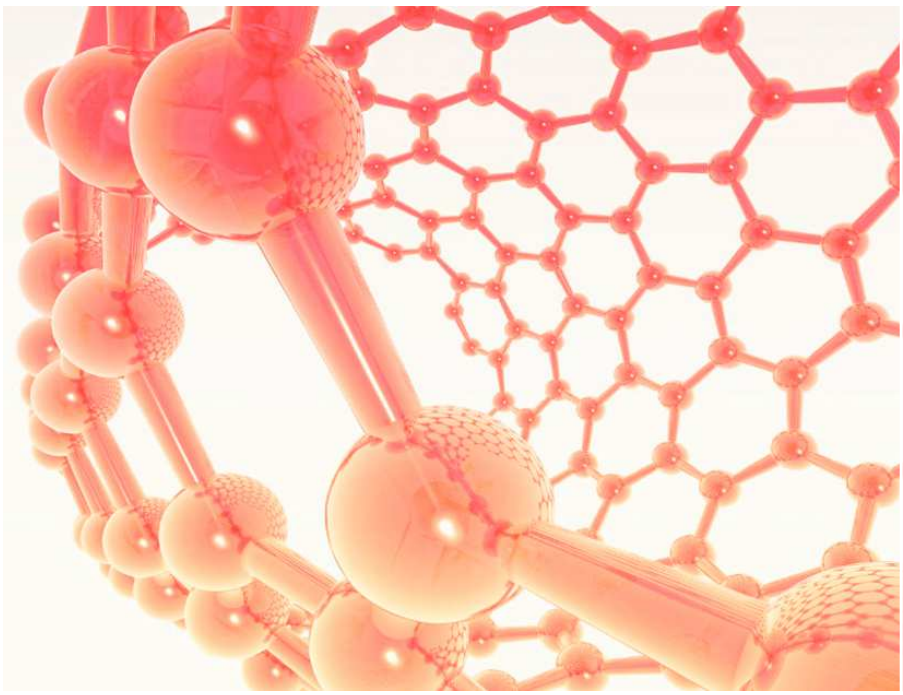
REACHnan

*LIFE REACHnano*

*Development of a web based REACH Toolkit to support the chemical safety assessment of nanomaterials*

# Guidance on available methods for risk assessment of nanomaterials



**Technical Guidances series - 2015**

ITENE LEITAT Technological Center member of TECNIO INVASSAT Institut Valencià de Seguretat i Salut en el Treball NIA

*LIFE11 ENV/000549*

www.lifereachnano.com

# Guidance on available methods for risk assessment of nanomaterials

This publication was published in 2015 with support from the European Union's LIFE Fund in the framework of LIFE programme environmental policy and governance entitled "LIFE REACHnano - Development of a web based REACH Toolkit to support the chemical safety assessment of nanomaterials" (LIFE11 ENV/ES/000549)



Printed in Spain



First Edition, 2015

This technical guidance has been produced by the REACHnano Consortium:

# Contents


1. Introduction and vision ..... 3
2. Scope and objectives of the guidance ..... 4
3. Overall view of Risk assessment and risk characterization methodologies under REACH .. 5
   - 3.1. Introduction ..... 5
   - 3.2. Application of in vitro approaches for hazard assessment ..... 8
   - 3.3. Application of in silico approaches for Risk Assessment ..... 10
   - 3.4. Exposure Assessment ..... 13
   - 3.5. Risk Assessment methodologies: methods and approaches within REACH ..... 17
4. Best practice recommendations for risk assessment of nanomaterials ..... 27
5. References ..... 27

# 1. Introduction and vision

Within the European Parliament and Council Regulation (EC) No 1907/2006 on the Registration, Evaluation, Authorisation and Restriction of Chemicals (**REACH**), risk assessment and risk characterization is conducted under the overall framework of the chemical safety assessment (CSA) process, by which a registrant shall identify and describe the conditions under which the manufacturing and use of a substance is considered to be safe during each life cycle step.

According to the European Chemicals Agency (ECHA), the main requirements under REACH regarding the Chemical Safety Assessment (see Figure 1) are:

- To perform a complete **Hazard assessment** based on the physicochemical, toxicological and ecotoxicological properties of the substance, in whatever size, shape or physical state;
- To define the **levels of exposure** under reasonable conditions of use;
- To **characterize the risk** by comparing the levels of exposure and threshold levels below which risks for human health and for the environment are considered to be controlled.

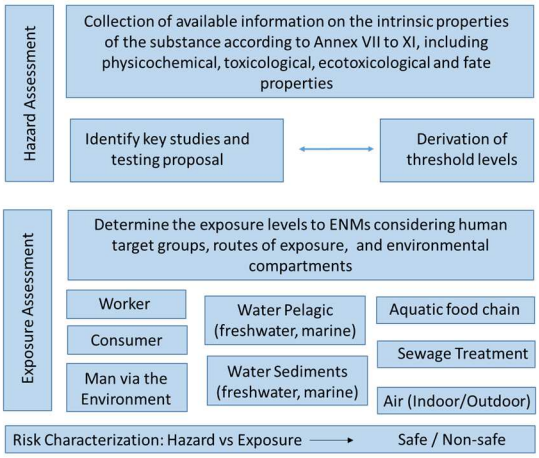



Figure1. Risk Assessment framework under

Under this framework, if risks are under control, the chemical safety assessment ends here. If risks are not under control, the chemical safety assessment has to be refined, either by obtaining more data on the properties of the substance, changing the conditions of manufacturing or use, or making more precise exposure estimations. The process is iterative and will continue until the risks are shown to be under control.

In the specific case of engineering nanomaterials (ENMs), as any chemical, they are regulated by REACH, being covered by the definition of a chemical "substance" stated in the regulation. The general obligations in REACH therefore apply as for any other substance, **requiring a risk assessment**, which should be performed as part of the chemical safety assessment process using pertinent information.

In the light of current knowledge and opinions of the EU Scientific and Advisory Committees and independent risk assessors, **current risk assessment methods are applicable**, even if work on particular aspects of risk assessment is still required due to the current lack of standardized approaches for determining the hazards and levels of exposure to the human health and the environment. In this regard, it has been acknowledged (e.g. by the OECD chemicals programme on cooperation on risk assessment) that the **existing risk assessment paradigm developed for traditional chemicals should also be applied to ENMs** (OECD, 2012). Nevertheless, these steps need specific considerations in practice when applied to ENMs (e.g. metric to use, exposure assessment methodology, etc.).

