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Thesis:

DEVELOPMENT OF A FRAMEWORK FOR REGULATORY RISK ASSESSMENT OF ENGINEERED NANO- MATERIALS

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ABSTRACT

Although nanotechnology may potentially contribute to socially beneficial applications, there are concerns that certain nanomaterials may pose novel risks for the human health and the environment (Allianz et al 2009).Engineered nanomaterials have different properties compared to their bulk counterparts, which enables unique applications, but may lead to novel mechanism of toxicity. Reviews of available data in the field of risk assessment of engineered nanomaterials(ENMs) recognizes substantial uncertainties, limitations and constrains in conventional risk assessment (RA) of chemicals when applied to ENMs, which raises health and safety concerns. (Hristozov et al 2012).

Since it appeared that no clear and universally accepted definition of a nanomaterial is present, and that a relevant measure for expressing hazard and exposure is as yet not known.(Pronk et al. 2009), therefore a need for more scientific knowledge and firm government regulations.

In addition to insufficiency of the standard information requirements to assess hazard and exposure, and other data gaps, it cannot be determined to what extent the nano-form of a substance corresponds to the bulk form of the same substance. To this end, a life cycle perspective is considered towards the analysis of nano specific fate, transport, toxicity and impacts of ENM on humans and environments. Presently, research institutes, regional and national governments and international organization are working on an integrated top-down strategy to guide bottom-up research activities on the state-of-the-art of the regulation of nanomanufacturing.

This thesis examines the current data on nano-enable materials, their suitability to the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation for safe use of Nanomaterials through datagap analysis. The process critically review the state of the art in the area of nanosafety assessment with a case study of use of nano-silver. Coupled with the data gap analysis, a comparative review of emerging methods and tools applied in the evaluation of effects of nanometerial on human and ecology is performed through, the Analytical Hierarchy process in SWOT analysis of the current applicable ecological and occupational tools. This is aimed at analysing the strengths, weaknesses, opportunities and threats related to available methods and tools. The result is used as the basis for developing a strategy for integrated risk assessment for nano-silver.

These activities will contribute to (1) a framework for integrated (human health and ecological) risk assessment of ENM, tailored to the current REACH regulations, (2) formulation of a roadmap for sustainable nanomanufacturing.

The Structure of The Thesis:

Chapter one deals with the process of human health and ecological risk assessment. Different stages of risk assessment, requirements for risk evaluation and available methodologies are described.

Chapter two reviews the existing tools for (ecological and occupational assessment), using Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis. Ranking of the tools are useful to derive quantitative information and their versatility for present and anticipated applications.

Chapter three presents two case studies of nano-silver containing products: HeiQAGS-20(textile material) and Biocides (agricultural chemical) , performed by regulatory bodies US Environmental protection agency (EPA) and European Chemical Agency (ECHA).respectively. Dealing with different processes that describe the preparation, forms of nanomaterials attributes and their chemical reactivity that lead to the Environmental fate and transport.

Chapter four discussions limitations, uncertainties and possible solutions at different stages of risk assessment process and proposes a new framework for sustainable nanomanufacturing

Chapter five presents a roadmap towards a decision making process for sustainable nanomanufacturing using the analysis of risk assessment.

OBJECTIVES AND OUTLINE

This thesis has the following aims:

1. Review critically the state of the art in regulatory risk assessment of Silver nano- particles

2. Perform a comparative review of emerging methods and tools for risk assessment of Silver nano-particles

4. Develop a framework for integrated (i.e. human health and ecological) RA of ENMs, tailored to the current regulations

3. Propose a roadmap for generation of sustainable nanomaufacturing based on this framework.

CHAPTER 1. INTRODUCTION

1 OUTLINE

In this chapter, the risk assessment process, definition, description and different methodologies are described. Explanation of required metrics in the conversion of test data from animals to human, and appropriate response of human and environment to chemicals (e.g ENMs) that defined the adverse or no- adverse effects as an indicator of risk on different endpoints. To this end, many concepts in the literature have enumerated the need for tools and frameworks, tailored at the identification and evaluation of the effects of chemicals in various forms and quantity. Using the REACH guidelines or other national and internationally accepted procedures, regulators (ECHA, EPA) safeguard human and environment against the use of chemicals and other associated materials such as ENM.

Determination of ***risk or no-risk*** through the assessment procedure helps to determine the magnitude of effect of ENMs and update all necessary information needed towards the safe use of nano-enabled materials.

Description of the ***risk or no-risk*** process is analysed in this thesis using a case study of nanosilver. Analysis of its toxicity in various environmental media (air, water and soil) in Europe, and effects on human when exposed (occupational and use of nano-enable materials) based on their physical and chemical properties.

Illustration is made on the existence of datagap and how this could be addressed via the proposed framework. Analysis of Strengths, Weaknesses, Opportunities and Treats (SWOT) of current applicable tools in risk assessment is employed. This process leads to the importance of life cycle thinking.(Life Cycle Analysis) to determine various stages of the life span of material (from cradle to grave) that contribute to the release of nanoparticles and the eventual hazardous effect on both the environment and human.

1.1.1 STATE OF THE ART

Nanotechnology is a field in the area of Science and Technology dealing with the design, production and use of materials at the nano-scale (i.e.1 to 100 nanometres) (Smalley et al 2007). Due to their new or enhanced physicochemical properties, some ENMs are suitable for a wide number of applications in many sectors, including information technology, energy production, environmental protection, biomedical applications, food and agriculture. For this reason the market of nano-products has been exponentially growing from the reported $147 billion in nano-enabled products in 2007 to the predicted $3.1 trillion by 2015. (Schmidt.C.W et al 2012)

The widespread use of nano-enabled products and continuous development of new generations of nanotechnologies may lead to intentional and/or accidental exposure to human and the environment.(OECD

2010).Chemical risk assessment (CRA)has been put forward as the most relevant approach to understand, evaluate and quantify these risks.(REACH 2008).CRA is a process by which scientific and regulatory principles are applied in a systematic fashion in order to describe the hazard, associated with environmental and/or human exposure to chemical substances. Furthermore, it describes the identification of a risk to humans or environment that may arise from exposure to a certain chemicals. The process identify relationship between dose and its effect on the target populations to determine hazardous properties of chemicals through impact assessment, exposure assessment and risk identification. (REACH 2007). According to REACH, a four-step process is involved in CRA:

(1) hazard identification,
(2) effect assessment,
(3) exposure assessment, and
(4) risk characterization.

The main outcome of the iterative process is a statement of probability, describing the effect of humans or other environmental receptors when exposed to a chemical agent, and to what degree they will be harmed. As it is internationally recognized and employed by major actors, such as the World Health Organization (WHO) and the Organization for Economic Co-operation and Development _(OECD), CRA represents the scientific foundation for many national and international regulatory guidelines ,e.g. in US, EU, Japan and Canada.

The present thesis is in line with the main aim of the European FP7-funded MARINA project (Managing RIsks of NAnomaterials)and SUN project aimed at defining reference methods and strategies for managing the risk of Engineered Nanoparticles.

1.1.2 RISK ASSESSMENT: DEFINITION, DESCRIPTION AND METHODS

Risk assessment can be defined as the process of Identification, Evaluation, and Estimation of the levels of risk of human health and environment as a result of exposure or contact(Direct or Indirect) to any agent of contamination, such as chemicals and other stressors.(EPA 2009)

Historically, chemical regulations evolved to meet the need to control health hazards, while environmental regulations evolved with the aim to protect or improve the environment. National, regional, and international regulations have been developed towards the regulation of chemicals(e.g EPA in USA, REACH in EU), considering different areas of economic development such as agriculture, energy production, clothing, food supplements and packaging, cosmetics and other consumer product where large scale of chemicals are been used. However, a major challenge is the inter-crossing between risk assessment, communication and management and

how to demonstrate inter-relationship between different fields (toxicology, epidemiology), testing strategies (in-vitro,in-vitro),standards(1-10tonnesper year, PH of substance, route of exposure), observation(MOE,DNEL,BMD,OELS,LCA), and sources of data(OSAR model, LD50,LC50,human data)
(Buzea et al 2017)

The scope and nature of risk assessment depends on (1) The amount of substance present in the environment comprising the endpoint(Water, soil and Air), (2)The degree of contact with the substance of contamination and (3) The hazardous strength of the chemical. Such assessment also range widely from scientific analyses of point of exposure (site specific studies) to a broader prediction of future harm to human health or the environment. (ENPRA/MARINA 2013).

1.2 RISK ASSESSMENT PARADIGM

The classical risk assessment process involve four main stages: Hazard Identification, Dose- Response assessment, Exposure assessment and Risk characterization. *Figure 1.* Many research studies in various fields have provided data required for the risk characterisation, which has serves as input to risk management decision-making. It has been agreed internationally that the existing risk assessment paradigm developed for traditional chemicals should also be applied to nanomaterials.(ECD 2012).

Risk assessment deals with both Environmental(Ecology) and Human health(Occupational).Evaluation of quality of data aimed at reducing waste. This requires data for the four processes aimed at assessing the toxicity effects of chemicals on the agent of exposure.

"The US National research council's NRC 1994 report on the science and judgement of risk assessment enumerate different challenges (datagap, extrapolation),and provides different solutions for incorporating new scientific knowledge and findings into the risk assessment process". (Faustman, EM; Omenn GS 1996). With the goal of improving the scope and capability of risk assessment, the US National research council NRC framework explicitly asks the question of what options are needed to reduce the hazards or exposures that have been identified ?,and how can risk assessment be used to evaluate the advantages of the various options ?. This approach could be useful to reduce the problem that has occurred with some risk assessments in practice (NRC 2009). It can be noted that various elements proposed in the framework are currently implemented in regulations such as REACH .

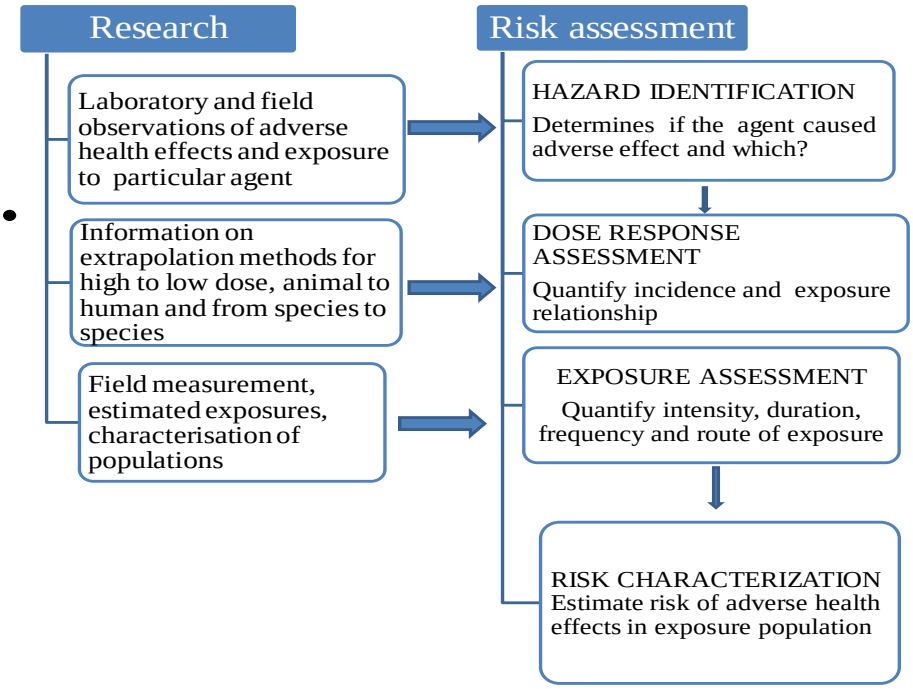

Figure 1. Risk Assessment for human health assessment (Adapted from *NRC 2009)*

1.3 HAZARD IDENTIFICATION

This involves the characterizations of hazard effect (toxicity) of the element at risk (Human and Environment). It describes the screening process through:

(1) Collection of data from both experimental data (In vitro and in vivo) and non-experimental sources (e.g. Quantitative Structure Activity Relationship, QSAR). These data could be collected from literature, databanks, websites and various available exchange forum. Relevant and available information such as substance identity, physico-chemical properties, exposure/ uses/ occurrence and application of substances, toxicity, ecotoxicity, environmental fate, chemical and biotic degradation are required with any other data that may assist in identifying the presence or absence of hazardous properties of the substance. While human data such as analytical epidemiology studies, descriptive or correlation epidemiology studies, and controlled studies in human volunteers can be used. However, this is strongly discouraged when good quality data are already available.(REACH 2008).

(2) Consideration of tonnage quantity according to regulations(e.g. chemical produced or imported that are more than 10,000 tonnes a year according to REACH regulation). However, this may vary in accordance with specific criteria for different endpoints.

(3) Evaluating available data and investigating the capacity of the available information meeting the required procedure under different guidelines (EPA or REACH). This stage deals with the following characteristics:

*** Relevance**(extent to which data or test is appropriate for hazard identification and characterization),

***Reliability**(quality of a test report or publication to preferably standardised method; Klimish code adopted by REACH) and

* **Adequacy** (usefulness of the data for hazard and risk assessment).

Evaluation of integration of all available information using the Weight of Evidence (WOE) approach is conducted to assess the relevance, reliability and adequacy of available information. Other factors such as adaptation to a standard information requirement through existing data, WOE, or Substance-tailored exposure driven waiving or testing are adequately justified, coupled with factors such as toxicokinetics.

(4) If all information collected is not adequate for the proposed assessment after the first second and third iterative procedures, new information may be generated using integrated testing strategy for endpoint-specific in order to meet the requirement of regulatory guideline.

1.4 DOSE RESPONSE ASSESSMENT

The underlying principles of risk assessment rely on the understanding of the causal relationships between exposure and effect. In order to better comprehend how exposure-related effects can be explained, the concept of dose-response assessment is used. This step describes the varying doses Vs incidence of adverse effects in exposed population.

To measure the extent with which a substance can cause an adverse effect on the human health and environment, required information with respect to the substances' fate in the body. Toxicokinetics (absorption, distribution, metabolism, and excretion) studies of chemicals via human and environmental endpoints is required for dose response assessment. Human endpoints (irritation and corrosion, skin and respiratory sensitisation, acute toxicity, repeated dose toxicity, mutagenicity, carcinogenicity, reproductive and developmental toxicity, and environmental endpoints (aquatic toxicity: either acute or chronic), sediment toxicity as well as any other available information on the toxicity of the substance, toxicity to sewage treatment plant micro-organism, degradation and bio-degradation, aquatic bio-concentration, bioaccumulation, terrestrial bio accumulation and toxicity, long term toxicity and terrestrial toxicity are applied in the REACH regulation. However, the standard requirement for these endpoints (Human and Environment) according to REACH are tonnage-dependent. Table 1

Table 1: Human Health Endpoints

Type	Standard information requie	Observation	Source of data
Irritation/corrosion (Local effect on skin, eye or respiratory system)	**1-10t/y** tonnage band: * PH of the substance required, existing acute toxicity by dermal route. **10-100t/y** tonnage band: In-vivo skin and eye irritation studies	Basis of setting occupational Exposure Limits (OELS. Strong oxidants are more irritant or corrosive depending on concentration, Chemicals predicted to be corrosive to the skin are automatically considered to be severely irritating to the eye.	Human data on Dermal and respiratory irritation.
Skin and respiratory sensitisation (caused agent that can activate the immune system)	1-10t/y tonnage: LLNA test required, PH of substance before In-vivo test, corrosivity of substance set at concentration and dose	LLNA correlated relatively well with the human data on skin sensation. DNEL based on the data from the LLNA or WOE	Human data(diagnostic clinical studies, workers medical surveillanceand case report) QSAR not yet available
Acute toxicity (Adverse effects which results from a single or short exposure)	Oral for 1-10t/y tonnage band, Dermal for 10-100t/y tonnage band. (Limit test of 2000mg/kg)	Pathological changes in organs and tissues(deaths are often observed)Corrosive substance cause acute toxicity	QSAR model, LD50,LC50 data. Human health on acute toxicity data(e.g poison information centre or clinical case report)
Repeated Dose Toxicity (general toxic effects that occur After daily dosing)	10-100t/y tonnage band (28 day study) 100-1000t/y tonnage (90 day study) Route of human exposure is used .	Changes in morphology, physiology, growth or life span. NOAEL or LOAEL can be obtained	QSAR currently not available.
Reproductive and developmental toxicity (Effects such as reduced fertility, Growth and developmental Retardation in the offspring)	10-100t/y tonnage band: Screening test for reproductive and developmental toxicity required. 100-1000t/y tonnage band: prenatal developmental toxicity study required	Derivation of DNEL fertility and development with underlying dose threshold, Reveals the effect of reproductive organs in test animals.	Human data and epidemiological studies
Mutagenicity (effects of induce or increase in the frequency of mutation)	1-10t/y tonnage band: In-vitro mutation in bacteria. 10-100t/y tonnage band: In-vitro studies in cytogenecity(chromosome aberrations) and Gene mutation in mammalian cells. 100-1000t/y tonnage band: In-vivo studies required.	Positive results in In-vitro studies test confirm the need for In-vivo mutagenicity. Negative results in In-vitro tests is considered sufficient evidence for non-mutagenic. Toxicokinetics is used to analyse if the test compound actually reach the target organ	Human data, QSAR data, toxicokinetics
Carcinogenicity(measure of any Occurrence of a genetic damage)	Standard set at the highest tonnage level above 1000t/y	WOE is required. In case genotoxicity mechanisms are involved, the effect is considered to have no-effect threshold. Carcinogenicity at tonnage lower than 1000t/y is based on mutagenicity and repeated dose toxicity studies	QSAR model, Read across/ category data, human epidemiological da ta

Source: ECHA (REACH guidelines) 2008

Environmental endpoints describe the effect of chemicals on Air, Water and Soil. For Water, aquatic toxicity (physiochemical properties of substance that is detrimental to aquatic organism in either a short or long time when exposed to the substance) is used. Two major exposure/ effect in relation with duration are acute and chronic effects. Where an acute effect defines an exposure over a short-term, a chronic effect defines a long-term exposure.

Acute toxicity: This is the toxicity to aquatic organisms when exposed to substances within a duration of an hour up to 2 or 3 days. This represent a short period in comparison to the duration of the life-cycle of the organisms. Acute toxicity effects are mostly expressed as median lethal or effect concentrations (L/EC_{50}) (a test concentration at which 50% of the organisms is affected or at which 50% effect is measured for a specifically defined endpoint) providing mortality as the endpoint. (e.g. growth rate effects of test chemicals on algae).

Chronic toxicity: This describes the toxicity to aquatic organisms when exposed to substances for a prolonged period of time (15-30 days). The duration may vary depending on the species used, but general long based on the life-cycle of the organism. Such chronic effects usually include different endpoints (e.g. survival, growth and reproduction). The highest tested concentration where an effect has not been observed (No Observed Effect Concentration or NOEC) is the most frequently used parameter, which may often be replaced by an EC_{10} that can be estimated based on the concentration-effect relationship.

For the assessment of aquatic short-term toxicity test, data on invertebrates at the lowest tonnage band of 1-10 tonne/year is used, however, data on fish at the higher tonnage band of 10-100 t/y are required. (REACH 2008). At higher tonnage bands, data on the long-term effects on invertebrates and fish are considered depending on the outcome of the Chemical Safety Assessment (CSA).

However, different protocols for testing pollutants in the water column (for different level of sediment) in freshwater and saltwater has been investigated.(Organisation for Economic Co-operation and Development (OECD), 2006, U.S. Environmental Protection Agency (EPA), 2000, U.S. Environmental Protection Agency (EPA), 2002a, U.S. Environmental Protection Agency (EPA), 2002b).Several regulatory bodies (EPA, ECHA) required risk assessment using standard assays and species for the detection of hazards in relation to specific scenario.

1.4.1 DOSE/ CONCENTRATIONCHARACTERIZATION

Research has showed that a progressive increase in dose results in an increased response for a typical dose response relationship. "At low doses there may be no response, but at some level of dose, the responses begin to occur in a small fraction of the study population or at a low probability rate" (EPA 2008). Both the dose at which response begin to appear and the rate at which it increases given increasing dose can be variable between different pollutants, individuals, exposure routes, etc.(EPA 2008).A steep dose response indicates toxicity concern, where a small increment in exposure could have a significant impact. A shallow dose response also indicates greater toxicity concern, since there is less certainty about the precision of the NOAEL(PMRA Canada 2008).

