaterials. Environmental concerns include e.g. throwaway nanomaterials may constitute whole new classes of non-biodegradable pollutants which are not studied sufficiently. It's possible that nanomaterials could bind certain common but harmful substances in the environment, such as pesticides or PCB's. In addition, Greenpeace sees that too little work has been done in order to ascertain the possible effects of nanomaterials on living organisms. In particular, the possibility of toxic elements attaching themselves to otherwise benign nanomaterials inside bacteria and finding a way into the bloodstream should be studied. Another possibility is that proteins in the

bloodstream will attach to the surface of nanomaterials, thus changing their shape and function, and triggering dangerous unintended consequences /163/.

Socio-political concerns Greenpeace has include e.g. genetic discrimination caused by nanomaterial based medicine usage, inequality between developed and developing countries increases because the nanotechnology related patents are owned by developed countries and some concerns of the misuse of nanotechnology for e.g. military purposes. Especially Greenpeace emphasizes the importance of public discussion with the scientific and policy communities to avoid public conflicts /163/.

Friends of the earth (FoE) has raised concerns about nanomaterials being used unlabelled in several consumer products including sunscreens and cosmetics. FoE sees that the safety testing of these products has not been sufficient and that consumers should be able to know weather products include nanomaterials or not. FoE concerns include e.g. the different chemical properties of nanomaterials compared to the bulk materials, the possibility of nanomaterials passing through the skin and accessing tissues and organs, potential next generation harm and worker safety. FoE emphasizes the need of transparency and labelling in addition to safety testing of products including nanomaterials /165/.

Natural Resource Defence Council (NRDC) is very concerned about the health affects of nanomaterials. NRDC proposes a three-part framework for regulating nanomaterials based on a precautionary approach to managing toxic chemicals. The framework consists prohibiting the use of unsafe or untested use of nanomaterials and conducting full life cycle environment, health and safety impact assessments before commercialization. In addition NRDC calls for public and worker participation in the development and control of nanotechnologies /164/.

Case Nano-fibrillar cellulose:
One report of Friends of the Earth concentrates on nanotechnology used in food, food packaging and agriculture. It raises concerns that nanomaterials are used in packing as a barrier, antibacterial substance or biodegradable packing in food packaging without labelling.
The report raises concerns about the potential toxicity of nanomaterials used in food or in close contact with food. It also states that "Early studies of public opinion show that given the ongoing scientific uncertainty about the safety of manufactured nanomaterials in food additives, ingredients and packaging, people do not want to eat "nanofoods" /166/.

5.4 NANOTECHNOLOGY IN MEDIA

A lot discussion around nanotechnology and sustainability assessment can currently be found in international media. This discussion concentrates on processing, emission and potential impacts of new products as well as on defining feasible methodologies for evaluating sustainability effects /179,180,181,182/.

Figure 4 summarises key words and topics extracted from discussion around nanotechnology and sustainability using various sources (NGOs, companies, r&d institutes, blogs, authorities etc.). The figure summarises the discussion using text mining; the most relevant topics have been extracted and appear in the figure. The size of a word depicts the importance of the topic, and the distance between words describes how strongly the topics are linked to each other. This picture indicates that potential of nanotechnologies is at the moment more relevant than e.g. risks.