The majority of standard endpoints used in **regulatory hazard assessment remain appropriate for ENMs in the context of supporting data for toxicological and environmental risk assessment**. However, changes on sample preparation and dosimetry have been foreseen for most of the tests (OECD, 2012). Parameters such as particle solubility and stability in the test media are essential parameters, among others, to be reported for (eco)toxicological studies, as the information obtained is necessary for exposure considerations. Indeed, mass comparisons of concentrations nanomaterials versus the concentration of the chemical fraction dissolving from the ENMs are needed to understand the source of the hazardous effects reported.

At present, the current view is that the general existing **test guidelines are applicable to nanomaterials but have to be adapted and extended** for some ENM specific issues. Consequently, validated in vitro tools bioassays, hazard assessment tools, and especially predictive models, remain to be developed and tested for ENMs.

Regarding exposure assessment under REACH, occupational, consumer, and environmental exposure to NMs should be characterised during the entire product life-cycle, and particular attention should be given to the potential release of nanoforms at different stages (manufacture, use and disposal).

In the specific case of nanomaterials, there are specific considerations to take into account. ECHA acknowledges that measuring ENM exposure is a complicated task and no single approach can currently be used nor recommended, given that the most appropriate choice depends on the substance-specific information and the measuring techniques available.

In occupational settings for example, evidence of technical measurement difficulties related to background nanoaerosols has been reported in several studies (ECHA Guidance, Appendix to Chapter R.14, 2012). Similarly, regarding environmental exposure, detecting and quantifying ENMs in complex matrices (e.g. soil or sediments) is challenging, particularly for those ENMs made of chemical constituents that are highly abundant in the natural environment (e.g. many metals and metal oxide nanomaterials, carbon materials, etc.).

In summary, for a **comprehensive risk assessment of ENMs**, relevant information is needed with respect to the intrinsic properties of the particle that may assist in identifying the presence or absence of hazardous properties, likelihood of exposure in a specific compartment (i.e. workplace, freshwater, marine water, sediments or soils, sewage treatment plant and air), and dose-response data (i.e. DNEL- Derived No-Effect Level, PNEC - Predicted No-Effect-Concentration). Major investments have been done so far on the characterization of the toxicological profile of the first generation of ENMs, including data on relevant human health endpoints such as acute toxicity, irritation and corrosivity, sensitisation, repeated dose toxicity, mutagenicity, carcinogenicity, and reproductive toxicity, as well as data with respect to the nanoparticles'fate in the body (toxicokinetics, i.e. absorption, distribution, metabolism, and excretion).

In contrast, research aiming to improve our understanding of the possible exposure arising from all stages of the production, use and disposal of nanoparticles is far less advanced. The amount of research activities focused on the evaluation of the likelihood of exposure has increased in the last decade in parallel with the increased interest of the industry on the production and use of ENMs for developing new added value products.

There are increasing concerns over possible human health risk associated with the use of ENMs as far as the production volumes of some ENMs are already exceeding thousands of tonnes, which directly implies an increase of the likelihood of the potential exposure to ENMs during production, use, and end-of-life treatments in the near term.

Hence, in the coming years, a remarkable challenge for the nanotechnology industry, the academia and the regulators will be the generation of new data on the (eco)toxicological profile of available ENMs, as well as on the current levels of exposure in workplaces and the environment.

## 2. Scope and objectives of the guidance

This guidance document is part of a series of guidance documents that are aimed at helping manufacturers and downstream users of ENMs to perform a complete risk assessment taking into account the requirements laid down on REACH regulation.

The development of the guidance was informed by research and technical activities undertaken as part of the REACHnano project, whose main purpose is to develop a web-based platform to support the chemical safety assessment (CSA) of nanomaterials according to the risk assessment procedures and information requirements laid down on REACH.

Overall, the guidance provided includes a thorough description of the **current available approaches for risk assessment of ENMs**, assisting REACH registrants in general, and manufacturers and downstream users of ENMs in particular. This guidance particularly assists companies in the selection of adequate methodologies for risk assessment purposes on a regulatory basis when dealing with ENMs. It is aimed at:

- Registrants responsible for REACH compliance within companies producing and/or using ENMs;
- Experts from industry associations and other stakeholder organizations informing companies about the requirements for nanomaterials under REACH, especially for risk assessment purposes;
- Experts from standardization (i.e. ISO committees) and/or regulatory bodies (i.e. ECHA);
- Researchers from academia, non-profit research organizations and private research institutions.

This guidance can be obtained via the website of the REACHnano project (http://www.lifereachnano.eu). Further guidance documents will be published on this website when they are finalized or updated.

Users are reminded that the information in this document does not constitute legal advice.

# 3. Overall view of Risk assessment and risk characterization methodologies under REACH

## 3.1. Introduction

The following sections summarize the main aspects considered by REACH when evaluating the hazards and exposure of substances at whatever size or form:

### a) Hazard Assessment within REACH

The Hazard assessment is the first step of the chemical safety assessment (CSA). Requires the collection and evaluation of all available and relevant information on the substance that may assist in identifying the presence or absence of hazardous properties of the substance. This includes information on the intrinsic properties of the substance according with the data requirements established by REACH Annexes VII to X.

For a comprehensive human hazard assessment, information is needed with respect to the substances' fate in the body (toxicokinetics, i.e. absorption, distribution, metabolism, and excretion) and on the following human health endpoints: acute toxicity, irritation and corrosivity, sensitisation, repeated dose toxicity, mutagenicity, carcinogenicity, and reproductive toxicity as well as any other available information on the toxicity of the substance.

Similarly for a comprehensive environmental hazard assessment, toxicity data for organisms living in each environmental compartment (i.e. freshwater, marine water, sediments and soil, sewage treatment plant (STP) and air) are needed, including acute and chronic toxicity to aquatic organisms, toxicity to sediment-dwelling (benthic) organisms, toxicity to sewage treatment plant (STP) micro-organisms and other relevant environmental properties such as aquatic and terrestrial bioconcentration and bioaccumulation.

Under REACH, registrants are obliged to collect and evaluate all available information on the intrinsic properties of the substance before conducting testing to generate such data. Annexes VI-X of REACH specify these minimum data requirements for a given substance according to its tonnage for registration purposes (REACH Article 12), which however may be adapted as appropriate.

The evaluation of this hazard information should aim at identifying the dose descriptor of the substance, ideally the DNEL (Derived No-Effect Level), which represents a level of exposure above which humans should not be exposed, and the PNEC (Predicted No-Effect-Concentration), which is the concentration of a chemical in any compartment below which unacceptable effects on ecosystem and its organisms will most likely not occur during long term or short term exposure.

The hazard assessment approach within REACH is depicted in the Figure 2.