There are two main steps in the dose-response assessment.

Step one involves, an assessment of all available data gathered through experiments for documentation of the dose-response relationship over the range of observed doses.

Step two consists of extrapolation to estimate the risk (probably of adverse effect) beyond the lower range of available observed data in order to make inferences about the critical region where the dose level begins to cause the adverse effect in the human population.

1.4.2 DOSE DESCRIPTORS AND THRESHOLD LEVEL

The dose descriptor gives the relationship between a specific effect of a substance and the dose at which the effect occurs. The evaluation of the dose response assessment is aimed at determining the No-Observed-Adverse-Effect Level(NOAEL) **figure 3**

The dose descriptors are used to derive the no-effect threshold levels for human health and the environment. DNEL represents a level of exposure above which humans should not be exposed. It is derived by dividing the NOAEL with assessment factors representing the uncertainties (e.g., with respect to extrapolation between species and among humans). REACH requires that a quantitative Derived Minimal Effect Level(DMEL) should be performed if DNEL could not be derived. The potential that a substance will cause an adverse health effect depends on the exposure pattern to the substance. This is usually defined by a combination of (1) the **population** likely to be exposed to the chemical, (e.g workers, consumers or humans exposed through the environment), or specific vulnerable sub-populations such as pregnant women or children, (2) the **frequency and duration** of the exposure, (e.g. single exposure or continuous exposure for eight hours), and (3) the **route** of exposure (dermal, inhalation or oral)

The relevant external dose units used to express DNEL for different exposure are

(1) Dermal: ***mg/ person/day or mg/cm^3 body area/day***

(2) Oral: ***mg/kg body weight/day***

(3) Inhalation: ***mg/ m^3***

Dose descriptors such as NOAEL, NOAEC, BMD, LD50, LC50, T25 are commonly used to derive the no-effect threshold levels for different endpoints in both human and environment.

Threshold response is characterized by a toxic effect occurring above an exposure concentration. Most environmental contaminants are threshold contaminants. Non- Threshold response exhibit effects at virtually all level of exposure(Any exposure results in some level of risk).

When establishing DNEL, the uncertainties in the assessment , including involving species difference, difference in sensitivity among human, quality of the dataset shall be taken into account(e.g).

However, data from different studies (e.g. in different species, with different durations) using more than one dose descriptor may be possible for a particular endpoint. But a major concern is how to determine beforehand, which of the dose descriptor that will produce the desired result, relevant to derive DNEL for a particular endpoint. REACH required that DNELs for more than one dose descriptor per endpoint should be derived prior to selecting the lowest DNEL for any endpoint using the weight of evidence approach. This requirement produced a quantitative assessment where DNEL(s) could be derived. However, the main emphasis is to assess the adequacy of control of exposure in the human population of interest by using other information other than a DNEL to qualitatively describe the potency of a health effect. The result is then used to develop an exposure scenarios with risk management measures.

1.4.3 EXRAPOLATION FROM ANIMAL TO HUMAN POPULATION

Extrapolation aims to translate the effect on (human and environment) of the hazard of a substance beyond the species on which the endpoint is tested. This provided allowance for application of tolerance within interspatial and extra spatial within species and environment.

Exposing animals to high concentration of a test chemical is used to determine the effect Vs dose in conventional risk assessment. Extrapolating from animal testing to predict the effect of a chemical on human population has been a major challenge in risk assessment process because of non-uniformity in the exposure patterns of the chemical as observed in tested animals compared with the human response to the same effect. Extrapolation of tested animal dose- effects to human effects is done to account for the following conversions: (1) High- dose to lower doses, (2) Test species to human, (3) single route of exposure in animal to multiple rate of exposure in human and (4) Constant, daily dosing in the animals to the continuous exposures in human population.

These extrapolations introduce uncertainty (e.g. involving species differences, differences in sensitivity among humans and quality of the data used) into the dose-response analysis. Other Challenges in obtaining dose-response relationships include

(1) Characterization of exposure or dose,

(2) Assessment of response, and

(3) Selection of dose-response model to fit the observed data.

For a non threshold effect such as non-threshold carcinogens, a semi-qualitative element is added to assess the likelihood that effects are avoided. Leading to the development of DMEL(Derived minimal effect level), a risk related reference which is considered to be a tolerable level effects.

1.4.4ASSESSMENT FACTOR

To address uncertainties introduced by the extrapolation of experimental data from animal to real human exposure scenario, assessment factors(AFs) are used, considering different factors such as:

* inter-species(sensitivity differences between experimental animals and humans)

* Intra-species (e.g. genetic polymorphism, age, gender, health status and nutritional status)

Therefore, an assessment factor that allow for differences in the experimental exposure duration, dose-response relationship and quality of data are needed. REACH regulation proposed the following equation to calculate Endpoint specific DNEL,

$$\text{Endpoint- Specific DNEL} = \frac{\text{NOAEL}_{corr} = \text{NOAEL}_{corr}}{AF_1 x\ AF_2 x \ldots\ldots x\ AF_n \qquad \text{Overall AF}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1.0)$$

NOAEL = No Observable Adverse Effect Level

AF= Assessment Factors

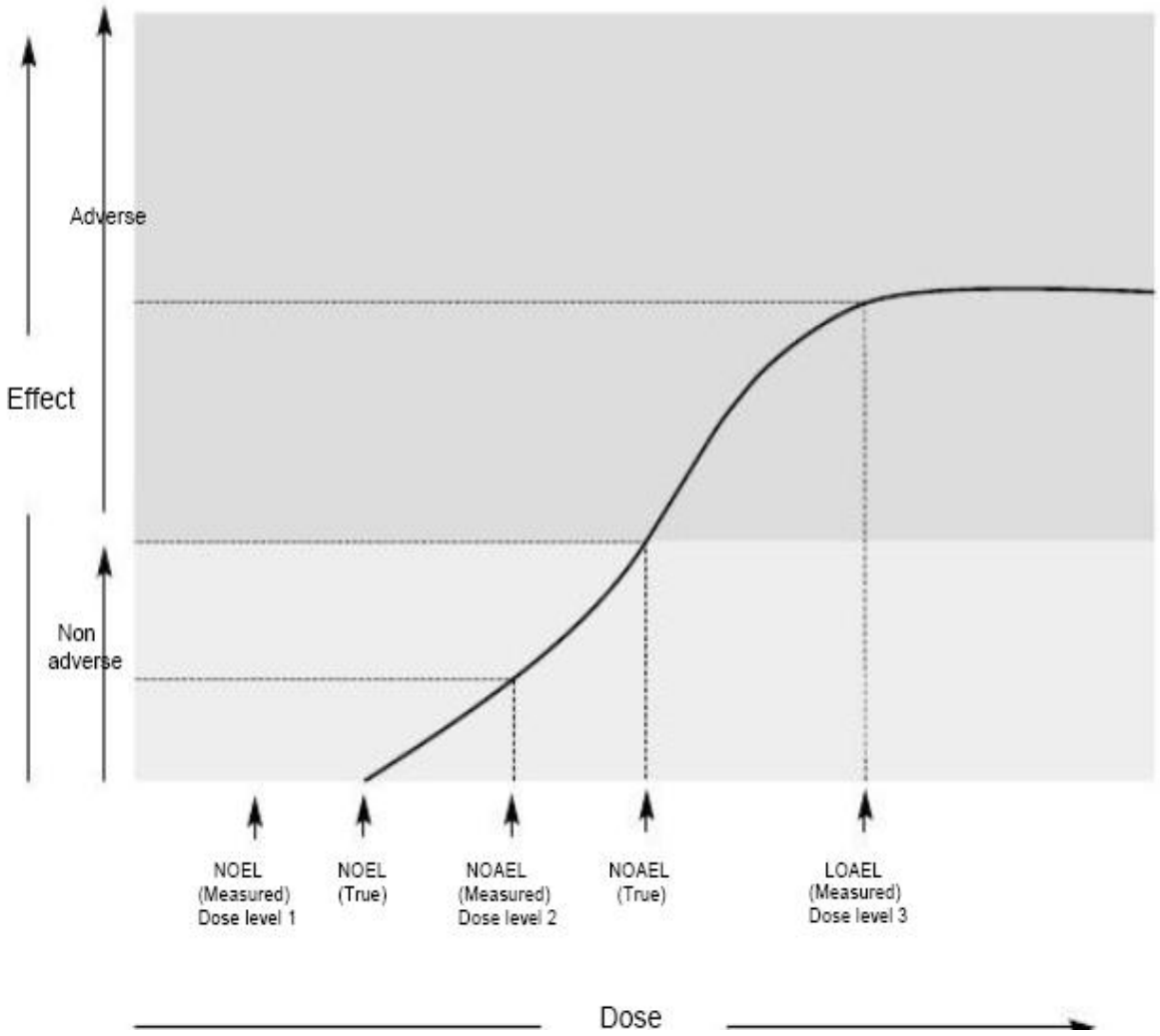

Figure 3: Dose- Response curve: No observed adverse effect level(NOAEL) and Lowest observed adverse effect level(LOAEL) are marked out. The identification of these levels of effect depend on the true dose-response relationship of the tested doses and the statistical power of the experiment.

1.5 EXPOSURE ASSESSMENTIN ENVIRONMENTAL RISK ASSESSMENT

This procedure covers any exposure that may relate to risks introduced due to how the chemical is handled or used, and quantifies incidence and exposure relationship. Exposure of the main endpoints is assessed by measuring the rate and measure of exposure concentrations, during and after the production of chemicals. With new chemicals, prediction could be made to assess their effects by estimating emissions, pathways and rates of movement of a substance and its transformation or degradation to derive the concentrations or doses to which human populations or environmental compartments are or may be exposed. It also describes the nature and size of the populations or compartments exposed to a substance, and the magnitude and duration of their exposure. Past or present exposure and anticipated future exposure can also be evaluated. Multimedia exposure models and measured data are often used, especially in environmental exposure assessment.

1.5.1 PERSISTENCE, BIO ACCUMULATION AND TOXICITY (PBT) ASSESSMENT IN ENVIRONMENTAL RISK ASSESSMENT

Persistence, Bioaccumulation and Toxicity (PBT) is a procedural assessment of substances based on their presence in the environment, rate of accumulation and aggregation in different environmental compartments and their toxicity effects. Chemical substances that have a PBT characteristics are more dangerous to the environment than others because of their inability to breakdown easily and possibility of transformation in different environmental media. (Blackstone et al 2013). Therefore, classification of substances using PBT required an exposure assessment (assessment of the expected exposures under the actual or anticipated conditions of use) (REACH 2012)

Several metrics are used by REACH to classify and label substances as either PBT or non PBT. (e.g secondary poisoning at work). Most applied metrics are often adapted to the relevant dose metrics for nanomaterials (Pronk et al 2009).PBT assessment is based on all the relevant information such as physiochemical, hazard and exposure in the context of the CSA for all substances above a volume of 100t/ year. A substance that fulfil all the three criteria for persistence, Bioaccumulation and Toxicity , and is present at a concentration of 80 % or more , is recognised as a PBT substance.

Persistence criteria: ranges from $T_{1/2}$>60 days in marine water, $T_{1/2}$> 40 days in fresh water or estuarine water, $T_{1/2}$> 180 days in marine sediments, $T_{1/2}$>120 days in fresh or estuarine sediments, $T_{1/2}$>120 days in soil.

Bioacumulation criteria: is based on BCF > 2000 L/Kg while

Toxicity assessment: is based on NOEC < 0.01mg/L for marine or fresh water organisms and carcinogenic chronic toxicity.

Human exposure to PBT contaminants has been reported in the literatures. Different studies have linked human PBTs exposure to many adverse effects including nervous system disorders, reproductive and developmental problems. Exposure of Plants and animals to PBTs in the environment is mostly through air, water and food interactions. Higher animals in the food chain(Birds) are mostly susceptible to be more exposed to higher toxic level, Figure 4. This is due to their ability to accumulate in animal tissues at a higher concentration than in water.(The Commission for environmental Cooperation (CEC 2013)

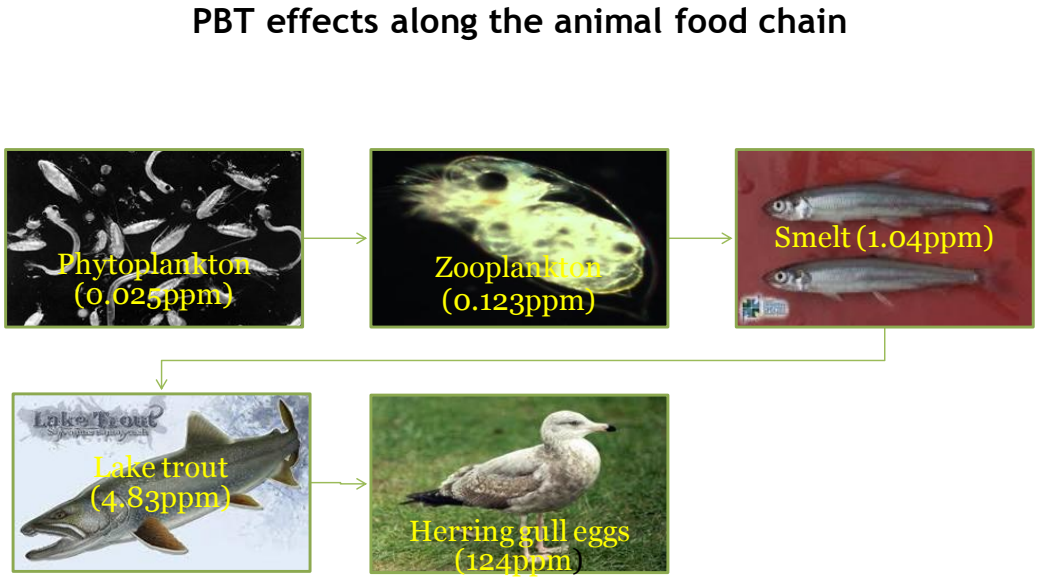

Figure 4 : PBT effects along the animal food chain

The EPA most recent published PBT chemical list identified sixteen chemicals and four chemical compound categories. Though limited in number, more may be identified through the ongoing research in risk assessment of nano-manufacturing.

1.5.2 EXPOSURE SCENARIO

Assessment of the expected exposures under actual or anticipated conditions of use of chemicals create exposure level to characterize the risk by comparing them with the outcome of the hazard assessment. This is achieved in two step procedure. First Step describes one or more initial exposure scenarios addressing how the substance is currently used throughout the supply chain a. The Second Step describes exposure estimate for different routes of exposure under the condition of use, e.g estimation of concentration of the substance indoor air at work place or at home, amount of substance in contact with skin when using an article, and concentration of a substance to be expected in the sediments of a water course.

To generate exposure scenario, there is need to understand operational conditions (OC) and risk management measures (RMM).ECHA 2012. Exposure estimation may be derived from either model or measured data. However it is important that the predicted exposure correspond to the OC and RMM.

Following procedures are employed to building the exposure scenario

(a) dialogue between substance manufacturers and downstream users,

(b) Dialogue between downstream users to downstream users further down the chemical supply chain.

1.6 RISK CHARACTERIZATION

Risk characterization compares the exposure level to standard hazard information such as Predicted – no- effect concentration PNEC or derived – no- effect level DNEL. Risk characterization estimates the incidence and severity of the adverse effects likely to occur in a human population or environmental compartment due to actual or predicted exposure to a substance. (C.J Van leeween; T.G Vermeire2007).It generally involves the integration of the previous three steps of :

1. Hazard identification.
2. Effects assessment,(the determination of the DNEL or PNEC)
3. Exposure assessment, (the determination of the PEC or human intake or exposure).

1.6.1Risk Characterization Ratio(RCRs) for environmental endpoints

Derivation of risk characterization ratio for chemicals is aimed at measuring the risk adequacy for each endpoints (environment and human population) that are likely to be exposed to the release of a substance. The purpose of the qualitative risk characterisation is to assess the likelihood that adverse effects are avoided when implementing the exposure scenario. (REACH 2008).

When suitable PEC or DNEL are available, risk characterisation ratios (RCRs) can be derived in order to decide if risks are adequately controlled for each environmental domain and for each human population known to be or likely to be exposed (REACH 2008). When these no-effect levels cannot be established for certain effects, a qualitative assessment of the likelihood that these effects are avoided when exposure scenarios are implemented shall be carried out .(ECHA 2012)

RCR is defined as the ratio of predicted environmental concentration PEC to predicted-no-effect concentration PNEC. (Box 1)

$$RCR = \frac{PEC}{PNEC} \text{ or } \frac{Exposure}{DNEL} \quad \text{.....(2.0)}$$

When PEC or PNEC cannot be derived, a qualitative risk characterisation should be conducted. The objective of the qualitative risk characterisation will be to assess the level of control over the risks generated by the substance.(REACH 2008) To compare the estimated exposure for relevant exposure scenarios with standard no effect level (PNEC, DNEL), relevant combination of exposure pattern with population exposed(workers, general population, consumers) and exposure route(inhalation, dermal, oral) are considered. Occupational exposure is categorized as a systematic long-term effects DNEL (dermal and inhalation) and systematic acute effects. Specifying the duration and route of exposure to humans that correspond to the DNEL. Table 2. Consumer exposure on the order hand is categorized as a systematic long-term effects DNEL (oral, dermal, Inhalation) and systematic acute effects.

Table 2: Duration and routes of exposure to humans corresponding to the DNEL

Exposure Pattern	Population exposed	Exposure route	DNEL unit
Occupational	Workers	Dermal	Daily deposition expressed in mg substance/cm^2 of skin
		Inhalation	Daily air concentration in mg substance/m^3
Consumer	General Population Humans exposed via the environment	Oral	Repeated exposure expressed as mg/kg/day
		Dermal	daily exposure expressed in mg substance/cm^2 of skin
		Inhalation	Daily air concentration in mg substance/m^3

Source: Guidance on information requirements and chemical safety assessment. Risk characterization (ECHA 2012)

Box 1: Human & Environmental risk Indicators

PEC—Predicted Environmental Concentration

(the concentration of the substance which will eventually be found in the environment)

PNEC—Predicted No Effect Concentration,

(the concentration of the substance below which adverse effects in the environmental compartment of concern are not expected to occur)

PEC/PNEC ratio—an indicator of risk.

PEC/PNEC < 1 = No immediate concern (No Risk)

PEC/PNEC > 1 = immediate concern (Risk)

PEC/PNEC is referred to as Risk Characterisation Ratio —**RCR**

Suitable PEC or DNEL is used to derive Risk Characterization Ratio(RCRs)

* RCR is used to decide if risk are adequately controlled for each environmental domain (REACH 2008)

* When PEC or PNEC cannot be derived, quantitative risk characterization is required (ECHA 2012)

1.6.2 RCRs for Human endpoints.

The risk to human can be considered to be adequately controlled if exposure level does not exceed the appropriate DNEL. Comparison is made between exposure and the no- effect level(NEL). Distinction is made between the effects exerted by the threshold and non- threshold mode of action.

For **threshold effects** for which a DNEL can be set, the RCRs is the ratio of the estimated exposure and the DNEL.

For **non – threshold effects,** a no-effect level and thus a DNEL cannot be established. If the DNEL could not be derived, REACH requires a quantitative assessment (DMEL) derived minimal effect level to be performed.

Intergrated framework for Risk Assessement RNMs

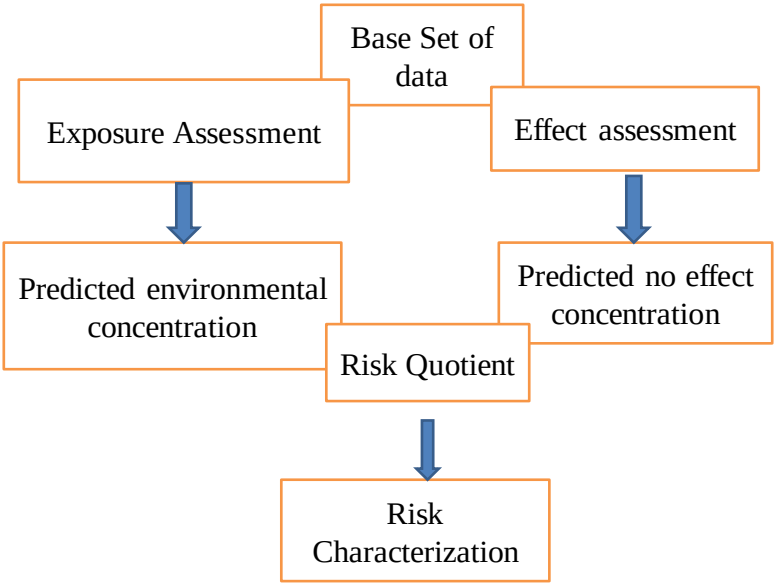

Figure 3: Risk characterization: a systematic procedure through which estimation of exposure and effects.(C.J Van Leeween; T.G Vermeire 2007)

1.7 ECOLOGICAL RISK ASSESSMENT

Ecological risk assessment is a process for organising and analysing data, information, assumptions, and uncertainties to evaluate the likelihood of adverse ecological effects (US-EPA, 1998). Application of problem formulation“ about why ecological effects have occurred, or may occur, from human activities.” (US-EPA, 1996) is considered. Figure 5. This is required for identification of the stressor, adverse effect and ecological value to be protected.