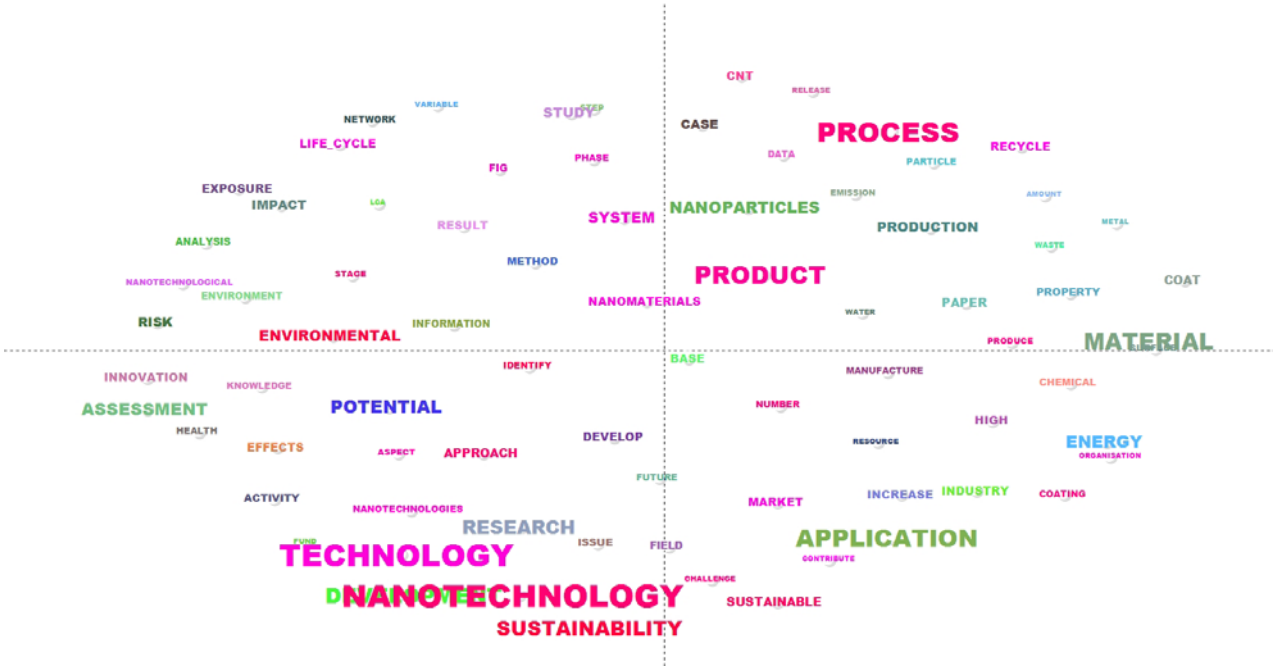

Figure 4. Nanotechnology and Sustainability Discussion, November 2009. Source: Pöyry

The Internet offers a vast material to be utilised in the analysis current and future trends and weak signals. Texts from various sources, e.g. general and scientific articles, blogs and discussion forums, can be processed with text mining. The result of the mining of texts from selected sources with the combination of different algorithms, is material that can be used to e.g. to describe general trends or to interpret the attitudes of the consumers towards nanotechnology-related issues.

To create an overview of how the consumers perceive nanotechnology we used our text mining toolbox and analysed millions of different kinds of online discussion forums, blogs and social networks during half year. The expressions related to nanotechnology having positive and negative sentiments were compared.

Figure 5 shows the results of the comparison; green columns describe the amount of positive sentiments and red columns the amount of negative sentiments, respectively. So far, the connotations have mostly been positive

Figure 5). However this promising start is now at risk of being reversed /183/. There is a very real danger that a scare – real or imagined – involving nanotechnology will hit the headlines, evaporating the current positive image of the 'nano' label.

Much of the public acceptance and commercial uptake of nanotechnologies in Europe will depend on three key challenges:
1. The future tone and course of the regulatory debate
2. The ability of companies to demonstrate specific benefits to consumers; and
3. Their success in branding and distinguishing specific products so as to foster acceptance of and trust in the new technology /184/.

It is important that the companies wanting to use nanotechnology successfully in the future will form an integrated strategy that helps them to effectively deal with all these aspects.