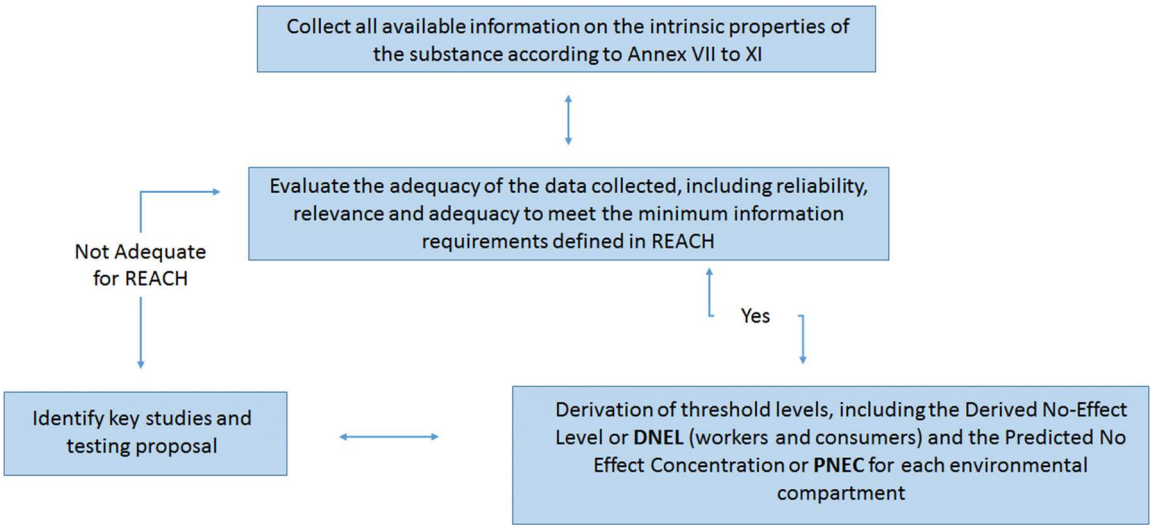



Figure 2. Hazard Assessment approach established by REACH

### b) Exposure Assessment within REACH

According to REACH Regulation (EC) No 1907/2006, the aim of the chemical safety assessment is to demonstrate the safe use of nanoparticles during each life cycle step when they are classified as hazardous to human health, the environment, presents a physicochemical hazard or is assessed as PBT[1] or vPvB[2] substance. Only in these cases, the chemical safety assessment will require an inclusion of an:

- Exposure assessment including the generation of Exposure Scenarios and exposure estimation and
- Risk characterization

If the substance does not fulfil any of the above criteria, the exposure assessment will not be needed. If yes, an exposure assessment shall be conducted comprising two main steps: i) development of **exposure scenarios (ES)** and ii) **exposure estimation**. Conceptually, the exposure assessment is the process of measuring or estimating the dose or concentration of the substance to which humans and the environment are or may be exposed, depending on the uses of the substance. Exposure is a key element in risk assessment since it is a precondition for the potential (eco)toxicological effects to take place. Exposure usually covers the three domains: occupational, consumer and environmental. Therefore, the emission/exposure of worker, consumer and environment resulting from all identified uses (IU) throughout the life cycle of the nanoparticles should be estimated. This includes the generation of sufficiently detailed information on uses, such as operational conditions (OCs) and risk management measures (RMMs).

The final exposure scenario will define the operational conditions and risk management measures required to ensure the safe use of the substance for each exposed population during all the life cycle stages of the substance, including waste stage and the article service life, where applicable. It is achieved through refinement of the operational conditions and risk management measures until the risks for humans and the environment are shown to be controlled.

The following figure shows the exposure assessment approach within REACH, including the scope of the exposure assessment in terms of human and environmental exposure.

[1] PBT: Persistent, Bioaccumulative, Toxic
[2] vPvB: Very Persistent, very bioaccumulative

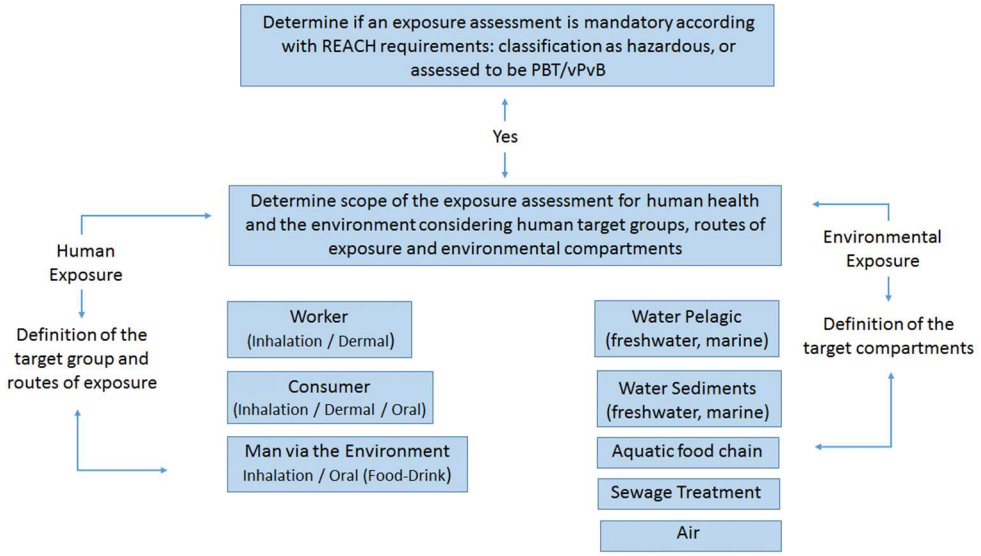



Figure 3. Exposure Assessment approach established by REACH

Ideally, the exposure assessment should be based on quantitative measurements of the levels of the exposure for each target groups and environmental compartment included in figure 3, however, in practice, the availability of reliable exposure data is scarce and mostly limited to the workplace, which implies that, in most cases, exposure levels has to be based on estimations conducted by means of exposure estimation models.

It is stressed that the following preferential hierarchy is applied to exposure data for the estimation of exposure levels:

- Measured data
- Data derived from modeled estimates which mimic the activities under controlled conditions (in cases where data lacking exist)

It is also needs to be kept in mind that the different routes of human exposure are: dermal, inhalation and oral. However, depending on the physical-chemical properties or the use pattern of the substance, some routes of exposure may be irrelevant. Thus, the most likely exposure route must be considered in occupational settings and therefore the determinants of exposure selected must be relevant for this route of exposure.

There is a wide range of exposure estimation models that can be used under REACH to obtain an initial estimation of exposure based on conservative models, taking the worst-case default values as input information for the different exposure parameters. This estimation is usually defined as Tier 1 estimation. A higher Tier estimation can be done using more sophisticated and detailed models, however, these higher Tier assessments (Tier 2 and 3) are meant to be carried out by experienced assessors able to carry out the assessment successfully. Occupational exposure estimation with modeling approaches are described in detail in sections 3.5.1 and 3.5.2 of this guidance.