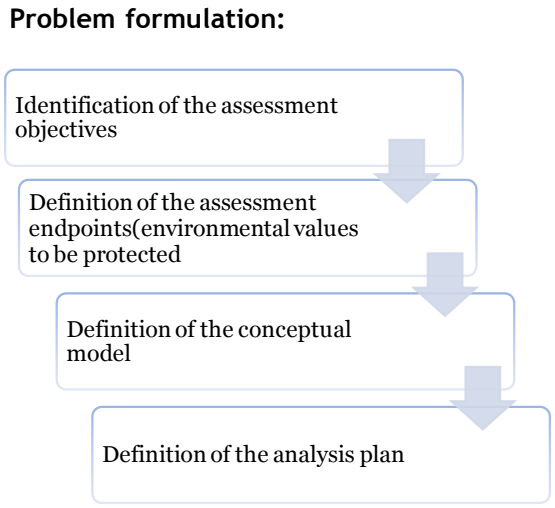

Figure 5: Framework for problem formulation

Information on sources and stressor characteristics includes:

(1) **Source type** (anthropogenic, natural, punctual, diffuse)

(2) **Stressor type** (chemical, physical, biological)

(3) **Magnitude of action** (chemicals concentration) and

(4) **Mode of action** (how stressor acts on organisms or on ecosystem functions: frequency, duration, spatial scale and exposure distribution)

Information on ecosystem characteristics includes:

(1) **Geographical location of the ecosystem affecting abiotic factors** (climate, geology, hydrology, soil and water type)

(2) **Structural characteristics** (abundance and number of species, food web relations)

(3) **Functional characteristics** (sources and energy processes, nutrient cycle)

(4) **Habitat classification and landscape context Sensitivity to the stressor** (sensitivity and exposure probability)

Information on ecological effects includes:

(1)**Type and quality of available information** (laboratory test, field experiments, modelling results)

(2) **Type of ecological effects are expected**

(3) **Conditions in which effects occur**

1.7.2 ASSESSMENT ENDPOINTS SELECTION

Three criteria used in the assessment endpoint selection are

***E*cological relevance** (e.g., may provide a fundamental habitat, activate nutrients biogeochemical cycles)

Sensitive to stressors , which includes

SENSITIVITY: how readily an assessment endpoint is affected by a stressor .*it depends on* stressors mode of action life stage of exposed organisms synergy with others stressors , and *is measured through* mortality, adverse effects on reproduction, community structure changes

EXPOSURE: frequency, duration and magnitude of exposure to stressor agents

Representative of management objectives i.e recreational or commercial relevance (direct or indirect) or public perception Essentially, it is required that assessment endpoints should include valuable ecological entity and should have measurable parameters.

The analysis phase involve the process of (1) Exposure characterization which assess the relationships between exposure conditions and stressors and (2) Effect characterization that assess the relationships between the concentration level of the stressor and the ecological effect. Risk characterization analyses the estimation of the ecological risk By the integration of exposure and exposure-effect profiles, using the line of evidence LOE, Interpretation of the adversity and estimation of the uncertainty associated to calculated risk.

In this chapter, the process of evaluation of risk assessment is explained. Given the required metrics, data conversion from animal test to human effects is described, with identification of adverse or no adverse effects in different endpoints(Human and Environment)

1.8 ILLUSTRATION: THE ECOLOGY OF *DAPHNIA MAGNA* AND THEIR ROLE IN SILVER NANOPARTICLE TOXICITY TESTING

Aquatic organisms such as Daphnia Magna are widely used in toxicity testing. They are used, depending on their life cycle and habitat, for different types of toxicity tests. Cladocerans and the fish *Pimephalespromelas* are fresh water organisms with habitat in the water column. These species are well suitable for use in water toxicity tests, due to their close proximity to the sediment. Figure 2

Cladocerans are polyphagous feeders and find their food in the seston (living and non-living particulate matter, suspended in the water column).

Daphnia magna plays a very important ecological role in freshwater habitats as a filter feeder that keeps algal blooms at bay oras, a major food source for a whole range of aquatic invertebrates or vertebrates. They are

sensitive to environmental conditions and many range of contaminants with a short life cycle that is observable in the laboratory.

Asghari et al 2012 investigated the toxicity effects of various sliver nanoparticles types(colloidal, ionic) on Daphnia Magna using a 48 hour immobilization test(acute toxicity test). The result concludes that the toxicity effect was dose and composition dependent when exposed to silver nanoparticles with abnormal physical orientation (swimming).

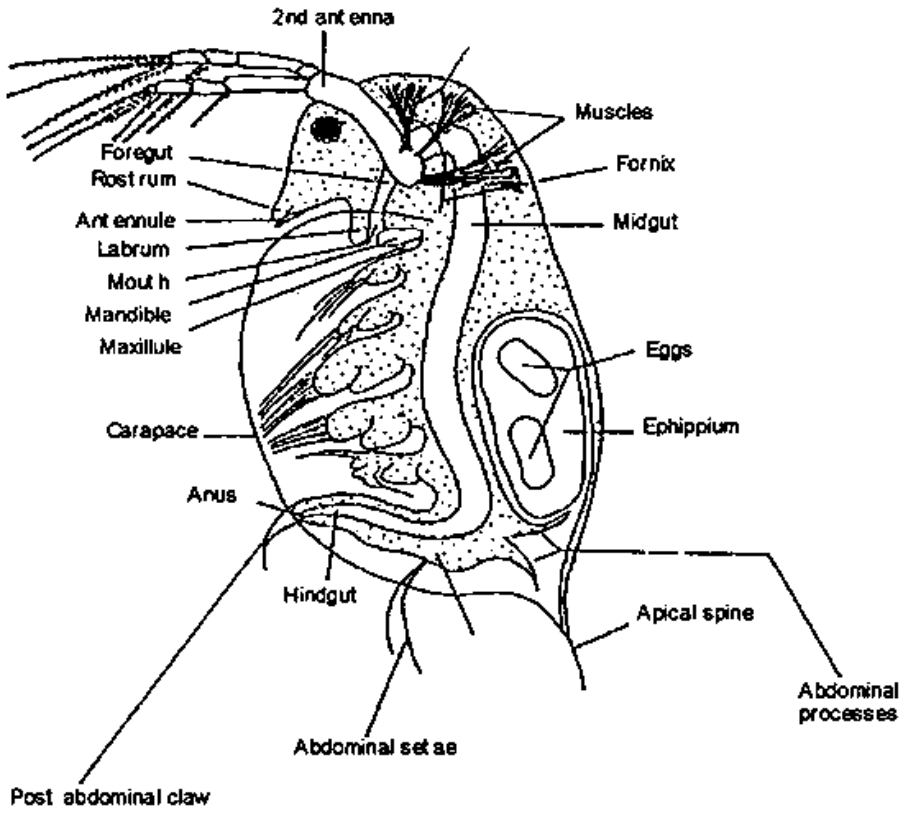

Figure 2: Anatomy of *Daphnia Magna* (Ebert et al 2005)

The reviewed test research on the effects of silver nanoparticles on human and environment is mostly based on acute test(short time of the whole life cycle), due to insufficiency of available data.

CHAPTER 2.0METHODS AND TOOLS FOR ECOLOGICAL AND OCCUPATIONAL NANO RISK ASSESSMENT

2.1QUALITATIVE REVIEW OF STRENGTH, OPPORTUNITY, WEAKNESS, AND TREAT OF APPLICABLE TOOLS

There is need to develop comprehensive and well-validated methodologies and tools for occupational and consumer safety evaluation of ENM (Hristozov et al., 2012). Available tools for both ecology and occupational as reported in the literatures with their description and applications. Table 3 and 4. These tools provide access to qualitative estimation, with continuous updating to meet the required procedure. They are mostly used as preliminary screening and research prioritization tools, aimed to identify relevant sources of risk in the lifecycle of synthetic nanomaterials and areas of knowledge deficits (Hristozov et al., 2012). Also, various robust methodologies are applied in the assessment of ENMs to deal with the limited data availability and uncertainty associated with each step of RA, e.g, Weight of Evidence (WOE) supported by Multi Criteria Decision Analysis MCDA. (Linkov et al. 2007; Tervonen et al. 2009; Zuin et al. 2010).

This chapter describes procedure towards achieving safety evaluation of occupational and ecology assessment of ENMs. Aim to support the development of a quantitative exposure model for relative ranking and prioritization of occupational exposure scenarios, specifically tailored for Nano-Silver.

Table 3:Nano risk/Ecological hazard assessment tools and their characteristic

Ecological Tools	Description
• Precautionary Matrix(Hock 2007)	• A ranking system supported by HHRA/ERA scope, Identify the need for precautionary measures in the lifecycle of the ENMs • Data Requirement: Physio-chemical properties, biological/environmental fate (i.e., stability under physiological/ environmental conditions), exposure data (e.g., frequency, magnitude) • REACH oriented (applicable in Industry)
• Risk based classification(Tervonen 2009)	• HHRA/ERA scope, Group ENMs in risk classes for screening level RA. Stochastic Multicriteria Acceptability Analysis (SMAA-TR) • Group ENMs in risk classes for screening level RA • Physicochemical properties, toxicity / ecotoxicity effects, environmental fate (i.e., bioavailability, bioaccumulation) • REACH oriented(applicable in Industries and academia)
• Worst case definition model(Sorensen 2010)	• HHRA scope, Assess the relative hazard of ENMs using Weight Of Evidence(WOE) • Non REACH oriented. (applicable by academia and Regulators • Physicochemical properties, exposure-related characteristics (e.g., stability), toxicity effects.

Source: Hristozov_et_al 2012

Table 4:Nano risk/ occupational hazard assessment tools and their characteristic

Occupational Tools	Description
• Nanosafer (2010)	• To estimate relative occupational risk and recommend risk reduction measure • physicochemical properties and/ or manufacturing processes data requirement • Estimated occupational risk level and recommendation on the most suitable exposure control. Applicable in industry.
• Control Banding tools (Anses 2010)	• provided a checking and corrective action steps (period assessment, data recording, routine monitoring and measurement with minimum prevention method). • Input data based on bulk material. Applicable in industry
• Stoffenmanager Nano1.0 (Duuren Sturman 2011)	• Estimation of relative occupational risk and recommend risk reduction measures • Physicochemical data/ exposure information • Estimation of risk level and recommendation on the most suitable occupational exposure control. Applicable in industry

Source: Hristozov_et_al 2012

2.1.1 SWOT ANALYSIS OF APPLICABLE TOOLS

Considering the overwhelming limitations to the risk assessment of ENMs, there is a need for timely governance of ENM, which requires flexible methodology. In this section, the Analytical Hierarchy Process (AHP) approach is applied on the most recent tools used in the risk assessment of ENMs. With reference to their Strengths, Weaknesses, Opportunities and Threats (SWOT), Ecological and occupational tools currently applied to ENM are examined, characterized and compared based on concept, quantification and scope."The Analytic Hierarchy Process (AHP) is a theory of relative measurement of intangible criteria. In the AHP, paired comparisons are made with judgments using numerical values taken from the AHP absolute fundamental scale of 1 to 9. A scale of relative values is derived from all these paired comparisons and it also belongs to an absolute scale that is invariant under the identity transformation like the system of real numbers. The AHP is useful for making multi-criteria decisions involving benefits, opportunities, costs, and risks. The ideas are developed in stages and illustrated with examples of real-life decisions." (Saaty et al 2005)

The objectives of AHP technique in SWOT analysis of the different tools are (1) to systematically evaluate SWOT factors (quantitative information about the versatility of different tools), (2) to examine their intensities(present or anticipated applications). Conclusion is made on the factors which predominate among the four SWOT groups.

This technique has the advantages of :

1. capability to make both quantitative and qualitative decision factors commensurable
2. flexibility with regards to the setting criteria to realize the desired objectives of the tools.

2.1.2OUTLINE FOR APPLYING AHP IN SWOT ANALYSIS

Using the hierarchical decision schema, the criteria properties of the tools are compared in a pairwise procedure within and between the SWOT group. Thus, a quantitative values(level of importance) of 1,2,3 …..5from verbal comparison are derived by pairwise comparison of factors.

*SWOT group(Strength, weakness, opportunity,threat)

*SWOT factors (individual criteria)

Table 5 SWOT analysis within groups of the ecological risk assessment tools

SWOT Group	Precautionary Metrix(Hock 2007)	Risk Based Classification(Tervonen2009)	Worst case Definition model(Sorensen2010)
Strength	*Considered the potential exposure throughout the whole life cycle of the examined nanomaterial. * Identification of high scenario (It can easily be updated) *Reliable near term risk estimation	* Preliminary grouping of ENMs in risk classes for screening(uncertainty characterization) * Surface properties are considered(agglomeration, reactivity charge, bioaccumulation , bioavailability, particle size and toxic potential * Inclusion of whole life cycle	* Boundary set-up for risk assessment * Clarification and identification of uncertainties *Multiple sampling procedure (sub models)with high variability
Weakness	*No quantitative and qualitative risk assessment, in accordance with the type and the quantity of available data. *No step for prioritization of relevant life cycle stages * No clear distinction between ecotoxicology and human toxicity parameters	* Assessment based on assumption * Uncertainties related to input parameters * No clear distinction between ecotoxicology and human toxicity parameters	* Identification of area at risk *Lack positive feedback mechanism * No clear distinction between ecotoxicology and human toxicity parameters
Opportunity	*Direct identification of the level of uncertainties *Inclusion of life cycle perspective *A template for the report of the risk assessment procedure and the communication of the risks is provided.	* Expected environmental impact determined * Documented application(C60, MWCNTs) *Adaptive iterative structured(Allow prior evaluation, grouping)	* Disclosure of significant sources of uncertainties * Point focused assessment (PUs and Crs) * Qualitative and subject to individual interpretation
Threath	*Error in score identification might not be easily detected due to the range used for the measurement *Unclear past and future aspects of the material life cycle *No documented applications, where the entire life cycle of an ENM or a nano-containing product is considered	* Limited data requirement * No quantitative estimation * Material specific	* No reference for uncertainties * low quantitative estimation * Non accountability of uncertaintie

2.1.3 PAIRWISE COMPARISON OF FACTORS WITHIN EVERY SWOT GROUP

Choosing the most important factors of the application of tools, pairwise comparison is made within the SWOT. The relevant criteria factors of the tools were identified and included in SWOT analysis for the ecological and occupational tools. Based on the compliance of the different tools to relevant criteria. *Table 4*, the SWOT analysis was constructed with three factors each for the SWOT group) Table 6 . For Standard AHP applications, it is recommended that the number of factors for each SWOT group should not exceed 10, because the number of pairwise comparisons needed in the analysis increases rapidly. (Saaty et al, 1980)

Comparison is based on the input criteria characteristics, namely, factor with greater weight and magnitude of the weight. The relative priorities of the criteria factors are computed using the eigenvalue technique in a iterative process through the multiplication of the matrix by itself, calculation of the eigenvector by summing up the rows and normalizing the sums, and repetition of step 1 and 2 until the difference of the new eigenvector is minimal

The pairwise comparisons matrix is constructed . With the relevant element according the following equations

- $a_{ij} = 1/a_{ji}$ and thus, ……(3.0)
- when $i = j$, $a_{ij} = 1$. ……(4.0)

The value of Z_i vary from 1 to 9,

- where 1/1 indicates equal importance and 9/1 indicates extreme importance

2.1.4 PAIRWISE COMPARISON OF (MOST IMPORTANT) FACTORS BETWEEN THE FOUR SWOT GROUP

Each tool is represented by the factors with the highest intensity value, generating four overall factors from each SWOT group. Comparison is made to calculate relative intensities between them. *Table 8,9 and10*. These factors represent the scaling factors of the four SWOT group, and are used to calculate the overall general priorities (Eigenvector) of the independent factor within the SWOT group which sum up to one. Numerical value for the factors are calculated to define the strategies.

The λ_{max} of a reciprocal matrix **A** is always greater than or equal to *n* (Saaty et al 1977). If the pairwise comparisons do not include any inconsistencies then, $\lambda max = n$. (where n=3 for this comparison)

The more consistent the comparisons are, the closer the value of computed λmax is to *n*.

Based on this property, a consistency index, CI, is calculated using the equation

$CI = (\lambda_{max}-n)/(n-1)$ ……...(5.0)

where n=3

*CI estimates the level of consistency with respect to a comparison matrix. Since CI is dependent on *n*, a consistency ratio CR is calculated.

*CR: measures the coherence of the pairwise comparisons. To estimate CR, the calculated average consistency index of randomly generated comparisons, ACI is used.

$CR = 100(CI/ACI)$ ……(6.0)

*ACI varies functionally, according to the size and range of the matrix 0,58 is used.