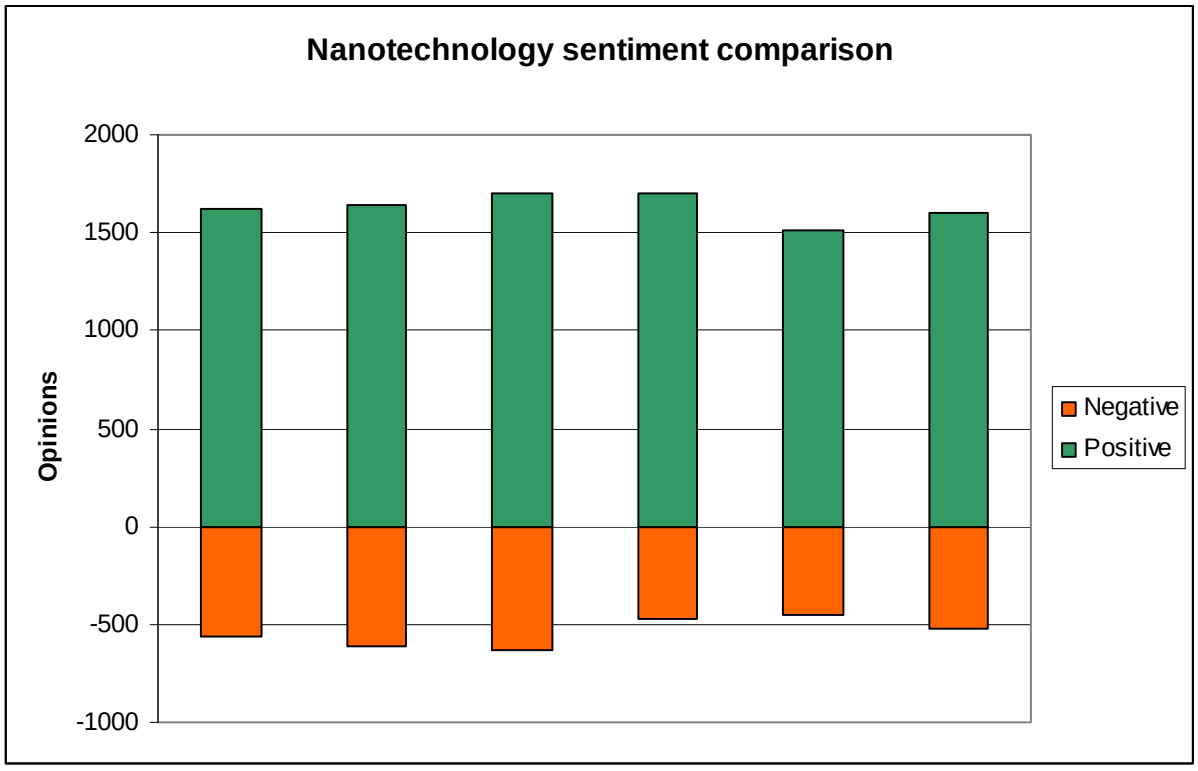

Figure 5. Monthly text mining sentiment comparison of nanotechnology from August 2009 to January 2010. Source: Pöyry

6 SUMMARY AND RECOMMENDATIONS

Nanotechnology industry is rapidly growing with expectations of substantial benefits that will have significant economic and scientific impacts. It is claimed that nanotechnology development is likely to require an additional two to ten million workers across the world by 2014. With this many potential workers and consumers exposed to nanomaterials now and in the future, risk assessment is essential. In addition, certain NGO groups have

raised concerns about the safety of nanomaterials. Concerns have reached different governments and designing legislation for ensuring nanomaterial safety seems to be ongoing. The public perception of nanotechnology seems to be at least neutral, even positive at the moment.

Cellulose as such is considered to be a safe natural material, but the charasteristics of NFC differ somewhat from cellulose. In addition, nanocellulose has three properties that are somewhat associated with pathogenicity in particles. First the nano form of cellulose could have more toxicity than larger sized particles. Second they are fibres and so might behave like asbestos and other pathogenic fibres which have toxicity associated with their needle-like shape. And third, they are expected to be biopersistant in humans.

There are lot of studies assessing risks of different nanomaterials. Nanoparticles can distribute from the site of entry to other sites in the body and it seems that nanomaterials can be found almost everywhere in the body after exposure. Some results suggest that certain nanomaterials might be toxic. Most of the studies focus on inorganic nanomaterials, like metals. The characteristics of NFC and FNFC differ from the inorganic nanomaterials and therefore the results and experiences obtained with inorganic nanomaterials can be considered as indicative only.

The testing methodology suggested for the risk assessment of NFC include in vitro cytotoxicity and immunotoxicity test to give indication whether NFC will cause cellular damage and whether their systemic effects will be likely. In addition a nematode model based test organism is used to investigate potential systemic effects and neurotoxicity. Because the exposure to NFC is likely to happen through inhalation, inhalatory toxicity study with animals is included.

7 ABBREVIATIONS

CASG Nano REACH Competent Authorities subgroup on Nanomaterials
CB Control Banding
CPC Condensation particle counter
CST Council for Science and Technology
DLS Dynamic light scattering
DNEL Derived No-Effect Level
DMEL Derived Minimum Effect Level
ECHA European chemicals agency
EDS Energy dispersive spectroscopy
ELPI Electronical low pressure impactor
ENM Engineered nanomaterial
ENP Engineered nanoparticles
FFF Field-flow fractionation
IR-CSA Information requirements and chemicals safety assessment
NFC Nano-fibrillar cellulose
NTA Nanoparticle Tracking Analysis
PNEC Predicted No-Effect Concentration
PMEC Predicted Minimum-Effect Concentration
RA Risk assessment
REACH The European Union regulation for the Registration, Evaluation, Authorization and Restriction of Chemicals
SCENIHR Scientific Committee on Emerging and Newly Identified Health Risks
SEC Size-exclusion chromatography
SEM Scanning electron microscopy
SMPS Scanning mobility particle sizer
TEM Transmission electron microscopy
TGD Technical guidance document

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