The predicted exposure levels are considered to be task-based and the exposure level characterizes a specific core scenario determined by a range of operative conditions and the control approaches applied to reduce or control the exposure.

### c) Risk Characterization within REACH

The last step in the chemical safety assessment process is the risk characterization, where the levels of exposure are compared with the threshold levels for each effect. When suitable predicted no-effect concentrations (PNEC) or derived no-effect levels (DNEL) are available, risk characterisation ratios (RCRs) can be derived in order to decide if risks are adequately controlled for each environmental compartment and for each human population known to be or likely to be exposed (REACH Annex I, 6.4).

These RCRs are calculated on the basis of the following equations:

$$RCR\ human\ health = \frac{Exposure}{DNEL} \qquad RCR\ enviroment = \frac{PEC}{PNEC}$$

Control of risk for a substance is demonstrated when the outcome of both the hazard assessment and exposure assessment are robust and where RCRs for all exposures (for all compartments, routes, populations and durations) related to all exposure scenarios and all end-points are below one: **RCR $\leq$ 1**, i.e the exposure levels do not exceed the appropriate DNEL/PNEC.

If the Risk Characterization shows that, based on the initial exposure scenarios, risks are not controlled; iteration will be needed, refining at any point of the assessment hazard and/or exposure data by the introduction of new risk management measures or the modification of operational conditions. The iteration should continue until the Risk Characterization shows that the risks are controlled.

## 3.2. Application of in vitro approaches for hazard assessment

As stated previously, the hazard identification consists on the integration of all the available information on the substance to identify the toxicological and ecotoxicological hazards and determine for each adverse health and environmental effect the dose descriptor of the substance.

In human health studies, as a result of the hazard assessment, derived no-effect levels (DNELs) are established. These are concentration levels below which a substance does not adversely affect human health. Under REACH, the registrants are required to address exposure via oral, dermal and inhalation exposure, taking into account identified local and systemic effects.

In environmental studies the dose descriptor used is the PNEC (Predicted No-Effect-Concentration) of a given environmental compartment, typically given in [mg/L] or [mg/kg]. This PNEC is the concentration of a chemical in any compartment below which unacceptable effects on the aquatic ecosystem and its organisms will most likely not occur during long term or short term exposure.

In the case of ENMs, due the multiple parameters affecting the toxicological profile, including, amongst many others, size, payload, composition and geometrical structure, it is essential to design an individual toxicology strategy reflecting current literature-based knowledge, and complemented with reliable testing strategies.

In the context of REACH, a battery of well-defined tests to assess a number of toxicological and ecotoxicological endpoints are necessary for regulatory approval. Table 1 identifies those properties “endpoints” especially applicable to nanomaterials according and the recommendations published by ECHA as part of the Guidance on information requirements and chemical safety assessment. It should be noted that a number of *in vitro* methods are currently applied to support the hazard assessment of ENMs, but none of them has been formally validated for ENMs.

The question that remains is whether or not the current regulatory testing regime and protocols are suitable for ENMs. There has been much debate over whether ENMs can be treated in the same way as chemical or pharmaceutical products, or if they require their own specific testing protocols because of their unique physico-chemical properties.

Table 1. Toxicological endpoints and in vitro test available for risk assessment under REACH.

| REACH relevant endpoints | In vitro tests available | ≥1 | ≥10 | ≥100 | ≥1000 |
|---|---|---|---|---|---|
| Skin irritation | Transcutaneous Electrical Resistance Test Method (TER) - OECD TG 430<br>In Vitro Membrane Barrier Test for Skin Corrosion (Corrositex) – OECD TG 435<br>In Vitro Skin Corrosion: Reconstructed Human<br>SpiSkin/EpiDerm/SkinEthic: OECD TG 431<br>In Vito Skin Sensitisation – OECD TG 442D<br>SpiSkin/EpiDerm/SkinEthic: In Vitro Skin Irritation - OECD TG 439<br>Skin Absorption: In Vitro Method – OCDE TG 428 | | X | X | X |
| Eye irritation | Bovine Corneal Opacity and Permeability Test (BCOP) - OECD TG 437<br>Isolated Chicken Eye Test (ICE) - OECD TG 438<br>Fluorescein leakage (FL) test method - OECD TG 460<br>EpiOcular (being considered for adoption) | | X | X | X |
| Skin sensitisation | In Chemico Skin Sensitisation- OECD TG 442C<br>In Vitro Skin Sensitisation - OECD TG 442D<br>h-CLAT = 7 human Cell Line Activation Test<br>OECD Guideline 428 (Skin Absorption: In Vitro Method) | X | X | X | X |
| In vitro gene mutation study in bacteria | Ames test for bacterial mutation - OECD 471 | X | X | X | X |
| In vitro cytogenicity study in mammalian cells or in vitro micronucleus study | In Vitro Mammalian Cell Micronucleus Test – OECD 487 | | X | X | X |
| In vitro gene mutation study in mammalian cells, | In Vitro Mammalian Cell Micronucleus Test – OECD 487<br>In vitro mammalian cell gene mutation test –OECD TG 476<br>In vitro mammalian cell gene mutation test – lymphoma assay | | X | X | X |
| Acute toxicity (oral) | There are currently no in vitro tests that have been officially adopted by the EU or OECD for assessment of acute toxicity | X | X | X | X |
| Acute toxicity (inhalation) | | | X | X | X |
| Acute toxicity (dermal) | | | X | X | X |
| Short-term repeated dose toxicity study (28 days) | Currently, no available alternatives to animal testing are accepted for regulatory purposes for detecting toxicity after repeated exposure. | | X | X | X |
| Sub-chronic toxicity (90d) | There are currently no in vitro tests that have been officially adopted by the EU or OECD for assessment of sub-chronic or long term toxicity | | | X | X |
| Long-term repeated toxicity (≥ 12 months) | | | | | X |
| Further repeated dose toxicity studies | The design of alternatives to in vivo testing for reproductive toxicity is especially challenging in view of the complexity of the reproductive process and large number of potential targets/mechanisms associated. | | | | X |
| Screening for reproductive/ developmental toxicity | Strogenicity (OECD TG 455 )<br>Strogenicity (OECD TG 457)<br>Steroidogenesis - OECD TG 456 | | X | X | X |
| Pre-natal developmental toxicity study | Currently, no available alternatives to animal testing are accepted for regulatory purposes. | | | X | X |
| Developmental toxicity study | | | | | X |
| Two-generation reproductive toxicity study | | | | X | X |
| Assessment of the toxicokinetic behaviour | | | X | X | X |
| Carcinogenicity study | Unscheduled DNA synthesis - OECD 482<br>Sister Chromatid Exchange - OECD 479<br>In vivo alkaline singlecell gel electrophoresis assay (comet assay) – OECD 489 | | | | X |
| Cell Viabiitity | Tetrazolium salts assay<br>Alamar Blue<br>Incorporation of [3H] thymidine into the DNA Cologenic assay | | | | |

A number of methods are also available to evaluate the effects of ENMs in the environment. Standardized ecotoxicological tests are suitable for the comparison of chemicals with respect to their ecotoxicity and for a first risk assessment. For refined risk assessments tests with more realistic environmental conditions are required.