Table 6: Average consistency index (ACI) based on random judgments depending on the order of matrix: (according to Saaty 1980)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0,00	0,00	0,58	0,91	1,12	1,24	1,32	1,41	1,45	1,49	1,51	1,48	1,56	1,57	1.59

Table 7: Calculation of Eigenvector for the SWOT groups

Strengths	s1	s2	s3	sum	eigenvector
s1	27.17	99.09	90.05	216.31	0.6342
s2	7.47	27.26	24.77	59.5	0.1745
s3	8.19	29.89	27.17	65.25	0.1913
sum				**341.06**	**1**
Weaknesses	w1	w2	w3	sum	eigenvector
w1	30.95	44.69	128.85	204.49	0.5173
w2	21.35	31.04	89.39	141.78	0.3587
w3	7.39	10.68	30.95	49.02	0.124
sum				**395.29**	**1**
Opportunities	o1	o2	o3	sum	eigenvector
o1	29.423	18.112	9.6335	57.1685	0.1729
o2	53.7813	35.5132	18.112	107.4065	0.3249
o3	82.8072	53.7813	29.423	166.0115	0.5021
sum				**330.5865**	**1**
Threats	t1	t2	t3	sum	eigenvector
s1	42.29	22.5143	103.635	168.4393	0.4146
s2	47.0675	25.99	114.845	187.9025	0.4625
s3	12.4172	6.6005	30.9501	49.9678	0.1229
				406.3096	**1**

Table 8*: PRECAUTIONARY MATRIX (HÖCK, 2011) Group Priorities based on the consistency ratios and consistency index of the SWOT groups and their factors with the greater weight with respect to each SWOT group*

SWOT Group	Priority of factor within the group(Eigenvector)	SWOT Factor	Consistency factor	Consistency Index
Strength	0,6342	Considered the potential exposure throughout the whole life cycle of the examined nanomaterial.	0,0028	0,48%
Weakness	0,5173	No quantitative and qualitative risk assessment, in accordance with the type and the quantity of available data.	0,0517	8,91%
Opportunity	0,6211	Inclusion of life cycle perspective	0,0486	8,39%
Threath	0,6268	Unclear past and future aspects of the material life cycle	0,0466	8,05%

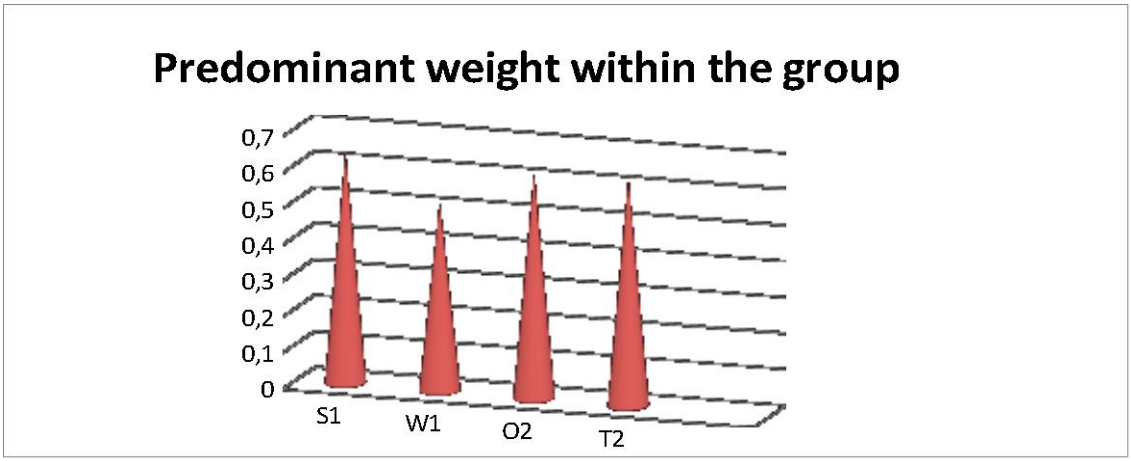

Figure 6 Comparison between weight of predominance between the group for the Precautionary matrix (Höck, 2011)

Table 9: RISK BASED CLASSIFICATION SYSTEM(TERVONEN, 2009) Group Priorities based on the consistency ratios and consistency index of the SWOT groups and their factors with the greater weight with respect to each SWOT group

SWOT Group	Priority of factor within the group(Eigenvector)	SWOT Factor	Consistency factor	Consistency Index
Strength	0,5173	Surface properties are considered(agglomeration, reactivity charge, bioaccumulation , bioavailability, particle size and toxic potential	0,0028	8.8%
Weakness	0,5740	Uncertainties related to input parameters	0,0498	8,6%
Opportunity	0,5965	Expected environmental impact determined	0,0273	4,72%
Threath	0,4835	Material specific	0,01415	2,44%

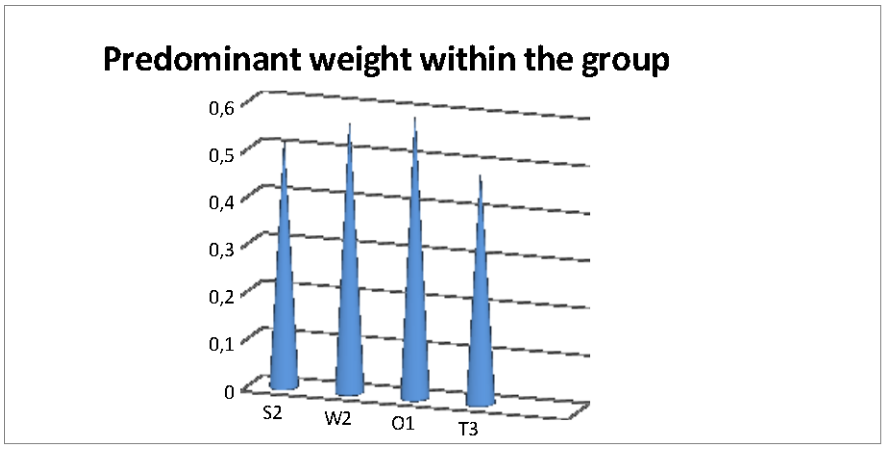

Figure 7 Comparison between weight of predominance between the group for the Tervonen (Risk based classification

Table 10: WORST CASE DEFINITION MODEL(SORENSEN, 2010)Group Priorities based on the consistency ratios and consistency index of the SWOT groups and their factors with the greater weight with respect to each SWOT group.

SWOT Group	Priority of factor within the group(Eigenvector)	SWOT Factor	Consistency factor	Consistency Index
Strength	0,4584	Multiple sampling procedure (sub models) with high variability	0,0333	5,74%
Weakness	0,5586	No clear distinction between ecotoxicology and human toxicity parameters	0,0028	0,5%
Opportunity	0,6211	Point focused assessment (PUs and Crs)	0,0052	0,89%
Threath	0,6268	low quantitative estimation	0,0078	1,33%

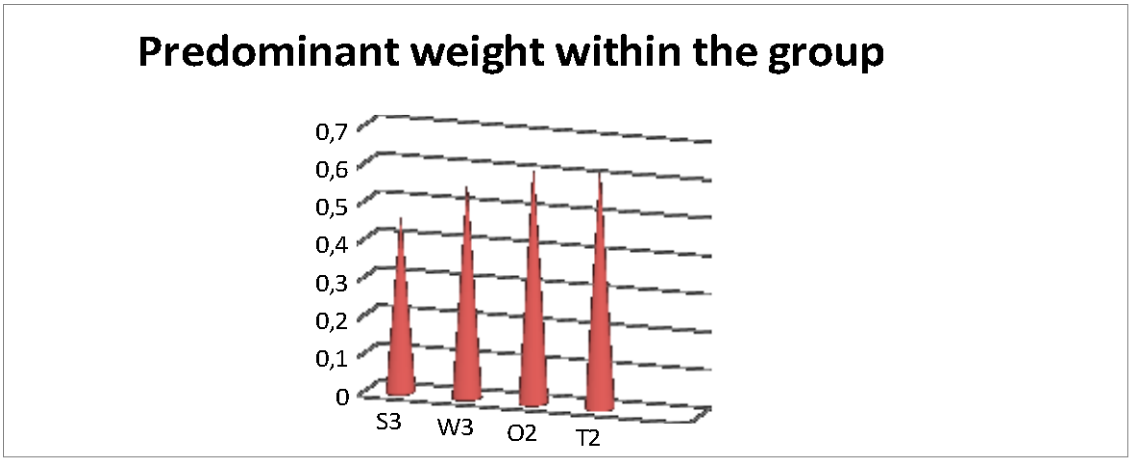

Figure 8 Comparison between weight of predominance between the group for the), Sorensen's(Worst case definition model)

Table 11 SWOT analysis within groups of the occupational risk assessment tools

SWOT Group	NANOSAFER 2010	CONTROL BANDING TOOLS(ANSES 2010)	STOFFENMANAGER NANO 1.0(Duuren-Sturman,2011)
Strength	*considered life cycle of the material *Include toxicology and surface area adjustment exposure *Construction of specific exposure scenario(Risk levels and prioritization band identify)	*Checking and corrective action steps(period assessment, data recording, routine monitoring and measurement) *Control band independent of the exposure band *Analysis of safety data sheet(contact with the supplier)	* Life cycle approach * Accounted for Near- field and Far-field exposure * Risk prioritization in exposure scenario
Weakness	*Input data based on the bulk material *Inconclusive scientific information needed(Bioavalability, degree of aglomeration) *No indication of intrinscic emission potentials of the materia	*Not adapted for large volume of data *Exposure based on assumption *Input data based on bulk material	* Bulk material based(Input data based on bulk properties of the material) * Information on the reactivity of NM is inconclusive. * Unconfirmed robustness
Opportunity	*Address both acute and work-day exposure *Clear contextual exposure information to construct the specific exposure scenario *E-learning tool and online report avalability(Easy accessibility to input data)	*Could be used in any work environment *Easy and simple to use *Minimum prevention method	* It indicate the intrinsic(physiochemical properties) emission potential of materials(distinction between ENMs in powder or granules) * Clear contextual exposure information to construct the specific exposure scenario * Particle characteristics based on parent material
Threath	*Exposure measurement based on assumptions *High input data requirement *Not validated	* High input data requirement * Non-inclusion of physical properties of ENMs * Inconclusive scientific information needed(Bioavalability, degree of aglomeration)	* High input data requirement(intensive data) * Assumption based exposure * Has not been tested

Table 12: OCCUPATIONAL TOOL NANOSAFER 2010.Group Priorities based on the consistency ratios and consistency index of the SWOT groups and their factors with the greater weight with respect to each SWOT group

SWOT Group	Priority of factor within the group(Eigenvector)	SWOT Factor	Consistency factor	Consistency Index
Strength	0,5051	Considered life cycle of the material	0,01866	3.22%
Weakness	0,6254	No indication of intrinscic emission potentials of the material	0,0067	1,15%
Opportunity	0,5326	Clear contextual exposure information to construct the specific exposure scenario	0,00256	0,44%
Threath	0,5400	High input data requirement	0,0025	0,42%

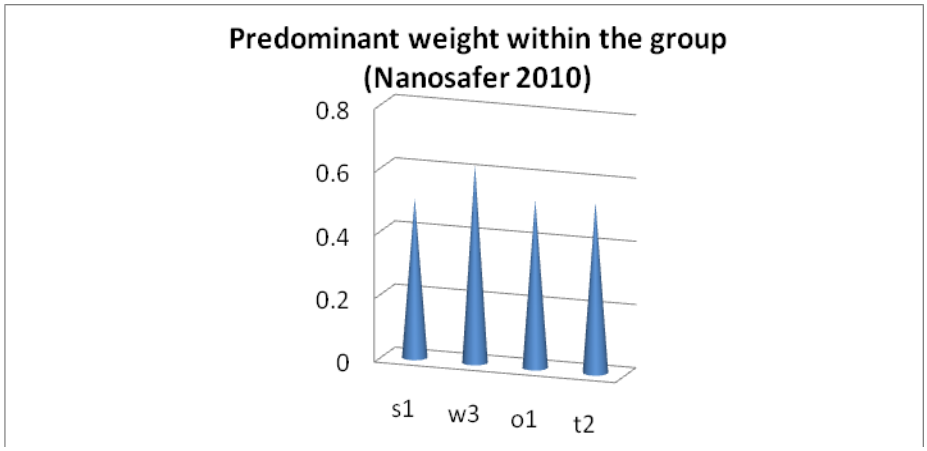

Figure 9 Comparison between weight of predominance between the group for the Nanosafe 2010

Table 13 OCCUPATIONAL TOOL ANSES 2010.Group Priorities based on the consistency ratios and consistency index of the SWOT groups and their factors with the greater weight with respect to each SWOT group

SWOT Group	Priority of factor within the group(Eigenvector)	SWOT Factor	Consistency factor	Consistency Index
Strength	0,5494	Checking and corrective action steps(period asessment,data recording,routine monitoring and measurement)	0,038	6,55%
Weakness	0,5472	Input data based on bulk material	0,245	4,22%
Opportunity	0,5407	Minimum prevention method	0,01	1,7%
Threath	0,4933	Non-inclusion of physical properties of ENMs	0,025	4,26%

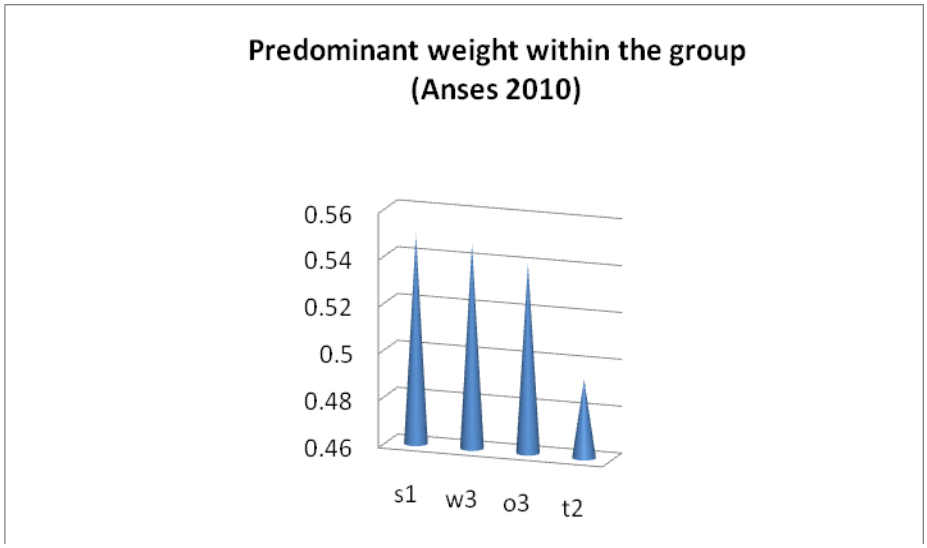

Figure 10 Comparison between weight of predominance between the group for the Control banding (Anses 2010)

Table114: OCCUPATIONAL TOOL STOFFENMANAGER NANO 2011.Group Priorities based on the consistency ratios and consistency index of the SWOT groups and their factors with the greater weight with respect to each SWOT group

SWOT Group	Priority of factor within the group(Eigenvector)	SWOT Factor	Consistency factor	Consistency Index
Strength	0,5469	Life cycle approach	0,0028	4,59%
Weakness	0,4444	Bulk material based(Input data based on bulk properties of the material)	0,0075	1,29%
Opportunity	0,5486	It indicate the intrinsic(physiochemical properties) emission potential of materials(distinction between ENMs in powder or granules)	0,0243	4,19%
Threath	0,5399	High input datarequirement(intensive data)	0,0466	7,58%

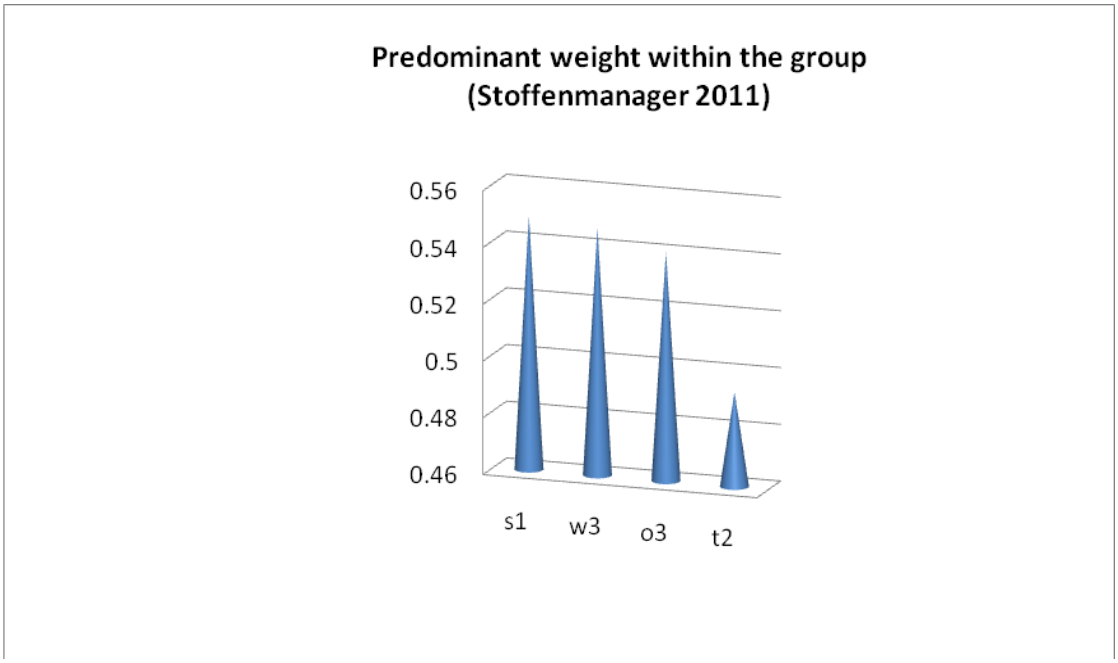

Figure 11 Comparison between weight of predominance between the group for the StoffenmanagerNano 2011

2.2 RESULT AND DISCUSSION

The results indicate that the life cycle of examined ENM is important for a qualitative and quantitative risk assessment. Using the available data, past and present future aspects of the material effect could be identified and coupled into any framework for an effective certification. Considerations of surface properties agglomeration, bioavailability, particle size and toxic potential could be a potential strategic alternative for a qualitative and quantitative assessment procedure. Since the strength of most of the applied tools come from the application of life cycle assessment with very low consistence value. Uncertainties related to input parameters are reduced with every stage of the material been evaluated. Therefore providing opportunity for measuring any expected environmental impact throughout the whole life cycle..

The AHP procedure is an iterative process, based on multiple sampling procedure with high variability.(e.g, It enhance a clear comparison between ecotoxicology and human toxicity parameters as applied to the tools). This serves as an iterative process of multiple sampling procedure. In addition, the needed pairwise comparisons were found useful, because they force the decision maker tothink over the weights of the factors and to analyze the situation more precisely and in more depth.

2.2.1 ECOLOGICAL ASSESSMENT TOOLS

The *Precautionary matrix (Höck, 2011), figure 6* with the greatest strength weight factor among the four groups. This explains how important the whole life cycle of the material is important to measure the ecological effect of the Nano materials. The tool's versatility covers the broad present and future applications. The Risk based classification(Tervonen 2009),*figure 7, where* the greatest weight factor is the opportunity, hence, describes the necessity of consideration of specific requirements for the characterization of ENMs(expected environmental impact of the Nano materials is the basis on which the assessment of ecological effect could be measured). While in the Worst case definition model(Sorensen 2010), *figure 8,* the greatest weight factor is the Threat with low quantitative analysis of the criteria used for measuring the ecological impact of ENMs.

2.2.2 OCCUPATIONAL ASSESSMENT TOOLS

For the occupational assessment tools, ***NANOSAFER 2010***: *table 12.* The strength and opportunity which predominate refer also to the consideration of life cycle of the material and clear contextual exposure information to construct the specific exposure scenario. The weakness and threat that may result from the use of the tool indicate a non-indication of intrinsic emission potentials of the material and high input data requirement which may produce a negative feedback.

The ***CONTROL BANDING TOOL(ANSES 2010)****table13*provided a checking and corrective action steps (period assessment, data recording, routine monitoring and measurement with minimum prevention method). These factors represent a constructive procedure towards the realization of a qualitative and quantitative data needed for effective assessment for the present and near future exposure analysis. Input data based on bulk material and non-inclusion of physical properties of ENMs represent a major setback which can be corrected.

The ***STOFFENMANAGER NANO1.0(Duuren-Sturman,2011)*** *table14* addresses the intrinsic (physiochemical properties) emission potential of materials (distinction between ENMs in powder or granules) with the inclusion of life cycle approach as the predominant opportunity and strength within the group. This represent the importance of the factors as regulatory procedure indicator. Bulk material based(Input data based on bulk properties of the material) and high input data requirement as the predominant weakness and threat.

2.3 CONCLUSION

In this chapter, a common strategic planning tool, SWOT, was used to determine the importance of different weight factors required for describing the relevance of the applicable tools employed for the formulation of different frameworks aimed towards the assessment of ENMs effect on the environment.

The objective of this study is to present a procedure, where weaknesses and threats of any tool can be identified. Moreover, it helps to avoid any error and limit future application deficiency. Strengths and opportunities obtained can provide improvement to any available tool for future qualitative assessment. This was done by linking SWOT with a decision analysis method, AHP. The result was a hybrid method, which produces the quantitative values for the SWOT factors.

One of the advantages of AHP analysis is the capability to handle decision making situations with some uncertainties and inconsistencies. The uncertainties and limitations in any tool make the conventional RA infeasible for ENMs. Therefore, risk assessment tools that can effectively generate results are needed to adequately inform regulators(Grieger et al. 2010; Hansen 2009; Hristozov&Malsch 2009).

It must be noted that, AHP calculations does not provides any direct information about the uncertainty of the priorities obtained. But contribute to the knowledge of the need to analyse materials over the whole life cycle. Thereby leading to identification of stage/stages that could contribute to the release of nanometerial into the human and ecological domain of the elements at risk

CHAPTER 3 REVIEW OF REGISTRATION OF CHEMICALS

This chapter describesthe procedure, process and requirement stipulated by different regulations towards the registration of chemicals and eventual approval for the placement of such chemicals in the market. Inadequacy in the system, techniques, tools for assessing risk with the uncertainties in predicting effects can make it difficult to discover potential risks, therefore, a need for more harmonized data to bridge the datagap. One of the problems encountered is the measurement of exposure levels in the environment, where model concentration are approximately in the order of 100µg$L^{-1}$with potential rise to about 1µgL^{-1} depending on applications, uses and behaviour of ENMs within the environment.(Hazardous Substance Advisory Committee HSAC, 2013)
Two case studies are examined
Case one: Registration of Biocides (Use of Sodium silver thiosulfate) in EU according to the REACH regulation.
Case two: Registration of HeiQ AGS-20 (a textile nanosilver material) in US (EPA regulation)

3.0 NANO MATERIAL UNDER REACH: NANO SILVER AS A CASE STUDY

The development of nanotechnology and the use of Silver nano-scale particles in many range of consumer products have raised concern about its impact on the environment and human health. Thus, it is required of any regulatory procedure to applya comprehensive analysis and framework to regulate the production and use of nanomaterials. Despite many research conducted on the toxicity of silver ions, there still remain uncertainties on the amount of Silver Nanoparticles (AgNp) and associated colloidal silver ion (Ag^{+}) ion released and the concentration that could result in toxicity. This section deals with nanosilver as a case study under the EU (REACH) regulation. Required procedures to distinguish different forms and nature of nanomaterials, mode of release to support the datagap analysis in the framework are explained.