Implementation of standardized ecotoxicological tests for the effects of ENMs in aquatic and soil environments may be problematic as aggregation/agglomeration is concentrations dependent.

Moreover, there is still a need for investigation of individual species differing in feeding mode (filter-feeding vs sediment-ingestion) and physiological complexity. Table 2 outline current recommended methods for ecotoxicity testing of ENMs under REACH.

Table 2. Ecotoxicological relevant endpoints and in vitro test available for risk assessment under REACH

| Tonnage level manufactured or imported | | ≥1 | ≥10 | 100 | :1000 |
|---|---|---|---|---|---|
| **Aquatic toxicity** | | | | | |
| Short-term toxicity testing on invertebrates | Acute immobilization- OEDC 202<br>Mortality (short term) - ASTM (E-1440-91) | X | X | X | X |
| Growth inhibition study aquatic plants (algae preferred) | Growth inhibition - OECD 201 | X | X | X | X |
| Short-term toxicity testing on fish | Acute toxicity - OECD 203<br>Short-term toxicity test on embryo stages - OECD 212 | | X | X | X |
| Activated sludge respiration inhibition testing | Respiration Inhibition Test - OECD 209 | | X | X | X |
| Long-term toxicity testing on invertebrates | Reproduction - OECD 211 | | | X | X |
| Long-term toxicity testing on fish | 21-day Fish Assay – OECD 230 | | | X | X |
| **Effects on terrestrial organisms** | | | | | |
| Short-term toxicity to invertebrates | Reproduction Test - OECD 222<br>Mortality /(short term) - ASTM E1706-05M | | | X | X |
| Short-term toxicity to plants | Lemna sp. Growth Inhibition Test - 221 | | | X | X |

A strategic approach is clearly required to answer the remaining questions with regards to the in vitro testing strategy required for the safety assessment and regulation of ENMs. A thorough study of which in vitro tests are the most informative, reducing false positive/negative results with ENMs is still needed.

## 3.3. Application of in silico approaches for Risk Assessment

Under this framework, REACH legislation promotes the use of computational approaches, commonly named **in silico**, which are all the approaches based on computer simulation or modelling used to predict the effects of chemical compounds without the use of the real chemical compound, but based on the chemical structure only.

There are different types of computational approaches to assess activity and/or toxicity of chemicals, all of them comprising a series of different tools whose commonality lies in the identification of a relation between chemical structure and exhibited activity or toxicity. These methods allow scientists to reduce the repetition of animal testing, by replacing further tests with computer modelling.

Similarly, for industrial manufacturers, importers and users of chemicals, in silico testing can help to avoid the costs and delays associated with animal experiments. Examples of common in silico methods applied for regulatory purposes are (Q)SAR, Read-Across and Virtual Screening. Quantitative Structure-Activity Relationship (QSAR) are computer-based models for the prediction of toxicological, biological and physicochemical properties.

Read-across is a very simplified version of QSAR model. Basically, the property of one or few chemicals is predicted on the basis of one or more similar compounds, using or not some chemical descriptors. Virtual screening is simulation based methods used to evaluate the binding between a chemical compound and a biological macromolecule, such as a protein.

The referred **QSAR models are in silico (computer-based) methods able to quickly predict and assess the toxicity of large numbers of chemicals** using a mathematical algorithm implemented in a software programme. These models are widely used to evaluate the safety of a wide range of chemical substances, and are considered as "alternative methods" by the OECD under the context of key regulations such as the Cosmetic Directive and REACH.

The high potential of this technique is widely recognized, and it is even accepted that in some cases QSAR could replace existing biological tests. In this regard, within REACH regulation, annex XI defines a set of criteria for applying (quantitative) structure-activity relationship models. In this context, the use of a QSAR model is valid if: the model is recognised as scientifically valid (using the OECD principles); the evaluated substance is included in the applicability domain of the model; results are adequate for classification and labelling and/or for risk assessment; adequate documentation of the methods is provided."

The compliance of such principles is extremely difficult in the case of ENMs due to the lack of sufficiently numerous and systematic experimental data on the physicochemical properties of the ENMs, limited knowledge on the mechanisms of the toxic action, and uncertain behavior of the ENMs in the environment, being necessary to generate reliable data for defined endpoints.

A number of models have been successfully developed and validated, however there are still some challenges to cope with. To perform QSAR studies, the molecular structures have to be characterized by descriptors that correlate with experimental properties.

Unfortunately, the calculation of such descriptor requires the systematic characterization of nanostructures using measured properties such as size, zeta potential or chemical reactivity, which currently are nearly absent in public data. In addition, there is no a "unique" set of molecular descriptors characterizing NMs features, and thus these descriptors must be studied for each case.

Secondly, there are no systematic studies of biological effects of ENMs available in the literature. Although some data exist on the associated toxicities of certain types of ENMs, little is known about the systemic distribution, metabolism, elimination, and health effects once the nanoparticles reach systemic circulation.

For the application of this methods in nanoscale substances, physical properties such as size, aspect ratio and surface area of ENMs have an important influence on the interactions of these materials with biological systems, thereby affecting their hazard potential. This problem has been studied and reviewed, and the necessity of new specific descriptors for NMs has been highlighted.

Moreover, there is not enough experimental data available, and the published data are frequently not systematic (e.g. experimental conditions are variable and in consequence they are not comparable) and thus useful for the development of specifically adapted QSAR models.

When there are not data enough to construct robust QSAR models, simplest alternatives such as Read-Across are possible. Read-across is based in the well-known "neighbourhood behaviour" principle: chemicals with common structural features will show similar physico-chemical properties, toxicological (human health/ecotoxicity) effects or environmental fate properties.

Thus, substances sharing structural similarities can be grouped together in a chemical category, and once a group has been established, it is possible to use information from the data rich members to fill data gaps. The table on the right describes the advantages/disadvantages of each technique to be considered when applying in silico methods to NMs.

The Figure 4 depicts the methodological approach commonly employed to develop QSAR models.

Table 3 summarizes advantages and disadvantages of the referred approaches.

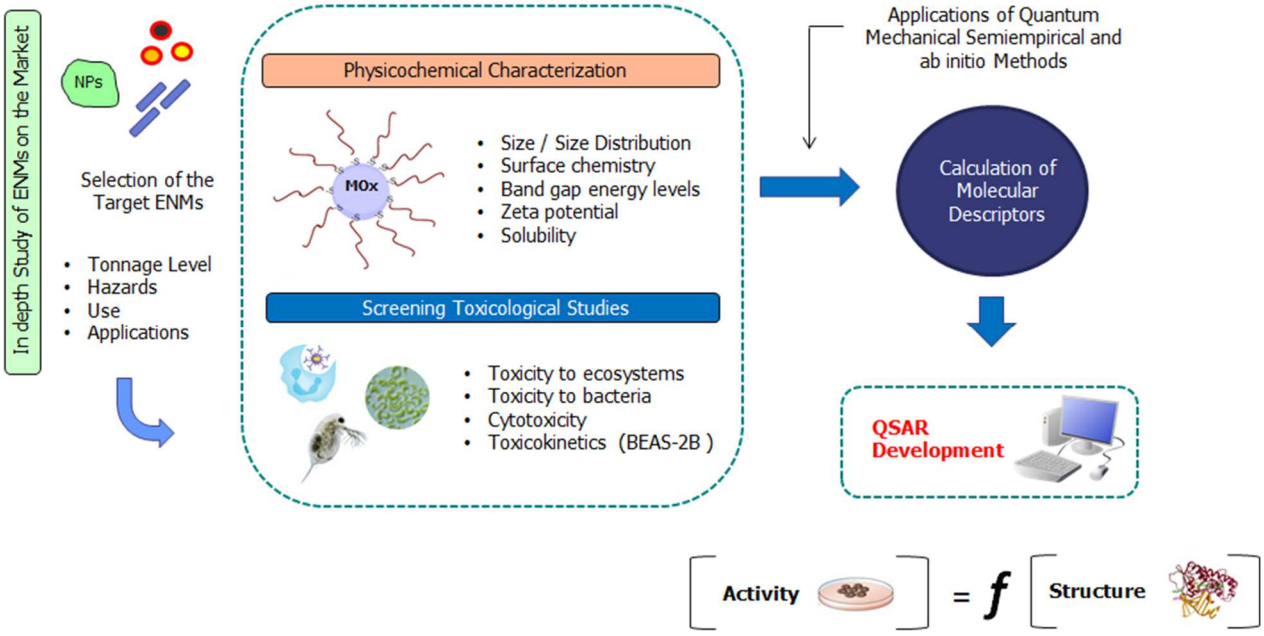



Figure 4. Conceptual representation of the steps to develop QSAR models.

Table 3. Main modeling approached available.

| | Advantages | Disadvantages |
|---|---|---|
| Docking | - Allows the study of molecular interactions.<br>- Different NMs can be compared amongst them. | - Requires the detailed knowledge of all the molecular structures implied: it is difficult to apply to NMs. |
| Molecular Dynamics | - Gives a dynamic perspective of molecular interactions. | - Long calculating time. |
| Quantum Chemical | - Gives exact values of molecular properties based on basic physical principles. | - Complexity.<br>- Computation expensive method. |
| QSAR | - Quick calculation once a QSAR model has been generated.<br>- Gives fair quantitative predictions of toxicity properties.<br>- Is has showed its efficiency in NMs. | - Does not provide direct mechanistic information.<br>- Adaptation of molecular descriptors to the ENMs features must be studied for each case. |

## 3.4. Exposure Assessment

### 3.4.1. Exposure Estimation under REACH

#### a) Occupational Exposure

In the case of conventional chemical compounds, REACH provides support for estimating occupational, environmental and consumer exposure although not everything is applicable to nanomaterials. Substances in the workplace may come into contact with the body and possibly enter into it by inhalation (inhalation route), by contacting and passing through the skin (dermal route), or swallowing (ingestion route). Ingestion or oral exposure may occur in situations where there is exposure to aerosols and where contaminated skin or clothing may lead to exposure due to contact with the mouth region. It could be minimized if good hygiene practices are followed (segregating working and eating facilities and adequate washing prior to eating, etc.). This route is generally considered less important, commonly arising from accidents or bad practices. Therefore, exposure by dermal and inhalation are considered the main routes of exposure.
The tools that are suggested by REACH for first Tier occupational exposure estimations are ECETOC-TRA (preferred) and the EMKG-Expo-Too while Stoffenmanager exposure model, RISKOFDERM dermal model and Advanced REACH tool (ART) are recommended for higher Tier occupation exposure estimations. In this guidance, a thorough description of the most used approaches by industry are explained in sections 3.5.1 and 3.5.2.

Within the context of the FP7 NANEX project, the estimates of ECETOC TRA and Stoffenmanager models were compared to measured concentrations of nanomaterials. The results showed no correlation between the estimated and measured values. The authors attributed this deviation to the fact that neither of the models is tuned to and calibrated for nanomaterial exposure situations. For the same reason it is theoretically expected that neither EMKG-Expo-tool nor ART would provide better estimates.

Apart from that it should be highlighted the fact that of all the above mentioned methods provide estimates in terms of mass per volume, for comparison with hazard after repeated or continous exposure, and not particle number or surface area which would be more appropriate for nanomaterial and this is the main factor that hampers their application to nanomaterials. However it should be also noted that if the exposure estimate is about highly aggregated/agglomerated nanoparticles whose aerodynamic diameter is in the micro-scale, all these tools could be used.

An exception is the case of RISKOFDERM method as the conclusions of NANEX project suggested that dermal modules might be suitable for use for nanomaterials. The reason is that the underlying equations do not appear to rely on nano-specific properties. However they also suggest that these methods like RISKOFDERM should be used with care as they are neither validated nor calibrated for nanomaterials and the output estimate is given in a mass-based metric.

#### b) Environmental Exposure

REACH regulation provides the tools for chemical safety assessments and reports. CHESAR (Chemical Assessment and Reporting tool) is the tool that is used for Chemical Safety Assessments (CSA) and Reporting (CSR). Before the latest version of CHESAR, environmental safety assessments in Europe were performed using the EUSES (European Union System for the Evaluation of Substances) tool while occupational safety assessments were performed with the ECETOC tools. CHESAR now includes both these tools as modules.

EUSES and CHESAR integrate functions to calculate PEC values. PEC calculation consists of two parts: On one hand is the calculation of the volume of the different natural compartments where the substance concerned is bound to end up in and on the other is the calculation/estimation of the quantity of this substance in each compartment. Chemical compounds/substances can be released throughout their life cycle.

EUSES model is focused on the European region and as such PEC calculations are based on the volumes of the natural compartments in Europe. Furthermore, emission factors for various life cycle stages are calculated based on known properties, uses and functions of a compound.