3.1 ENVIRONMENTAL RELEASE OF SILVER

Many Industrial processes such as mining, photography, and jewelry manufacture have led to an increased levels of silver being released into the environment (U.S. EPA, 1987). More concentration of nanoparticle occur near sewage outfalls, electroplating plants, mine waste sites, and runoff from silver disposal sites. Analysis of the risk to freshwater ecosystems of silver nanoparticles from textiles and plastics predicted that an increase of 15% of the total silver released into water in the European Union would come from biocidal plastics and textiles. Where the predicted mass flow of Silver into the natural water range from 20-130 tons/year and in solid waste/ Sewage sluge ranges from 80-190 tons/year. This compare with the mass flow of biocidal Silver in natural water (20tons/year) and solid waste/sewage sluge (29tons/year).(Blaser et al. 2008).

Distinction is made between different forms of substance based on their size distribution, chemical characteristics and reactivity. For Silver, the nanoform is one that measures between 1-100nm, while higher size dimension(greater than 100nm) are classified as the ionic and bulk form.(Kulinowski2008) provides an excellent review of the different types of silver and some of their characteristics Table 15.
Figure 12 illustrates the differences between the chemical reactivity of ionic, nanoform and bulk forms of silver.

DISSOLVED FORM : The dissolved form of silver is silver ions. Dissolved ionic silver is the chemically and biologically most active form of silver and is highly toxic in this form.
BULK FORM: Silver can be bound to larger particle, sediment, colloidal particle, or macromolecule.

NANOSILVER: May be present in colloidal form, dissolved in water or as a suspension in the form of silver chloride. Nanosilver particle can be produced using physical or chemical methods. Because of their very small size nanosilver particles can potentially pass through biological membranes and reach more and different organs and tissues in the body. Nanosilver acts as a reservoir for the delivery of dissolved silver ions, which have a strong bactericidal effect. Nanosilver particles have also been shown to be toxic independent from released silver ions.

Table 15: Difference form of Silver and their attributes

Term	Diameter(nm)	Attributes
Elemental/Metallic (Ag)	0.288	Not found in nature as a single Atom but in alloy form. The form found in ornaments, jewellery, coins and utensils.
Ionic /Silver ion (Ag+)	0.258	A single silver ion formed when a silver atom dissolved in water. May have +ve or -ve charge. Ionic silver is much smaller than nanosilver.
Nanosilver/ Nanoparticle silver (Nano-Ag)	1-100	May release ion or be toxic on its own.
Colloidal (Depends)	1-1000	A mixture of different sized particles, suspendedin fluid, may contain nano particulate silverorsilverions or both.
Inorganic silver compounds/silver salts (AgCl, Ag_2O)	Depends	Not easily dissolved, can be ionised
Organicsilver compounds(Depends)	Depends	Covalent, almost impossible to dissolve.

Adapted from Kulinowskiet al (2008) ICON (Environmental Impacts of Nanosilver)

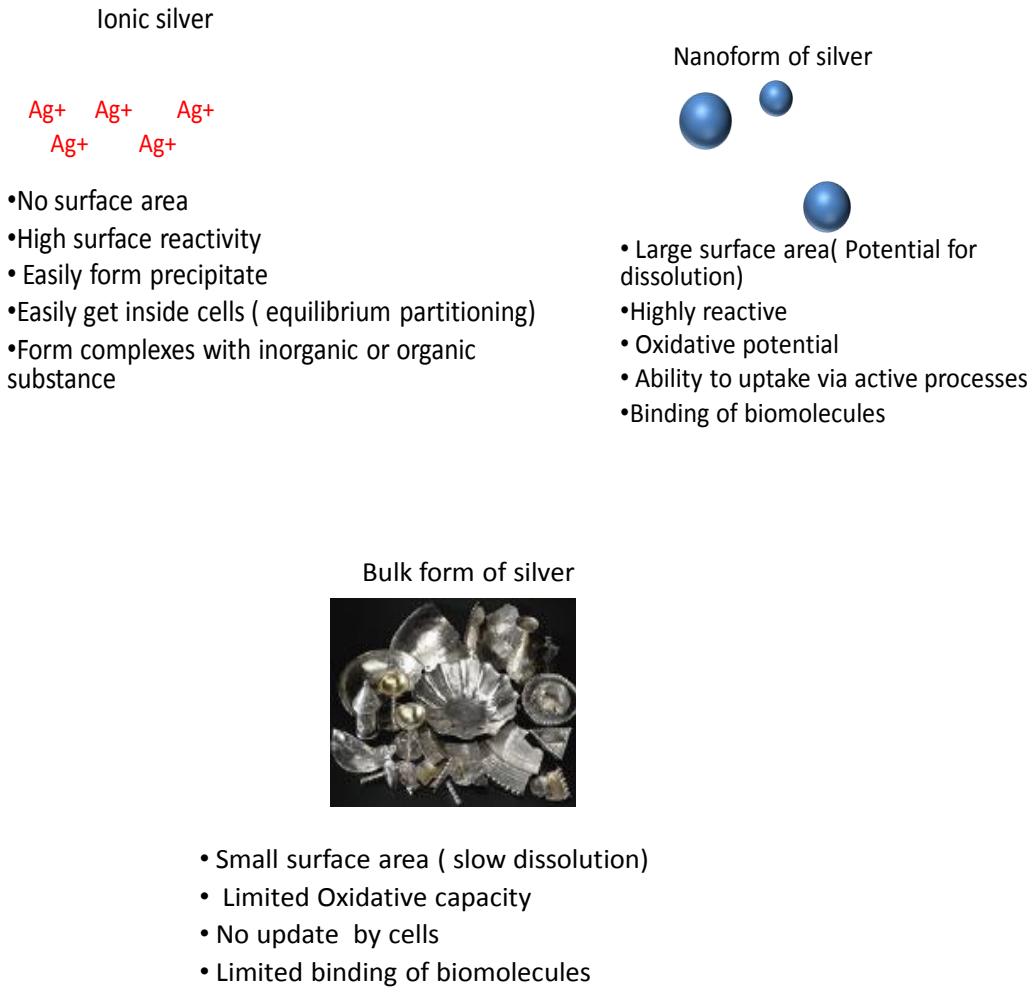

Figure 12 : Differences between the chemical reactivity of ionic, nanoform and bulk forms of silver. Schwirn et al 2014

3.2 METHODS OF PREPARATION OF NANOSILVER

Different studies show that, while ENM has the ability to penetrate cell walls and membranes, toxicology studies conducted on the dissolution effect of silver nanoparticles in the different media have reported different mechanism.(release of Ag+ through dissolution, inhibition of oxidative enzymes and surface binding).The physiochemical properties of nanomaterials depends on the particle's structure (size, shape, composition and surface structure).The main parameters affecting the quality of obtained nanomaterials are :

(1) the reaction method.

(2) quantitative and qualitative composition of reagents used. (Kowalski et al 2011). Therefore, a need for sensitive methodology that investigate particulate different structure. This can be explain by the methodology used for the preparation of the nanomaterials. Table 16lists different methodologies employed for the production on

nanosilver, the mode of preparation and advantages. Where the selection of methods present an impact on the final characteristics of the intended particles.

Table 16: Different methodologies employed for the production on nanosilver

Method	Mode	Advantage
Reduction of Silver nitrate(Panigrahiet et al 2004; Song et al 2009)	Using$NaBH_4$ on Initial concentration of silver salt	*Low tendency of nanoparticles to aggregate * easy to conduct * Low cost reagents and equipment * Large scale production
Photochemical reduction. (Kempa et al 2006)	Immediate reduction of silver cation using Ultra violet light.	* Nanoparticle formed are trapped kinetically. * Size range can easily be detected and produced
Laser Ablation (Pearce et al 2004)	Liquid –Solid interface reaction	* Purity and stability of the product * ability of migration of particles from solid to liquid
Vacuum ion spraying (Kowalski et al 2010)	Silica matrix bombardment	* High purity of nanoparticles
Electrochemical method	Anodic metal dissolution, reduction of intermediate salt in cathode	*Exceptional purity of nanoparticles * ability to control shape and size of particle * simple and affordable equipment.

3.3FATE AND EFFECTS OF SILVER AND NANOSILVER ON HUMAN

Research into the release of silver ions from medical devices such as catheters has shown that excess silver ions will form protein silver complexes, which are deposited into the liver, kidney, lung, brain and skin (Landsdown 2007).

Workers in industries using silver or increasingly nanosilver are most vulnerable to occupational exposure and strict occupational health and safety standards must be implemented and their compliance subsequently monitored.

Many nanosilver toxicity studies have focus on bacteria and complex animal species such as fish, rats, mice, and quails. *In vitro* studies demonstrate that nanosilver is toxic to mammalian liver cells (Hussain et al. 2005), stemcells (Braydich-Stolle et al. 2005) and brain cells (Hussain et al. 2006). A study on bioluminescent bacteria showed that nanosilver particles can disrupt cell membranes resulting in cell toxicity and cell deformation (Hwang et al. 2007). *Table 14* provides a summary of studies relating to animal nanosilver particle toxicity.

A number of researchers have shown that nanosilver particles can destroy the ability of bacterial DNA to replicate (Berger 2007) or can damage DNA. (Ahamed et al. 2008) used two lines of mammalian embryonic cells to investigate the effect of uncoated and coated silver nanoparticles (25nm) on DNA. Irrespective of being coated ornot, both types of silver nano particles induced cell death and caused DNA damage. However the polysaccharide coated particles caused more severe damage, perhaps due to different surface chemistry and decreased agglomeration.

One of the important factor in the antibacterial properties of nanoparticles is their shape. For example, the reaction of triangle silver nanoparticles in a lattice plane displayed the strongest biocidal action against *Escherichia coli* compared with spherical and rod-shaped nanoparticles and with AgNO3. (*Pal et al 2007*) Concluding that truncated triangular silver nanoparticles resulted in almost complete inhibition of bacterial growth at a total silver content of1μg. Therefore, speculating that the action of the silver nanoparticles is broadly similar to that of silver ions, where a bacterial cell in contact with silver nanoparticles takes in silver ions, which inhibit respiratory enzyme(s). This effect facilitating the generation of reactive oxygen species and consequently damage the cell. From this study, we can infer that different type of surface lattice of nanoparticles seems to influence the antibacterial properties of silver nanoparticles, although further studies are needed to confirm the lattice structure properties of nanoparticles to explain the process of dissolution to other forms, or whether other factors contributed the enhanced antimicrobial activity of the triangular particles.***Figure17*** summarises the mechanisms of antibacterial actions of silver.

Table 17: Examples of proposed mechanisms of (nano) silver toxicity

EFFECT OF TOXICITY	REFERENCE
DNA loses its ability to replicate. Brindha et al(cited by yang et al 2009) Implied that replication errors in general may play a part in inducing cancer.	Feng et al 2000, Yang et al 2009
Protein essential to ATP become deactivated	Tamanaka et al 2005
Membrane bound enzymes become deactivated, leading to structural change and cell death	Sondi and Salopek; sondi 2004
Inhibition of a respiratory enzymes, accelerate the generation of Oxygen species and hence damages or kill the cell	Pale t al 2007
Molecular mechanism: increate silver ions(even at very low concentration) can penetrate the cell membrane because they disturb the cell wall protein,once in the cell this leads to loss of energy and cell death.	Dibrov et al 2002

Source : Friend of the earth 2009

3.4FATE AND EFFECT OF SILVER IN THE ENVIRONMENT

Heavy metals such as Silver presence in the environment can be harmful to both human and environment. Silver metals are widely distributed all over the world through the increased production of nano silver materials and their eventual release into air, water and soil compartments. The molecular or ionic form of the nanoparticle are mostly released during the production, use and disposal in the life cycle of the material.

The environmental fate of nanomaterials is generally very complex, but this complexity cannot be sufficiently investigated with the currently available standard tests.

Silver biocide products may contain silver in ionic, colloidal or nanoparticle form, and to complicate things further, these may either be in free or bound form. Irrespective of the form of the silver used, a major characteristic that will affect the bactericidal effect of the silver is the concentration of silver ions released.

The fate of ENMs in water is determined by factors such as aqueous solubility, chemical reactivity and their interaction with certain biological processes (EPA 2009). Due to the characteristic low masses, ENMs generally settle more slowly to the bottom of water than larger particles of the same material. But their high surface-area-to-mass ratios provided an avenue to readily be absorbed to soil and sediment particles. Consequently, EMNs are more liable to removal from the water column compared with the bulk materials. (Oberdörster et al 2005). Certain ENMs might be subject to biotic and abiotic degradation, this process could lead to their removal from the water

column. For example, abiotic degradation processes of hydrolysis and photocatalysis (Dennekamp et al 2002). It is also recorded that ENMs exposure to sunlight allowed light-induced photoreactions which also could account for their removal and for changing their chemical properties.(Dennekamp et al 2002). In contrast to the removal processes mentioned above, some insoluble ENMs can be stabilized in aquatic environments.(Hristozov,D., Malsch.I., 2009)

The toxicity rate of silver in water is determined by the concentrations of silver ions. This is currently typically low in wastewater treatment systems and in the natural environment, partly due to silver's tendency to form strong bonds with various ligands such as chloride, sulfide, thiosulfate, and dissolved organic carbon. (Blaser et al. 2008).The fates of silver nanoparticles in wastewater may vary due to the tendency of being converted into ionic form, forming a complex with other ions, molecules, or molecular groups, agglomerating (Limbach et al. 2005; Zhang et al. 2008)

*"indicative evidence of the harm of silver nanoparticles at low concentrations on aquatic invertebrates, which suggest that the environmental release of silver nanoparticles will be detrimental for the environment and that any industry/ institute using silver nanoparticles should consider taking the necessary steps to reduce or eliminate the potential exposure of the environment to these nanoparticles. There is sufficient evidence to suggest that silver nanoparticles may be harmful to the environment and therefore the use of the precautionary principle should be considered in this case" (*Aitken et al. 2009).

Because of widely use of consumer products containing AgNPs (plastics, textile, paints etc). It is more likely that silver nanoparticles are released to the environment and consequently may damage ecologically important bacteria and other living organisms. Research on silver nanoparticles presence in sewage sludge conducted in Germany reveal that AgNP in sewage sludge can be toxic to soil microorganisms. It was calculated that at maximum of 30mg or more of silver nanoparticles per kilogram of sludge used in agricultural land as fertilisers could cause harm(Schlich et al 2013). As presented in *Table 18***,** silver contamination has been studied in several EU locations in different medium given different level of Predicted Environmental Concentration(PEC)

Table 18 : Selected EU studies of silver contamination in the environment.

Location	Anthropogenic source	Medium	Level (PEC) measured	Form of Silver measured
Germany Schlich et al 2013	Agricultural fertiliser	* Agricultural land *Effluent from municipal waste treatment plant	0.0015mg/kg dry soil 30 mg/kg dry sludge	Nanoform Bulk(Silver nitrate)
Switzerland(Rhine River) Sabina et al 2006	HeiQ Material Textile, Plastics	* Water * Sediment	0.8mg/kg (textile) 0.8mg/kg(plastics)	Silver ion (Ag+)Nanoform
Flander Belgium Ghent Belgium (River Leie)	Industrial waste Agricultural pesticides	*Soil *Water * Food	0.86mg/kg sludge	Nanoform Bulk (Silver nitrate)
Finland Mukherjee et al 2011	Sewage Treatment plant	• Soil • Water • Sediment	0.65mg/kg sludge	Nanoform

3.5REGISTRATION OF BIOCIDES (USE OF SODIUM SILVER THIOSULFATE) ACCORDING TO THE REACH REGULATION

The EU law on the production, placement in the market and the use of biocidal product such as disinfectants, household pesticides or wood preservatives has recently be revised. This change in regulation brings positive reforms in a well harmonised framework that provides a better protection of human and the environment. The new EU regulation on biocide prohibits the use of active biocidal substances that has extremely hazardous profiles in biocidal products. This approach agreed with the Pesticide Regulation revision (EC) 1170/2009 which strengthened the precautionary principle towards preventing adverse effects from hazardous substances on human

and environment. The regulation is an important improvement since new environmental properties are now taken into account in biocide registration : PBT, vPvB

The EU commission implementing regulation (EU) No 1195/2013 published on 22nd November 2013 amended the annex regulation (EU) 540/2011 in accordance with the regulation (EC) 1107/2009 on the placement of plant protection (Sodium Silver thiosulfate) on the market. Since the dossier for the inclusion of the active substance(sodium silver thiosulfate) was completed and satisfactory according to the EU directive 91/414/EEC, that the effect of the active substance on human, animal and the environment have been assessed for the proposed use by the applicant. This assessment concluded that plant protection product containing sodium silver thiosulfate is therefore approved by the commission.

The regulation stipulated that a reasonable period(Six months) should be allowed to elapse before approval in order to permit EU Member state (Annex I of article 29(1) regulation (EC) No 1007/2009) and all interested parties to prepare themselves to meet the new requirement resulting from the approval.

Re-evaluation of the plant protection product required that existing authorisations are to be amended or withdraw according to the regulation (EC) No 1107/2009. Verifying that the conditions in Annex I to the regulation are met.

Because of the unique behaviour, toxic properties, and possible risks of biocidal products that contain nanomaterials (e.g nanoscale silver compounds), the regulation specified that special risk assessment methods must be adopted for these active ingredients and products. Also, products treated with nanomaterials must also be clearly labelled for them to be qualified for simplified authorisation.

Specific provision for the approval of sodium silver thiosulfate is that member states shall pay serious attention to the following:

(a) the protection of operators and workers;

(b) limiting the possible release of silver ions through disposal of used solutions;

(c) the risk to terrestrial vertebrates and soil invertebrates from the use of sewage sludge in agriculture.

(d) Conditions of use shall include risk mitigation measures, where appropriate.

3.5.1 RE-REGULATION DECISION FOR SILVER IN THE EU REACH REGULATION

The EU REACH and CPL regulatory procedure emphasised a safe approach to the regulation of production and use of nanomaterials. This is directed towards achieving a high level of protection of human health and the

environment. It also promote further integrated and responsible development of competitiveness and safety aspects in nanotechnologies in a scientifically sound manner (REACH aims (Article 1.1.).

Even though there are no specific provisions for NMs, in the regulation, the EU Commission document CA/59/2008 reflects how REACH applies to ENMs. Regarding NMs as the "substance" covering substances at nano scale as well as bulk form and agglomerates and aggregates. However, it must be noted that substance definition in REACH does not provide clues for differentiating substance identities on the basis of physicochemical characteristics, since the shape, size and specific properties of nanomaterials may change over the life cycle of the substance.

The European chemical agency's sub group on NMs (CASG Nano) presented advisory activities concerning NM substance identification (materialised in RIPoN 1), physico-chemical properties, human health toxicity, ecotoxicity, fate, degradation and aquatic environmental testing. Studies have demonstrated that the mass concentration is not the most relevant parameter to correlate with measured effects, thus upsetting the traditional interpretation of the measures of (eco)toxicity. It is clearly shown that at equal mass, NMs are more toxic than products of the same chemical composition but of greater size. Correlation between the specific surface area of nanomaterials and toxicity has been identified by many studies, therefore, there seems to be a consensus in the scientific community to agree that several factors contribute to the toxicity of NMs. (ECHA 2012).

The suitability of the REACH regulation for safe use of nanomaterials is investigated. Since it appeared that no definition of a nanomaterial is present, and that a relevant measure for expressing harmfulness and exposure is as yet not known.(M.E.J. Pronk et al 2009).

In addition to insufficiency of the standard information requirements to assess hazard and exposure, proper characterisation of the nanomaterial becomes difficult. Also through the current available data, it cannot be determined to what extent the nanoform of a substance corresponds to the non-nanoform of the same substance. This made it unclear whether current risk reduction measures and extrapolation methods in risk assessment, as established for non-nanomaterials, are applicable to nanomaterials. Is the nanoscale and the bulk form of the same material's physiochemical characteristics? The 'sameness' analysis indicate whether the substance at nanoscale could be considered as a specific physical form of the bulk substance. Comparison between properties of substance nanoform and the bulk form and between one nanoform to another have been investigated. It was found that a nanoform of a substance exhibit a different properties compared to the bulk form while one nanoform of a substance exhibit the same properties with the same nanoform of the same substance.