Regarding the application of EUSES for ENPs, it should be noted that data on the properties, uses and functions of these new compounds are not available. In fact, calculation of PEC's for nanomaterials demands for approaches capable of handling inherent uncertainties of both properties and behaviour under various conditions (fate).

In the current context, there are still substantial gaps that need to be bridged before achieving robust and comprehensive environmental assessments of ENMs using the abovementioned models.

Nowadays, environmental exposure assessment for ENMs is mainly based on probabilistic approaches or on the use of different scenarios to provide estimates. Probabilistic Material Flow Modelling (PMFA) raises as a modelling approach that could provide a framework for building models to derive probabilistic PEC calculations for any new compound including of course the ENPs. A basic MFA based environmental model for nanomaterials is depicted in Figure 5.

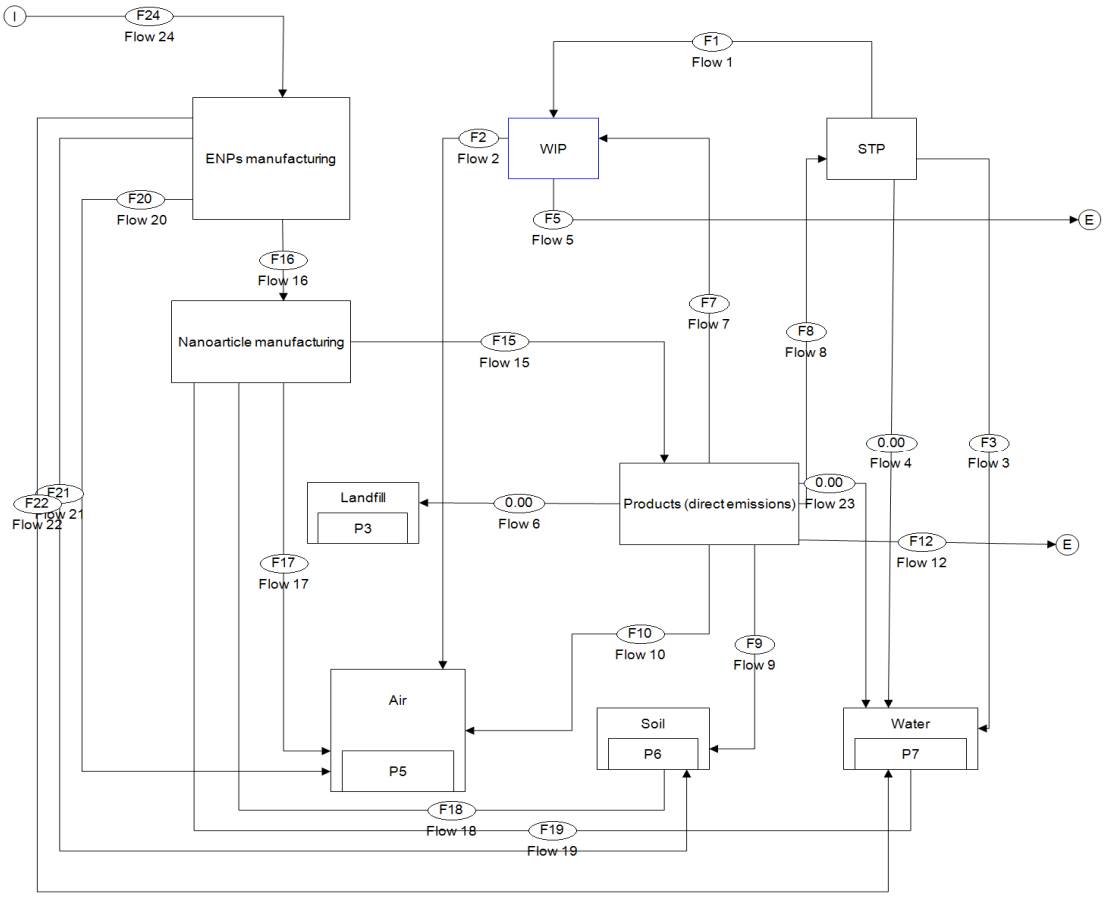



Figure 5. MFA environmental model example.

Under this approach, the input source represents the production volume of a nanomaterial (or the imported amount). Synthesized nanomaterials are then used in the manufacturing of the nanoparticle (for example nanocomposites, sunscreens, electronics etc).

Once the nano-articles have been manufactured then they begin their service life. During service life, nanoparticles may be released during their use (for instance sunscreens) or due to the weathering of products. Nanoarticles at some point will reach their end of life and they will become waste. Waste containing nanomaterials can be recycled, incinerated (WIP) or disposed of in landfills. Nanomaterials that are contained in waste water enter the sewage treatment plant (STP).

Any of these processes could be a source of emission of nanoparticles to the environment which in turn could end up in one of the natural compartments (air, soil or water). From the mathematical point of view, the model depicted in Figure 5 is an input-output stationary model that contains 9 processes, 23 internal flows, one input flow, and 3 output flows (air, soil and water) and 3 elimination flows. Production volumes, imported and exported quantities, applications and behaviour in the environment are generally known for most conventional chemical compounds and based on these fate mass transfer coefficients can be assigned to the different flows. For the calculation of this type of models, matrix algebra is typically applied and the solutions can be found by computing inverse matrices.

However in the case of new compounds and ENMs the same process still remains a challenge as most of the data remain unknown. In PMFA, instead of discrete values, density functions should be assigned to both volumes and transfer coefficients. Density functions describe the relative likelihood of a continuous random variable to take on a given value within a range. In other words, both volumes and transfer coefficients should be assigned first with a range of values and then the distribution of these values in terms of probability should be constructed.

Probability density functions are selected depending on the data availability on the experience. For instance uniform distribution is typically chosen when no data for suggesting other type of distribution are available. Triangular distribution may be chosen when the most probable outcome is known or can be guessed. Lognormal distribution on the other hand is typically chosen when sufficient data are available. Other distributions are PERT or discrete.

The combination of probability density functions of the various nanomaterials volumes and transfer coefficients is then performed with Monte Carlo (MC) simulation methods. Monte Carlo is a computer-based method capable of producing system probability distribution. Monte Carlo method consists in picking randomly values from the different probability density functions of the system and produce outcome scenarios (iterations). By repeating this process, a high amount of times it finally builds a distribution of possible outcomes.

It should be noted that although probabilistic modelling offers a powerful way to effectively deal with uncertainty in systems, the output of any MC simulation is extremely sensitive to the choice of probability density functions.

In view of the current uncertainty, Multi-Criteria Decision Analysis (**MCDA**) is an alternative approach for risk assessment within the context of nanomaterials environmental exposure. MCDA methods cannot predict PEC and therefore cannot directly replace the probabilistic approaches although they can serve as the basis for qualitative risk assessment and ranking of options to support decision making.