3.5.2 CONCLUSION

It can be concluded that products covered by the EU pesticide and biocides directive (91/414, 79/117, 76/769/EEC) and regulations 396/2005, 98/8/EC need to be assessed, reformed and updated. Current legislation may have to be modified in the light of available new information (threshold use). Considering the dossier already registered for silver which does not contain information on nanosilver. This has caused a delay in the registration of nanosilver until 2018 despite its widespread use in consumer products. A case study on a hypothetical registration of nanosilver under REACH showed that REACH is not sufficiently implementable for this particular nanomaterial (Pronk, 2009, Wijnhoven, 2009a). Recent analysis (Christensen, 2010) concluded that no definite conclusions for regulatory decision making could be taken due to lack of data on exposure and hazard. Based on existing data, concerns were raised that repeated inhalation in the workplace, possible consumer inhalation and (uncontrolled) nano-silver drug may cause risk. (Malkiewicz et al 2011)

The main challenge is in form of implementation of legislation via use of regulatory instruments among the EU member states. Supporting implementation documents, related to risk assessment adopted within the context of current legislation, will have to be reviewed in order to ensure that they effectively address risks associated with nanomaterials, and to make the best use of the current available information. However, national, regional and local authorities and agencies will have to pay special attention to risks in relation to nanomaterials where production and marketing are subject to pre-market control. In order to properly develop, modify or in particular to implement legislation, the scientific knowledge base needs to be improved. This Communication therefore pays attention both to legislation, implementation and bridging the knowledge gap.

3.6 REGISTRATION OF HEIQ AGS-20 (A TEXTILE NANOSILVER MATERIAL) IN US (EPA REGULATION)

In a decision document released by the Environmental Protection Agencies On 1 December 2011, EPA proposed a procedure in granting a conditional registration for the (HeiQ material: AGS-20). The new product which contains an active ingredient made of nanosilver will be used as a textile preservative mainly for coating and control of odour of frequently used clothing, removing stains and reducing the textile material degradation.

According to a preliminary report by EPA, It was stated that Human and environmental exposure to nanosilver from AGS-20 products could occur in three forms,
(i) composite particles of nanosilver-silicate as an active ingredient in AGS-20, (ii) Silver nanoparticles that break away from the composite particle and (iii) Silver ions released from the treated textiles" (EPA 2010a). However, there remains a significant data gap in both the release of AGS-20 particles from treated fabric and the release of silver nanoparticles from the AGS-20 particles with few data been submitted on the safety of the Silver nanoparticles utilized in the AGS-20 substance.
The EPA acknowledged that the agency lacks information to conduct a complete assessment of the potential risks to human health and the environmental associated with the use of AGS-20, therefore, existence of considerable uncertainty about the risk assessment. Therefore, need for more extensive product chemistry, toxicology, exposure, and environmental data are necessary to provide an accurate assessment of the risks.

Beginning with the identification of the core elements at risk (Workers, Consumers and the Environment) as a result of exposure to the active ingredient (nanosilver) used in production of the product, a screening level regulations of the risk assessment from exposure to the nanosilver ions is needed. Using the available re-regulations for bulk silver materials, EPA concluded that the human health or ecological risk from the exposure to Silver ions from AGS-20 treated textile products is not of concern. But more data are still needed since the assessment is based on assumptions and need for a case-by case approach to hazard and exposure assessment.
In its risk assessment of the use of AGS-20 on the elements at risk, EPA adopted the use of conservative assumption by overestimating the dose of nano-Silver, and maximising the values of risk uncertainty factors.
Because of unavailability of long- term human or environmental toxicity studies for AGS-20 or for the nano – Silver that might break away from it, there is requirement for a new data development in areas such as (i) route-specific toxicity for occupational exposure, (ii) product characterisation and (iii) Stability tests for nano- Silver breakaway from the AGS-20 product.

Using the intermediate term toxicity data available for analogous form of nanosilver in the scientific literature, evaluation of the risk from occupational and consumer exposure could be carried out.

For purposes of risk exposure to HeiQ AGS-20, short term acute animal toxicity test conducted using high level dose confirmed that there were no mortalities or abnormalities in test animals after oral, dermal, and inhalation routes. But more qualitative and quantitative understanding of different procedure to convert from Animal to Human in the toxicity test is required. From the tested result, it can be concluded that regulatory procedure should be based over the life stages of the product rather than the immediate use.

Bearing in mind that the evaluation and assessment provided from the available scientific literature on the analogous form of nano-silver did not evaluate toxicity over all life stages for potential effect, therefore, there is a need for size property based criteria of different size properties of the nanosilver in different environments and transformation of nano-silver in the life stages. These obviously should be part of risk assessment. Therefore, it is necessary to evaluate the stages in the life cycle of nanomaterials needed for risk assessment.

The EPA adopted the use of maximum 10-fold database uncertainty factor to evaluate the risk from exposure to the nanosilver that might break away from AGS-20). Need for more analysis of different strategies (MOE, DNEL) for correction from animal testing to human effect process in extrapolation from a short-term study to a chronic scenario is required.

Due to limitations of the USA regulatory risk assessment by treating nanomaterials like their bulk versions when they have the same molecular identity, Major data gaps in the limited currently available information preclude any reliable risk characterization. (David Q. A; Olga V.N, Houlihan, J. September 2010; Comments to EPA request premarket safety testing for nanomaterials)

“Although the test results provided useful information, the guidelines on which they are based have not been adapted generally for use with nanoscale particles. EPA anticipates that these guidelines will require revision going forward in terms of their application to nanoscale materials” (EPA 2011)

CHAPTER 4.0A FRAMEWORK FOR REGULATION OF NANO-SILVER

Nanosilver products are licensed for use by regulatory authorities if they comply with scientific criteria on quality, efficacy and safety. The concern of the regulatory authorities (e.g USA- OSHA, EU-ECHA) is the safety of the consumer, individuals handling the material and the environment to which the material is released.

This chapter presents a framework that is based on current regulations and knowledge in nanomaterial ecotoxicity, human health toxicity, and ecological toxicity, and aims to guide risk informed sustainable nanomanufacturing for nanosilver. Definitive data based on standardized nanomaterials and testing methods are required to deal with various uncertainties and perform comprehensive risk assessment. While waiting for such studies, frameworks are required to rank the potential environmental or human exposure and toxicity of emerging nanomaterials from environmentally relevant products (Cummins et al 2010).Data gaps related to potential exposures and hazard of nanosilver are broad and there is little information about nanosilver in the environment related to fate, transport and transformation (including what may be released from product producing nanosilver- enable products). (FIFRA 2009).Therefore, a need for a framework that could integrate available fragmented data to assess product risk.

This chapter describes the limitations of existing risk assessment frameworks and provides recommendations on research directions to assess the release of nanoAg to the environment(air, water, soil) using available data. Proposed adjustment needed for updated regulation of nanomaterials (release quantities of Silver nano material from different products) are compared to current regulatory law by REACH. Considering nano silver release and exposure; the product's life cycle is used to determine which stage in the product life cycle present opportunities for release of silver ions to the environment. Through identification and prioritization of data gaps, this study provides means for reassessment of exposure and potential hazard effect of nanoAg. This framework is based on selective data from exposure studies from various literature sources and current knowledge on nanomaterial ecotoxicity, to estimate qualitative risk assessment of ENMs tailored to the current regulations.

4.1 RISK ASSESSMENT OF ENGINEERED NANOMATERIALS: LIMITATIONS AND UNCERTAINTIES

"Although the conventional RA framework is a valuable approach, it may fail to adequately estimate the health and environmental risks from ENMs in the near term due to overwhelming methodological limitations and epistemic uncertainties" (Hristozov et al 2012).

Review of available data from RA of ENMs by the Scientific Committee on Emerging and Newly Identified Health risks (SCENIHR 2008) and European Food Safety Authority (EFSA),2009, identified different limitations and uncertainties in the stages/phase involved in the process of risk assessment. Limitations and uncertainties related to methodologies and strategies in their applications identified in the various stages of the risk assessment process present a draw back in their applications. Table 19 presents identified limitations and uncertainties at different stages or risk assessment as reported in the literature.

Important challenges include establishment of validated methods and instrumentation for detection, characterization and analysis of hazards of nanomaterials. (EC 2012). Comprehensive scientific interrelationship among limitations and uncertainties in risk assessment process is mostly derived from incomplete knowledge or data or system. (Scholz et al 2010)

While a set of physicochemical properties (surface area, shape, solubility etc) that influence the uptake and effects of ENMs in human and environment have been adopted, the literature reports on the failure to properly characterize the investigated ENMs and difficulty in identifying a suitable dose metrics. (Hansen et al 2009; Hristozov and Malsch 2009). "Risk assessment methodologies for the evaluation of potential risks of substances and conventional materials to human and the environment are widely used and are generally applicable to nano-materials, however, specific aspects related to nano-materials still require further development. This will remain so until there is sufficient scientific information available to characterise the harmful effects of nano-materials on humans and the environment." (SCENIHR 2009)

Table 19: Identified Limitations uncertainties and possible solutions

Phase	Limitations	Uncertainties	Suggestions
Hazard Identification	Non- standard testing methods(different species)	Physicochemical properties influencing the uptake (size, shape, surface area)	Nano (eco) toxicity results should be reported with comprehensive physicochemical profiles of the material.(OECD,ISO, Hanser 2009; Hristozov and Malsh, 2009)
Dose Response	Dose response testing	Dose expressed as "mass". *ADME are size and shape dependent. Influencing their toxic effect.	Use of multiple metric to avoid irregularities(surface area or particle number concentration for better correlation). ISO/TC/229;OECD,2011 (Oberdoster,Stoeger,;Wittimaack,2007)
Exposure Assessment	Datagaps in identification relevant exposure scenario(consumer exposure, occupational exposure No selective detection and monitoring of ENMs concentration(distinction between engineered and natural nanomaterials)	Lack of statistical data about the number of workers exposed to ENMs, the duration and the frequency of the specific tasks performed. Sampling techniques that allow distinction engineered and natural nonomaterials.	Aitken, 2011 ; Savolaine,2010 Development of portable and cheap instrument able to identify ENMs and perform real time measurements of workers exposure. Brouwer, Clark ,2010
Risk Characterization	Irregularities in characterization process	Sum or over extimation in the other phase of assessment(Identification, dose-response,exposure assessment)	Consideration of more than only one exposure metric to perform an appropriate characterization and quantification(Hansen, 2009).

*ADME(Absorption, Distribution, Metabolism and Excretion)

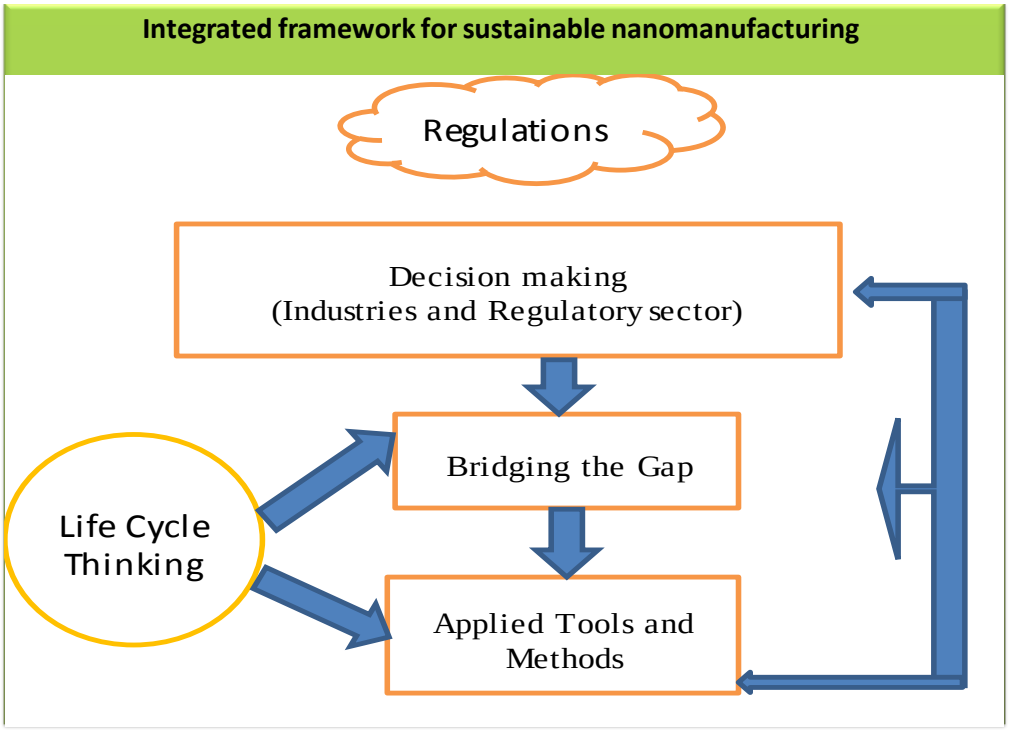

Figure 13: Integrated framework for **sustainable nanomanufacturing**

Bridging data gap: The process enhances identification of possible limitations and uncertainties in the risk assessment procedures. All risk assessment procedures are subject to limitations and uncertainties.While the level of uncertainty depends on many factors such as (quality and quantityof evidence, data variability, Lack of statistical data, hypotheses used and conclusions made at each stage of the process, determinable uncertainty may be reduced by the acquisition of better evidence. (HSAC 2013). Suggested possible solutions are recommended to fill the existing datagap. Bridging datagap help to reduce uncertainty due to lack of knowledge by adequate scoping of the problem. Application of conceptual modelto ascertains the source, pathway or route of exposure, the target organism under consideration, andthe possibility of any particular vulnerability or sensitive target organism that need to be considered; thereby providing concise and corrective documentation.

Applied method and tools:

Applied method in the risk assessment of nanomaterials is an iterative set of practices, structured with detail scope and coverage to estimate relative hazard and risk associated with the production of ENMs using available data. Applications of different methodologies could facilitate regulatory decision making. This serves as a preliminary hazard/risk screening, aimed to help industry in identifying relevant sources of risk in the lifecycle of synthetic nanomaterials and pinpoint areas of knowledge deficits.(Hristozov et al 2012) Tools as applicable in risk assessment represent a procedural process aimed at generating specific type of output for a robust and reliable estimation of effect of release of nanomaterials on human and environment. (e.g WOE, MCDA). Such approaches have the capacity to recognise uncertainties in the risk assessment of ENMs. Using existing data and information, their applications in a methodological framework could, facilitate the process of risk assessment, and delivery of reliable future risk estimations.

Life cycle thinking: Life cycle thinking represent a comprehensive quantification of ecological and human health impacts of a product or system over its complete life cycle. The cradle-to-grave assessment of the health & environmental impacts is vital for successful & safe commercialisation of nano-technologies while current existing standards for LCA are fully suitable for use on nanomaterials & -products.(Hischier et al 2007)

Decision making process: A starting point to conceptualize sustainable nanomanufacturing is by utilizing criteria rankings for a typical ENM manufactures', consumer's and regulators preference profiles among three nanomanufacturing alternatives (Baseline, low-end and high-end technologies). (Subramanian et al 2014) Therefore, meeting the mandate of both regulators and manufactures, towards a sustainable nanomanufacturing management for the desired benefit to human and environment. Manufacturers are to duly provide registration dossier (detail information on compounds that are manufactured or imported in quantities of one tonne or greater per year) This detail information are used to assess the risk that may arise from the use of the substance, thus, ensuringtimely and appropriate risk management.(European Commission(Environment Directorate-General) (2007). Regulators should work towards a broader regulatory management aimed at reducing risk providing a more safely use of chemicals. It is also required that different regulatory bodies should engage in harmonization of chemical management strategies. (e.g ECHA collaboration with pear agencies in EU and other countries for multilateral work on chemical safety) (ECHA 2013). This framework can support this requirement from the manufacturer and regulators by providing a platform where data from both organisations (manufacturers and regulators) can be integrated for an active decision making.

4.2 BRIDGING DATAGAP: CASE- BY CASE SAFETY APPROACH

This step aims to do a thorough assessment of state of the art knowledge on environment and human health risk to develop strategy toward future data collection and risk management. Nanomaterial properties, their intended use with the focus on material base properties, available methods, and tools to assess the intrinsic properties of the nanomaterial. This section is aimed at updating available dataset, where **assumptions** and **uncertainty factors** are compared.

The second regulatory review of nanomaterials by the EU regulatory body towards ensuring the use and safety of nanomaterials adopted the case-by- case approach to risk assessment.(EU October 2012). Strategies that are based on indications of potential risks(exposure, hazard)were used.. Distinction is made between toxic and non toxic materials with possible risk associated with different specific uses of nanomaterials. It was suggested that threshold response should be considered in both hazard identification and risk characterization. It is assumed that response which are threshold dependent and non- threshold dependent required different methodologies. (PMRA, Canada 2007, 2008). While threshold response dependent present a dose level that substances will elicit a response and a non threshold response dependent present an increased risk at any level of exposure, therefore required different risk assessment methodologies that do not use uncertainty or safety factors. Figure 14

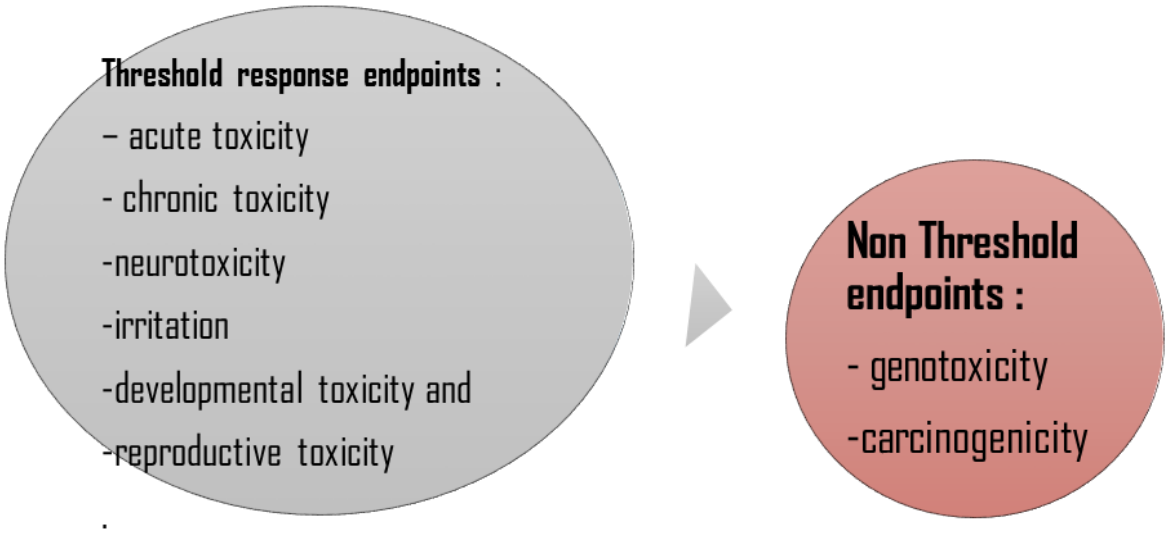

Figure 14 Threshold and non- threshold response endpoints.

Due to non-availability of data on the exposure effect of new compounds, many research proposed the use of assumptions, extrapolations or safety factors to fill such datagaps. (Kandlikar et al 2006). This procedure help to stimulate the compounds' environmental fate under various possible conditions. (Gottaschalk et al 2010).

E.g to calculate DNEL, the NOAEL is divided by an assessment factor(caused by extrapolation between species and humans).

DNEL=NOAEL/ Assessment factor

An investigation of the exposure of nano-TiO_2 in Switzerland using probabilistic material flow model. (Gottaschalk et al 2010).and a report of the UK department of Environmental Food and Rural Affairs(DEFRA) on the current exposure of engineered nanoparticles using emission factors to predict the exposure concentration in Air, Water and Soil are examples of predictive exposure studies. Since limited data were available on the fraction of each product that comprised ENPs, the assumption was made that there are no other sources of the engineered nanoparticles of concern other than cosmetics, personal care products and paints. Therefore, need to update the data as soon as new information becomes available.(Boxall A.B.A,; Chaudhry, Q,;Sinclair, C,; Jones,A; Aitken, R,; Jefferson,B,; Watts,C, 2007)

4.3 LIFE CYCLE THINKING: LIFE CYCLE ASSESSMENT OF NANO-SILVER

The second step defines three criteria to assess which stage in the product life cycle present opportunity for release of ionic form of the material to the environment. Possibility of any risk is identified for effective regulations. Life Cycle assessment is important for effective analysis of the effects of nanomaterials in different stages of its life cycle to determine release and exposure characterization.

- Type of properties (nanoform, bulk or ionic)
- Nature and behaviour in different medium(air, water, soil)
- Release and exposure

This is important for effective analysis of the effects of nanomaterials in different stages of its life cycle to determine release and exposure characterization.

The type phase identifies the characteristics of nanomaterial in different forms. The nature phase identifies their physical and chemical properties in different medium (air, water and soil) to describe the natural and artificial altering of their physiochemical properties during synthesis, use and disposal. The system phase describes the release and exposure of nanomaterials over the whole life cycle.

Life cycle assessment (LCA) is a methodology used to assess the potential environmental impacts of a product/service system over its life cycle. The term 'life cycle' includes the extraction and processing of raw materials, production, transportation and distribution, use, and end-of-life (re-use, recycling, recovery and final disposal) (European Environmental Agency 1997)

The description of nanoscale, materials include the external dimension and internal structure. LCA comprehensively quantifies both ecology and human health impacts of a product or services over its complete life cycle. (Walser et al 2012). LCA is modelled by implementing various input and output flows over the production, use and end- of –life. The ISO standards14040 -14044 define the goal and scope of LCA and Life cycle inventory(LCI) respectively. At present, few published data on nanomaterial and nano-enabled product exist. Reviewing the current state of LCA application in ENMs, It is possible to conclude that certain challenges occur across different studies. There are high datagaps with less robust and holistic procedures, that evaluate the nano-specific fate, transport and toxicity impact of ENMs on human health and environment through the stages in life cycle. Also, there is scarce availability of inventory data with no characterization of factors for release (input&output)of the system assessed.(Dong. Y., Laurent.A.,Hauschild. M.Z (PROSUIT, 2013)

Comprehensive analysis of ENMs over its life cycle serves as a technical and scientific support for the risk assessment process aimed at evaluating the ecology and human health effect, when released into environmental. The LCA of nanomaterial will complement the risk assessment process, where functional units of LCA act as supportive feature of a RA. Different stages of nanosilver products life cycle have been studied to investigate:

- Which stage of the product life cycle present opportunities for release of silver ions to the environment,
- Which stage is needed for the evaluation of risk assessment.

The analysis of the life cycle of a product determines stage (production, transport/storage, use, and disposal/recycling) or environmental compartments (Water, Air, Soil) or type of nanomaterial production technology that allow the release of silver ions. Depending on the product life cycle, humans and ecology may be directly exposed to nanomaterial. For LCA on nanosilver, several challenges had to be dealt with, including

- How to adapt conventional regulatory- oriented risk assessment methods, often based on conservative assumptions, in order to estimate cumulative chronic toxicological risks and potential impacts in comparative applications such as LCA
- How to account in a generic but accurate way for complex functions, such as intermittent dissolution character of nanosilver, ionic silver and bulk silver.
- How to structure fate(transport in the environment, exposure, and resulting intake) in a consistent way following impact pathways from production- based rather than subsistence based exposure.

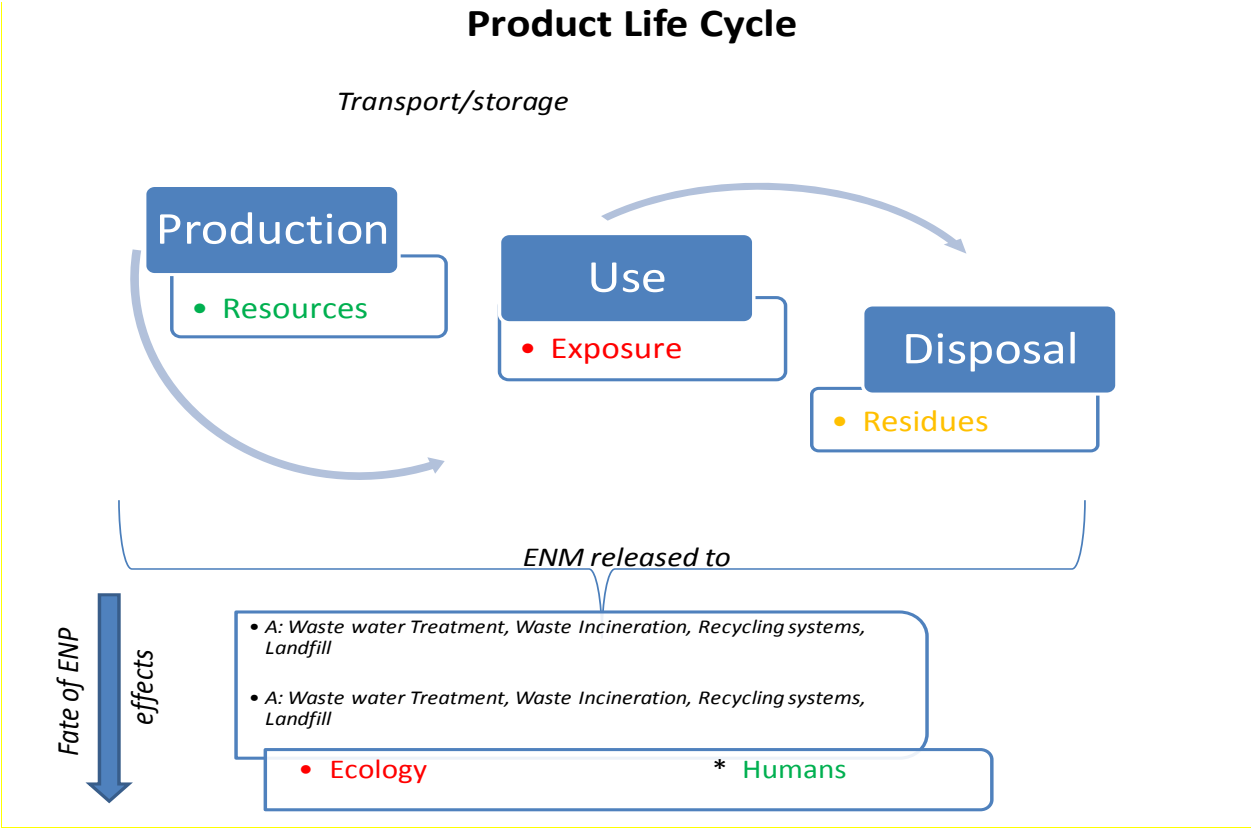

Figure 15: The life cycle of a product defines the ***fate****(emission, fraction transferred, concentration), exposure(dose response severity) and hazard effect. Adapted from* (Som et al 2011)

The assessment of toxic impacts of nanosilver particles in the environment is still in the early research stage. Therefore, there is need for further research on issues that have not been considered in the current LCA studies. This includes:

- Classification and characterization of nano-specific effects of nanosilver
- Reduction of data gaps that describe the distinction of silver particle release rates between different nanosilver coatings and
- Reducing uncertainties in the derivation of bioavailability of dissolved and colloidal (bulk) bound phases

Another factor considered is the dissolution factor of the silver particles. Research has shown that during the washing of nanotextiles both dissolved and particulate silver are released. (Som et al 2011*)* . Further, the comparison of the environmental impacts between a nano Silver T-Shirts with conventional T- Shirts treated with triclosan was investigated by Walser et al. Using two different environmental performance technologies(FSP and

PlaSpu) with respect to the production, use and disposal phase. Result shows a significant differences in environmental burdens between the two nanoparticle production technologies. Comparing to the "cradle-to-grave" climate foot print (2.55kg of CO2- equivalent for a conventional T- shirt) the flame spray pyrolysis (FSP) technology result in release of 2.70kg of CO2 equivalent while the plasma polymerization with silver co-sputtering (PlaSpu) result in release of 7.67-166kg of CO2. Conclusion was made that the use phase pollutes the environment most if commercial technologies are used, while the production phase shows high sensitivities to the applied silver mass (long term toxic emission to the aquatic compartment through silver mining). But nanosilver T – shirt produced with the non commercialised PlaSpu technology have higher climate change impacts during production than in use.

Using the benchmark of 95% w/w, Walser et al reported that silver in the effluent is calculated to be 29% w/w (dissolved) and 71% w/w (associated). This allows the silver to form strong complexes with water ligands, consequently minimizing its aquatic toxicity. Considering the characteristics of nanomaterials relevant for their environmental behaviour (fate, ecotoxicology and toxicology, agglomeration/deagglomeration and solubility)some researchers argue that nanosilver have the tendency to agglomerate to microscale particles and thus present no specific nanorisks for the environment and human health. (Som et al 2011). Meyer et al 2011;in relating the impact during manufacturing and release of silver nanoparticles(antimicrobial agent)used in the production of socks, conclude that more environmental and human health impacts are more in the use phase(washing of socks) because of higher release of ionic form of nanosilverthan in the manufacturing phase.

4.3.1 Type of properties

Because of huge variety of existing nanomaterials and possible increase in application for registration of more in the future, there is need for update in the present REACH regulations. Due to the difference in chemical composition, size, shape, crystallinity and surface modification of nanomaterial, taking into account this plurality of physico-chemical characteristics and resulting changes in the hazard profile, an approach must be found to adequately cover nanomaterials under REACH.(Schwirn et al. 2014).For example, Derivation of occupational exposure limits OELs in the US for CNTs with graphite, reported a great divergence in the derivation of OELs for both nanomaterials and bulk materials. It was reported that OELs for bulk form are hundred times higher than their nano-form. (Schulte et al 2013). Another property that differentiate the nanoform and bulk form of nanosilver is the oxidative potential. While the nanoform has a higher oxidative potential, the bulk form possesses a limited oxidative potential. "Oxidative stress constitutes one of the principal injury mechanisms through which ENMs can induce adverse effects".(Fenoglio 2013)

Scientific literatures proposes two approaches which could be conceivable used to cover nanomaterials under REACH. (1) treating nanoparticles as substances on its own, (2) treating nanoparticles as specific forms of a substance. Figure 16a & b.

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4.3.2Nature and behaviour in different medium(air, water, soil)

Distinction is made between the toxicity vs physical properties relationship and toxicity vs chemical properties relationship. This procedure is aimed at providing a specific data towards the characterization of different nanomaterial in different medium. It should be noted that nanomaterials functionality at the different forms present different characteristics, are likely to have new biological reactions associated with their new forms. Table 20

Table 20:Physical and chemical properties of nanomaterials

Physical properties	Chemical properties
• Size • Shape • Surface area • Surface change • Dispensability • Density	• Surface reactivity • Solubility • Molecular structure • Chemical composition • Porosity

Source: (Buzea et al 2007)

4.3.3 Size specificity: The translocation properties of nanomaterials, where size characteristics allow them to transport to different compartment of the system and causing different reactions as they move from different organs or environmental boundaries. Small size provides a larger surface area leading to a large surface activity in different medium. Also, the number of particles(weight number) of small size particles provides a higher reactivity.

4.4 REGULATION POLICY: HARMONIZATION OF DIFFERENT OELs

This step describes the need for new or updated regulation. (e.g Occupational Exposure Limits). Based on the second step, evaluation of existing regulations to check and balance any incomplete information is performed. Consideration of specific requirement for regulation of nanomaterials (nano Ag) to account for nanoscale version of already listed chemicals under different regulations.

Occupational exposure limits have been developed by various governments, organizations and even manufacturers of chemicals(including different forms of products). This is useful to quantitatively measure the risks related to exposure of workers to different chemicals. (NIOSH 2013),thereby reducing work related health risk. Examples of different OELs includes the National Institute of Occupational Safety and Health(NIOSH) which recommends 2.4mg/m^3 for fine Titanium oxide(TiO_2) and 0.3mg/m^3 for ultrafine(including engineered nanoscale) TiO_2 and 1µg/m^3 for Carbon Nanotubes (CNTs) with standard time weighted average(TWA) of 10hours/day during a 40 hour week.

However, in the United State, most of the OELs developed have been essentially voluntary guidelines or recommendations which include the following exposure limits: (1) OSHA's permissible exposure limits (PELs), (2)The ACGIH's TLVs, (3) The AIHA's Workplace Environmental Exposure Level (WEELs), (4) NIOSH's Recommended Exposure Limits (RELs), (5)The EPA's New Chemical Exposure Limits (NCELs)and (6) Internal company standards developed by chemical manufacturers and users. (Mmccluskey et al 2010)

Other examples include the OELs guidelines by the UK Control of Substances Hazardous to Health (COSHH) regulations which adopted two main occupational exposure limits (1) Occupational Exposure Standard (OES); set for substances of known concentration at which there is no significant risk of health and (2) Maximum Exposure Limit (MEL); set for substances with serious health effects of which OES could not be set. (e.g Carcinogens). Germany's Commission for the Investigation of Health Hazards of Chemical Compounds(The MAK collection).

In the absence of harmonized government or consensus on the standardization of OELs, Bayer MaterialScience (BMS) a subsidiary of Bayer AG established an exposure limits of 0.05mg/m^3 for its product

Baytubes(a multiwalled CNTs) and 2.5µg/m^3 for Nanocyl CNTs on a mark of 8 hour/day exposure. (NIOSH 2013)

For more than five decades since OELs have been developed, main issue has been the possibility of harmonizing various OELs into a standardized form that can measure any occupational health risk. Such initiative is the agreed European Union chemical hazard classification system.(Topping et al 2001) that can lead to a possibility of globally harmonized system. This framework supports the harmonization process through bridging the datagap to inform manufacturers and regulators of the need for an integrated sustainable nanomanufacturing. This is more elaborated in the next chapter.

CHAPTER 5.0 SUSTAINABLE NANOMANUFACTURING

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Nanomanufacturing covers a wide range of materials, products and processes at different levels of technology and scale. (Liddle et al 2014). Assessing products in the intended area of applications and associated driven factors of environmental, social and economic dimensions is the main purpose of the development of sustainable nanomanufacturing.

Common classification of emerging technology's sustainability measure, according to the environmental, social and economic domain is not as clear cut in the literatures. Life cycle impacts of nanomaterials are Integrated into the sustainability dimensions in the development of nanotechnology. This is aimed to address the complexities in the dynamic nature of the **D**emand, **N**eed and **U**se of nano- materials to meet the sustainability responsibility. Figure 17.

This chapter present sthe process that promotes sustainable nano-manufacturing, a point of reference to which the risk assessment of nano material products are examined to allow quantification of the extent, duration and severity of the effect.(e.g workplace)

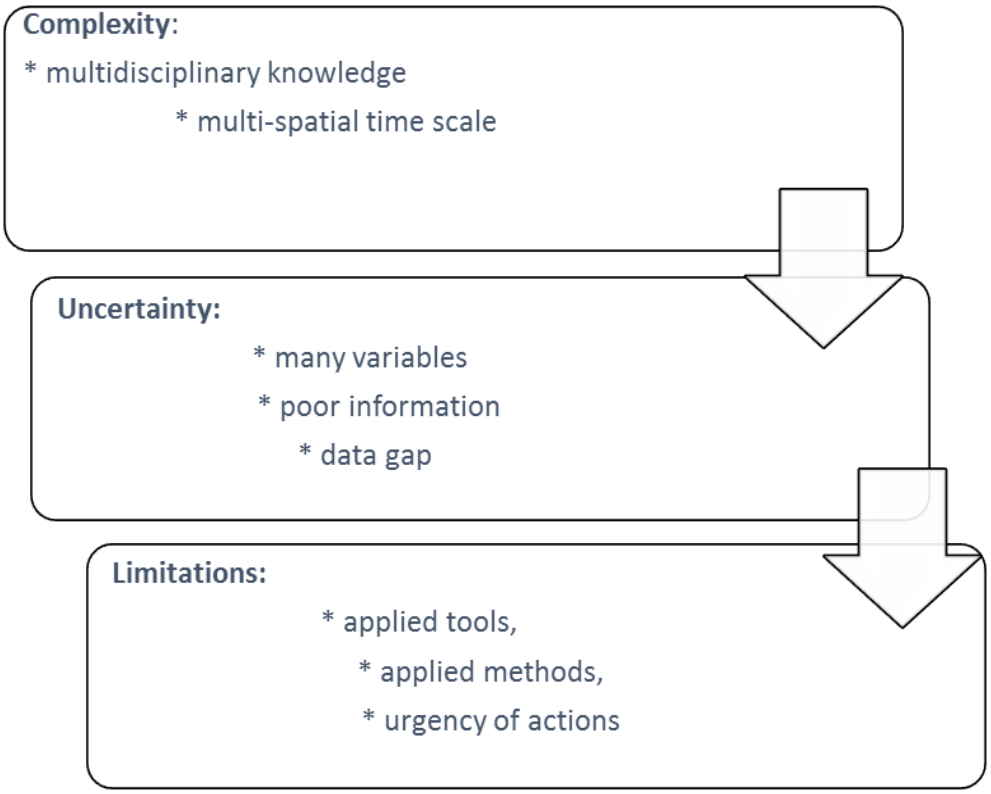

Figure 17: Analysis of possible drawback to evaluation, definition and measurement of sustainable nanomanufacturing

5.1 DEFINITION, MEASUREMENT AND EVALUATION

To define sustainable nanomanufacturing, the impact on the three dimension of economic, social and environmental using green chemistry and green engineering metrics is presented. Table 21. Definition and measurement of sustainability of emerging technology such as nanomaterial products is a complex task. (Subramahian et al 2014).Sustainability definition and measurement of"greenness" of nanotechnology are useful in bridging the knowledge gaps at both temporal and spatial scale in the manufacturing process that meet the three dimension of economic, social and environment. The outcome (data information) are used by organizations, government, and producers in the decision making process towards an effective sustainability practice .

Table 21: Suggested Sustainability metrics for nanomaterials.

Category	Green Chemistry& Green Engineering
Economy	*Renewable feed stocks, * Particle size *Energy efficiency, *Material yield
Environment	*Less hazardous chemical synthesis *Design for degradation
Social	*Real time analysis of pollution prevention, *Work environment index

Source: Mulvihill et al 2012

Some definition of sustainable nanotechnology from the literature include:

(1) Policies and strategy that meet the need of the present society without compromising the ability of future generation to meet their own needs.(Bergeson et al 2013)

(2) Principles that lead to efficient manufacturing and production process, having in mind the safety of human and environment with less energy consumption, less toxic materials over the whole life cycle of the product.(Mulvihill et al 2012)

(3) Management practices that integrate occupational and health of workers and intending users of nano enable products. (Schulte et al.2013)

5.2 EMERGING METHODS AND TOOLS FOR SUSTAINABILITY ASSESSMENT OF PRODUCTS

Various assessment tools tailored at evaluating and measuring nanomanufacturing sustainability have been developed. These tools are classified based on (1) indicators that describe the process of manufacturing such as life cycle assessment, product material flow analysis and product energy analysis. (2) Indicators that describe the measurement of manufacturing effects such as ecological footprint, environmental sustainability index and sustainable national income. (3) indicators that assess the interaction and risk of the manufacturing such as environmental impact assessment, risk assessment and risk analysis. Though various sustainable manufacturing practices, such as Life Cycle Assessment , Risk Analysis and Assessment are currently applied in industries to enhance the green chemistry alternatives(Dhingra et al 2010).The applicable tools required for quick and accurately characterization of products at the relevant scales of one to hundreds of nanometers have yet to be developed. (NNI SI 2010).

The risk assessment research group of inter-university consortium on environmental chemistry at Ca' Foscari University Venice demonstrated the development of a Triple Bottom Line (TBL) approach to define sustainable nanomanufaturing, using a Multi Criteria Decision Analysis(MDCA) in a pedagogical context (Subramanian et al 2014) figure 18. TBL is used to derive different baselines(High-end and Low-end) manufacturing techniques. Using different preference weighting schemes for manufacturers and regulators. Result of the model states that manufacturers tend to choose the High-end technology alternatives(associated with higher risk) while regulators opt for Low-end technology alternatives (associated with low risk). However, It was noted that regulators tend to change from Low-end to High-end alternatives due to small increase in societal values (safety of workers and users of products), since their main goal is to reduce human and environmental risk. Therefore, " sustainability should be linked to technology management processes to capture evolving technology and understand of its benefits and risks" (Subramanian et al 2014)

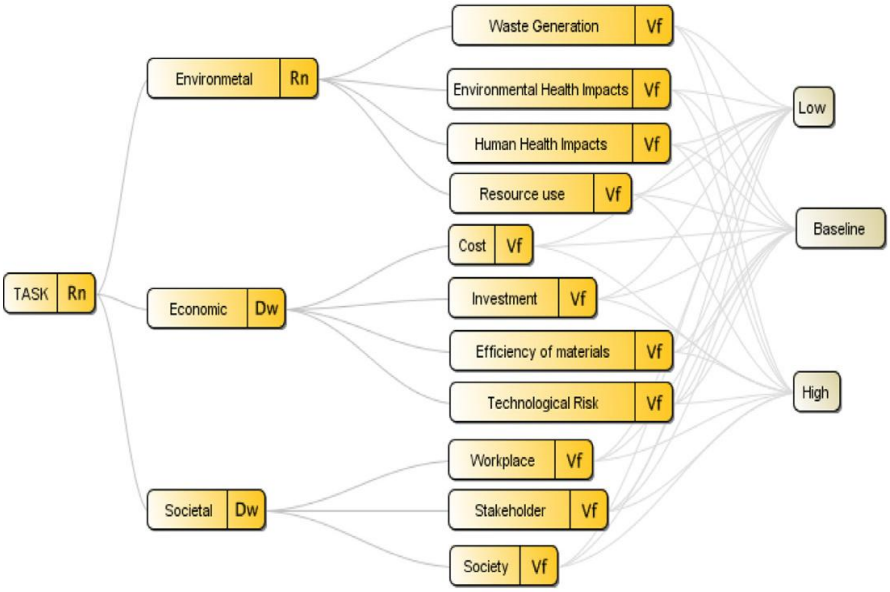

Figure 18: Multi Criteria Decision Analysis using TBL approach for measuring and monitoring sustainable nanotechnology. Adapted from (Subramanian et al 2014)

5.3 GREEN CHEMISTRY AND GREEN ENGINEERING

Different ranking methodologies have been employed in the classification, standardization and certification of products and services based on their intended effect on the three sustainability (Environment, Social and Economic) dimensions. But there exist a significant differences in the methods applied in the literature. This arises in the dimensions in which the intended impacts are taken into consideration and issues regarding the definition of truly comparable products. ***Bachmann et al 2012.***

"Green chemistry and engineering seek to maximise the efficiency and minimize health and environmental hazards throughout the chemical production process" (Mulvihill et al 2012). Achieving "greenness" in nanotechnology development, process and manufacturing require a drastic changes in resource use and energy consumption. This can be achieved through the incorporation of life cycle inventory analysis of LCA.

Product **m**aterial flow **that is b**ased on process requirement and emission measurements are used to check mass flows. However, **v**ariation in the difference steps over the whole process of manufacturing can influence the material flow inventory. Manipulation of materials at the nano-scale may therefore demand a high purity in the starting material. (Krishnan et al 2008).

Calculation of the energy requirement for the production of materials is part of the main output through the various stages of the life cycle of a product for a sustainable manufacturing process. Many research has identified that different materials required different energy for their production.(Krishnan et al 2008). Figure 19.

For nanomaterials, data on the material processing of different nanomaterials observed distinctive difference in the energy requirement for the production of different materials e.g 0.1-1.0 tetrajoules/kg for Carbon nanotubes and 9.7 megajoules/kg for Silicon wafer nanoscale semiconductors, depending of the size. (Gutowski et al 2010). The Economic Input-Output life Cycle assessment (EIO –LCA) reported a hybrid approach to evaluate upsteam energy requirement of different forms of particulate nature observed an exponential increase of a specific manufacturing energy with increasing precision. But an inverse trend when comparing specific manufacturing energy and rate of material removal. (Krishnan et al 2008)

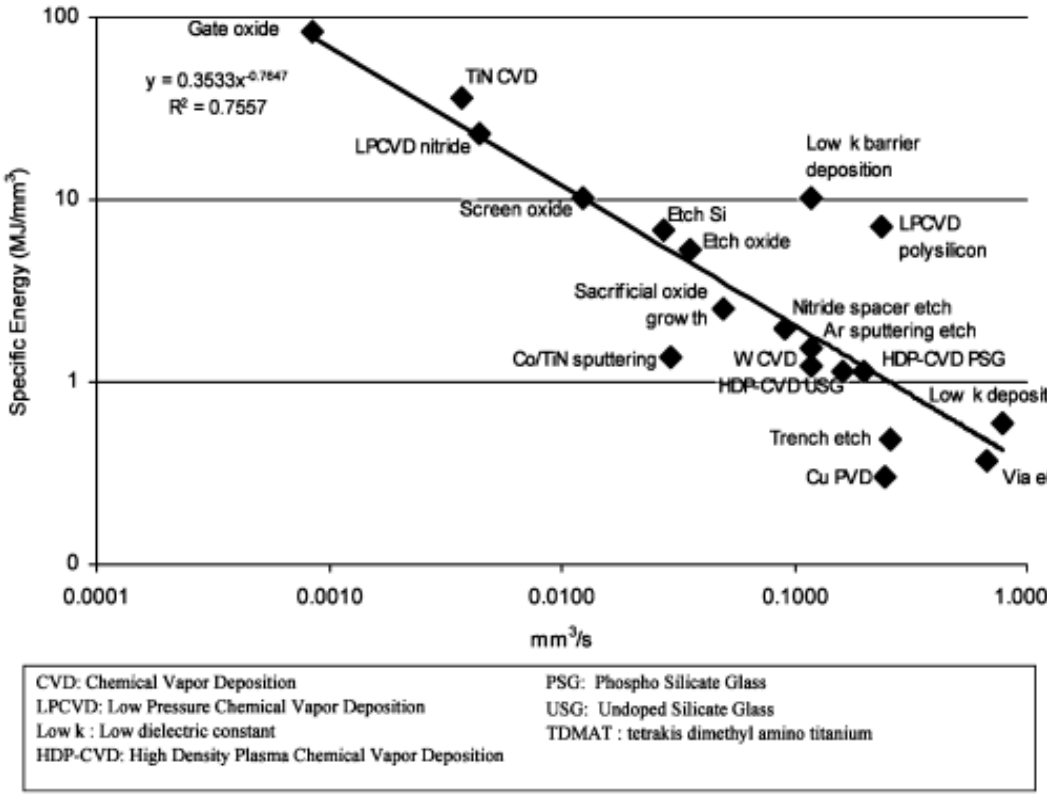

Figure 18: Specific processing energy for different products measure in MJ/mm^3 vs process rate mm^3/s.Source :(Krishnan et al 2008)

CONCLUSION

It had been recognised that many methodologies used in the production of nanomaterials are characterized by low process yield, low purity, use of toxic, acidic or basic chemicals and generation of Green House Gases(GHG). (Sangul et al 2008).

The framework for risk assessment of nano-Ag is tailored to practically guide a risk informed sustainable manufacturing with integration of available data from different sources. Thereby can be used in re-assessment and ranking of potential environmental and human exposure and risk.

Therefore, for effective sustainable nanomanufacturing, sustainability assessment tools require incorporation of green chemistry and engineering to life cycle assessment and risk assessment. This will improve the process of cradle to grave analysis of nano products. However, it should be noted that despite the awareness and promotion of sustainable nanomanufacturing, processes and products, significant toxicology and physical hazards can still exist. This might in part due to nonexistent of opportunity to fully incorporate health and safety into the sustainability paradigm (Schulte et al 2013).

Presently, the ability to tune and maintain nanoscale assembly processes is severely limited by the lack of truly nanoscale, real-time, techniques. (NSTC 2010).Therefore, other issue to consider in the sustainable nanomanufacturing process is the need for a design of scalable nanomaterial production and scalable process techniques for efficient and safe production of nanomaterials. It is important to do rsik assessment of ENMs because, the results can lead to sustainable nanomanufacturing, thus contributing to innovation and economic growth.

Finally, the sustainable nanomanufacturing must be thoroughly understood from both scientific and practical standpoints for effective risk assessment analysis. This framework can support the requirement from both manufacturers and regulators, by providing a platform, where data can be integrated for effective decision making.

REFERENCES

Agboola.E.A., Pike.R.W., Hertwig.T.A., Lou.H.H 2006 Conceptual design of carbon nanotube processes DOI 10.1007/s10098-006-0083-2

Aitken R.A., Bassan A., Friedrichs S., Hankin S.M., Hansen S.F., Holmqvist J., Peters S., Poland C.A., Tran L. (2011). Specific advice on exposure assessment and hazard/risk characterisation for nanomaterials under REACH (RIP-oN 3) final project report.

Asghari S., Johari S.A., Lee J.H., Kim Y.S., Jeon Y.B., Choi H.J, Moon M.C., Yu i.J. 2012, Toxicity of various silver nanoparticles compared to silver ions in Daphnia Magna. DOI: 10.1186/1477-3155-10-14

Bergeson L.L., 2013: Sustainable Nanomaterials: Emerging Governance Systems. DOI.org/10.1021/sc4000863

Benn, T. M. and P. Westerhoff (2008). "Nanoparticle silver released into water from commercially available sock fabrics." Environmental Science & Technology 42(11): 4133-4139.

Blaser, S. A., M. Scheringer, et al. (2008). "Estimation of cumulative aquatic exposure and risk due to silver: Contribution of nano-functionalized plastics and textiles." Science of the Total Environ-ment 390(2-3): 396-409.

Blackstone.R 2013: Introduction to Persistent, Bioaccumulative, Toxic (PBT) Compounds in the Environment. http://www.enn.com/sustainability/article/46592

Bogumiła R,; Andrea H,; Andreas L,; Kenneth A. D,;Iseult L.(2013) Mechanisms of Silver Nanoparticle Release, Transformation and Toxicity: A Critical Review of Current Knowledge and Recommendations for Future Studies and Applications. doi:10.3390/ma6062295

Boxall, A. B.; Tiede, K.; Chaudhry, Q.,2007: Engineered nanomaterials in soils and water: How do they behave and could they pose a risk to human health? *Nanomedicine*2007, *2*,919–927.

Brouwer D., Berges M., Virji M.A., Fransman W., Bello D., Hodson L., Gabriel S., Tielemans E.(2011). Harmonization of Measurement Strategies for Exposure to Manufactured Nano-Objects; Report of a Workshop. Annals of Occupational Hygiene

Buzea C., Ivan I., BlandinoP.,Robbie K. , 2007: Nanomaterials and nanoparticles: Sources and toxicity. Biointerphases vol. 2, issue 4 (2007) pages MR17 - MR172

Clark K., Van Tongeren M., Christensen F., Nowack B., Gottschalk F., Friedrichs S., Schmid K., Aitken R.J., Vaquero C., Gkanis V. et al. (2010). NANEX WP7 report: Scientific integration and gap analysis

Ciceri, N.D, Gutowsk, T.Gi, Garetti M."A Tool toEstimate Materials and Manufacturing Energy for a Product."IEEE, 2010. 1–6. Web.©2010 IEEE.

Dennekamp, M.; Mehenni, O.; Cherrie, J.; Seaton, A. Exposure to ultrafine particles and PM 2.5 in different micro-environments. *Ann. Occup. Hyg.* 2002, *46,* 412-414.

Dong. Y., Laurent.A.,Hauschild. M.Z (PROSUIT, 2013): Recommended assessment framework, characterisation models and factors for environmental impacts and resource use. EC 7th framework project 227078

ECHA 2012,Workshopon nanomaterials proceedings European Chemicals Agency, http://echa.europa.eu/"

EEA 1997: Life Cycle Assessment (LCA), A guide to approaches, experiences and information sources. Environmental Issues Series No 6 http://www.epa.gov/nrmrl/std/lca/pdfs/Issue20report20No206.pdf

EFSA (2009). The Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety. Scientific Opinion of the Scientific Committee.

FIFRA SAP, 2009. FIFRA Scientific Advisory Panel Meeting held November 3 - 5, 2009 on the Evaluation of Hazard and Exposure Associated with Nanosilver and Other Nanometal Pesticide

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

Hansen S. (2009). Regulation and risk assessment of nanomaterials – Too Little, Too Late? PhD Thesis. Technical University of Denmark.

Ghiazza.M, Carella, Oliaro-Bosso.S., Corazzari.I,, Viola.F, FenoglioI Nanomaterials Journal of Physics: Conference Series 429 (2013) 012024 doi:10.1088/1742-6596/429/1/012024

Gottschalk F;Scholza, R,W;Nowack, B 2010: Probabilistic material flow modeling for assessing the environmental exposure to compounds.Environmental Modelling & Software, 2010, 25, 320–332

Grieger K, Linkov I, Hansen SF, Baun A. 2011. Environmental risk analysis for nanomaterials: Review and evaluation of frameworks.Nanotoxicology 0:1–17.

Hakkert, B.C (2009). Nanomaterials under REACH :Nanosilver as a case study RIVM report 601780003/2009

Hristozov, D. R., Gottardo, S., Critto, A. &Marcomini, A. Risk assessment of engineered nanomaterials: a review of available data and approaches from a regulatory perspective. *Nanotoxicology*6, 880–898, doi:10.3109/17435390.2011.626534 (2012).

Hristozov D., Malsch I. (2009). Hazards and risks of engineered nanoparticles for the environment and human health. Sustainability 1: 1161-1194

ISO TC/229 Nanotechnologies (2011). Standards and projects under the direct responsibility of TC

Kandlikar, M., Ramachandran, G., Maynard, A., Murdock, B. and Toscano, W. A., 2006.Healthrisk assessment for nanoparticles: A case for using expert judgment. Journal of Nanoparticle Research 9 (1), 137–156.

Kempa T., Farrer R.A., Giersig M., FourkasJ.T.: Photochemical synthesis and multiphoton luminescencje of monodisperse silver nanocrystals. Plasmonics2006, 1, 45.

Khan, S; Mukherjee, A; Chandrasekaran, N. (2011). Silver nanoparticles tolerant bacteria from sewage

environment. J Environ Sci 23: 346-352. http://dx.doi.org/10.1016/S1001-0742(10)60412-3

Krishnan.N, Boyd. S, Somani. A, Raoux.S,Clark.D,Dornfeld.D "A Hybrid Life Cycle Inventory of Nano-Scale2008 42 (8), 3069-3075

Kulinowski et al (2008) ICON (Environmental Impacts of Nanosilver)

Kurttila, M.,Pesonen M.,Kangas J., KajanusM.Utilizing the analytic hierarchy process AHP.in SWOT analysis a hybrid method and its application to a forest certification case. Elsevier: Forest Policy and Economics 1 2000. 4152

Saaty T L (1980)*The Analytic Hierarchy Process*, McGraw Hill International.

Malkiewicz, K; Pettitt , M; Dawson, A. K;(2011) Nanomaterials in REACH

Mccluskey, G.J 2010Occupational exposure limits: Caution Poison Gas. /40/421

Mueller, N. C. and B. Nowack (2008). "Exposure modeling of engineered nanoparticles in the environment." Environ. Sci. Technol. 42(12): 4447-4453.

Mulvihill.M.J.,Beach.E.S.,Zimmerman.J.B.,Anastas.P.T.,2012GreenChemistryandGreenEngineering:AFramework for SustainableTechnology Development. doi:10.1146/annurev-environ-032009-095500

NSTC Committee on Technology Sustainable Nanomanufacturing 2010: Creating the industries of the future

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

Oberdorster G., Maynard A., Donaldson K., Castranova V., Fitzpatrick J., Ausman K., Carter J., Karn B., Kreyling W., Lai,D., et al. (2005). Principles for characterizing the potential human health effects from exposure to nanomaterials: Elements of a screening strategy. Particle Fibre Toxicology 2: 8

Oberdörster G., Oberdörster E., Oberdörster J. (2007). Concepts of nanoparticle dose metric and response metric. Environmental Health Perspectives 115: A290.

OECD 2012 Important Issues on risk assessment of manufactured nanomaterials. ENV/JM/MONO(2012)8

OECD 2010: Small sizes that matter: Opportunities and risks of Nanotechnologies
http://www.oecd.org/science/nanosafety/37770473.pdf

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

Panigrahi S, Kundu S, Ghosh S.K., Nath S, Pal T.: General method of synthesis for metal nanoparticles. Journal of Nanoparticle Research 2004, 6(4),411.

PMRA. 2007. Regulatory Proposal PRO2007-01.*Use of Uncertainty and Safety Factors in the HumanHealth Risk Assessment of Pesticides*. Ottawa, Canada.

PMRA. 2008. The Application of Uncertainty Factors and the *Pest Control Products Act* Factor in the Human Health Risk Assessment of Pesticides.

Pronk M.E.J 2009: Nanomaterial Under REACH (Nanosilver as a case study) National Institute For Public Health and the Environment. http://www.rivm.nl/bibliotheek/rapporten/601780003.pdf

SCHENIR (2009). Risk Assessment of Products of Nanotechnology, Brussels

SCHER, SCENIHR,SCCS EU 2013 New challenges for risk assessment. DOI:10.2772/37863

Schlich, K., Klawonn, T., Terytze, K., Hund-Rinke, K. (2013) Hazard assessment of a silver nanoparticle in soil applied via sewage sludge. Environmental Sciences Europe.25:17.DOI:10.1186/2190-4715-25-17. www.enveurope.com/content/25/1/17

Schlich, K.; Klawonn, T.; Terytze, K.; Hund-Rinke, K. Effects of silver nanoparticles and silver nitrate in the earthworm reproduction test. *Environ*. *Toxicol*. *Chem*. 2013, *32*, 181–188.

Schmidt.C.W 2012: Nanotechnology-Related Environment, Health, and Safety Research: Examining the National Strategy Environ Health Perspect . Apr 2009; 117(4): A158–A161.

Scholz, R. W. (2010). Environmental Literacy in Science and Society: From Knowledge to Decisions. Cambridge University Press, Cambridge (forthcoming).

Schulte.P.A., McKernan.L.T., Heidel. D.S., Okun A.H.,Dotson.G.S., Lentz. T.J., Geraci, C.L Heckel.P.E., Branche.C.M 2013;Occupational safety and health, green chemistry, and sustainability: a review of areas of convergence Environmental Health 2013, 12:31 http://www.ehjournal.net/content/12/1/31

Schwirn,K., Tietjen,L., Beer,I 2014 Why are nanomaterial different and how can they be appropriately regulated under REACH? Environmental sciences Europe, 2014, 26;4doi:10.1186/2190-4715-26-4

Smalley.R.E, Institute for Nanoscale Science and Technology (Rice University, Houston, TX); http://www.springer.com/978-1-4020-6119-6

Som C,(2011) Environmental and health effects of nanomaterials in nanotextiles and façade coatings, Environ Int (2011), DOI:10.1016/j.envint.2011.02.013

Som, C., Berges, M., Chaudhry, Q., Dusinska, M., Fernandes, T. F., Olsen, S. I., and Nowack, B.(2010). The Importance of Life Cycle Concepts for the Development of Safe Nanoproducts. *Toxicology*, In Press, Corrected Proof.

Song K.C., Lee S.M., Park T.S., Lee B.S.: Preparation of colloidal silver nanoparticles by chemical reduction method. Korean Journal of Chemical Engineering 2009, 26, 153.

Stoeger T., Schmid O., Takenaka S., Schulz H. (2007). Inflammatory Response to TiO2 and Carbonaceous Particles Scales Best with BET Surface Area. Environmental Health Perspectives 115

Subramanian,V.,Semenzina.E.,Hristozova.D.,Marcominia.A.,Linkova.I.Sustainablenanotechnology:Defining,measuringand teachinghttp://dx.doi.org/10.1016/j.nantod.2014.01.001

Topping, M Occupational exposure limits for chemicals. Occup Environ Med 2001;58:138–144

U.S. Environmental Protection Agency Nanotechnology White Paper; U.S. EPA: Washington, DC, USA, 2007; Available online: http://www.epa.gov/osa/pdfs/nanotech/epa-nanotechnology-whitepaper-0207.pdf (accessed 10 September 2009).

Walker W.E., Harremoes P., Rotmans J., Sluijs J.P., Asselt M.B., Janssen P., Krauss M.P. (2005). Defining Uncertainty: A Conceptual Basis for Uncertainty Management in Model-Based Decision Support. Integrated Assessment 4 (1)

Walser.T, Demou.E, Lang.D.J,Hellweg. S (2011) Prospective Environmental Life Cycle Assessment of NanosilverT-Shirts. America chemical society: Environ. Sci. Technol. 2011, 45, 4570–4578

Wittmaack K. (2007). Dose and Response Metrics in Nanotoxicology: Wittmaack Responds to Oberdoerster et al. and Stoeger et al. Environmental Health Perspectives 115