There are various MCDA methods like for instance Weighted Sum Method (WSM), Multi-attribute utility theory (MAUT), Analytical Hierarchy Process (AHP) Elimination and Choice Expressing Reality (ELECTRE), Preferance Ranking Organization Method for Enrichment of Evaluation (PROMETHEE), goal programming etc.

Purpose of any MCDA method is to provide a coherent framework for evaluation different alternatives based on a multi-perspective synthesis. In general, an MCDA (Multi-Criteria Decision Analysis) problem is defined on m alternatives and n decision criteria. Each of the decision criteria is assigned with a relative weight or importance coefficient wj that represents the relative importance of the criterion Cj and thus its influence on the overall assessment (final score). Furthermore, aij is the performance value of alternative j when it is evaluated in terms of criterion Cj. The range of the performance values should be established by the users.

MCDA for instance could be used for the selection for comparing various nanomaterials (m alternatives) in terms of potential environmental harm: Different inherent to the material parameters that may affect its behaviour in the environment like for instance agglomeration, reactivity/charge, critical functional groups etc) may be represented by n decision criteria. How decisive these parameters will be in the behaviour of the particles is represented by the weights (wj) while the performance of each of the alternatives in terms of criterion Cj is reflected through the performance values (aij). Having all these parameters defined, a particular score can be obtained highlighting the alternative that makes trade-offs among the decision criteria. Of course, the previously mentioned example can be further developed by adding more criteria that can be related to other aspects like for instance the occupational risks or even economic and social criteria.

The main advantage of MCDA is that they can support decision making combining not only hazard-related parameters but also parameters related to other aspects like for instance economic or stakeholder preferences. However, the definition of most of its components relies on the opinions of experts and spatial and temporal parameters which are of crucial importance in the case of the environmental fate of nanomaterials cannot be taken into consideration. But MCDA can still provide guidance and highlight aspects that need to be addressed before full-scale environmental exposure and risk assessments.

Finally, **Bayesian Networks** theoretical principles could serve as the basis for building quantitative tools for predicting environmental exposure and risks associated with the use of nanomaterials. Bayesian networks is a probabilistic graphical model that represents a set of random variables and how they depend on each other through a directed acyclic graphs (DAG). Each variable consists of a certain set of mutually exclusive states. The links between the variables represent their relationship. For instance, a link from variable A to variable B indicates that A can cause B. (A is a parent of B and B is a child of A). Each variable has a Conditional Probability Table (CPT) associated with it. Parents have a very simple probability table which gives the initial probability distribution of the variable.

The main advantage of the Bayesian networks as a modelling tool is their adaptive nature which allows for continuous improving and refining. Bayesian networks are decomposable and each part of the model can be treated separately.

This is of crucial importance in the case of nanoparticles provided that more data and knowledge will be available in the near future. It is worth to note however that Bayesian networks based models may result in complex networks and therefore their parameterization may be too complicated.

### 3.4.2. Quantitative exposure assessment under REACH with measurements

The evaluation of the exposure to ENMs at the workplace is a key priority within the nanosafety research community. A number of approaches have been published to date, all of them are based on four main steps, including 1) identification of the potential sources of emission (e.g. dedicated questionnaires), 2) definition of the measurement strategy, including instrumentation and metrics, 3) evaluation and characterization of the background levels of ENPs, describing sources of ENPs and characteristics, and 4) data processing.

The development of adequate instrumentation has been paid much attention in the last few years, including portable and non-portable instruments that monitor ENMs in quasi real-time and instruments that sample (time-aggregated) ENMs on a substrate, followed by off-line analysis using techniques such as inductively coupled plasma-mass spectrometry (ICP-MS), energy dispersive X-ray fluorescence (ED-XRF), atomic force microscopy (AFM), electron microscopy (EM), and X-ray diffraction (XRD).

A suite of real-time devices are already available, and new devices are likely to become available on the market in the near future.

The most employed devices include: portable condensation particle counters (CPCs) in the size range of 10 to 1000 nm, portable optical particle sizers (OPS) in the size range of 0.3 to 10 µm, transportable surface area monitors in the size range of 10 nm to 1000 nm, and high sensibility particle sizers depending on the time resolution needed such as the SMPS-Scanning mobility particle sizer (<30 s) or the FMPS-Fast mobility particle sizer (1 s).

Figure 6. Exposure assessment using direct-reading instruments

The combined use of these instruments will provide valuable information on the levels of release and exposure to ENPs, including particle number concentration (particles/cm3), size distribution and surface area (µm2/cm3), all relevant metrics for risk assessment (see figure 6)..

Apart from the direct reading instruments, the collection of air samples in adequate filter media is necessary to determine the chemical composition of the airborne ENPs. To this end, appropriate air sampling filter media must be selected depending on the type of ENM and desired analytical information. In this sense, traditional open-faced cassettes (37 mm) are used.

A thorough description of the most relevant approaches accepted to perform a reliable quantitative assessment of the levels of exposure is provided within the Guidance on Exposure Characterization for airborne ENMs.

In the case of environmental concentrations, despite major research efforts, little is known about the concentration, fate, and toxicity of ENMs when released into the environment. These knowledge gaps are partly due to the lack of techniques available for collecting, preserving, and storing samples containing ENMs, as well as to scarce understanding of nanomaterial properties and their behaviour in the environment.

So far, environmental scientists have not been able to fill these gaps for a number of reasons, including the fact that ENMs consist of highly diverse types of substances, and their exposure concentrations are often very low in natural systems. Moreover, suitable analytical methods are still under development.

A wide range of analytical tools are available, however, the most commonly used detection and characterisation techniques are not suitable for the study of ENMs. Nevertheless, recent studies have shown promising results when using field-flow fractionation coupled with analytical detection methods (e.g. FFF–ICP–MS and FFF–ICP–AES) 14 /16 for the detection of ENMs in liquids.

Few studies have been conducted in real natural systems, and possible existing forms and the proportion of ENMs in the environment remain unclear. For instance, there are no specific studies on the transport and fate of airborne ENMs in the atmosphere, although some options for read-across from studies on the transport of unintentionally produced or naturally occurring ultrafine particles are expected. The transport behaviour of ENMs in soil systems is essential to reveal the impact of ENMs, yet to date, there are different understandings of the transport of ENMs in porous media.

### 3.5. Risk Assessment methodologies: methods and approaches to characterize the risk within REACH

According to REACH regulation, risk assessment will enable industrial partners to establish the conditions of manufacture and use which are needed to control risks to workers and environment throughout the life cycle of the ENM. The conditions of safe use will be then communicated through the supply chain via the exposure scenarios and attached to the extended safety data sheet.

Risk assessment is based on a structured methodology which can identify the hazards and exposure in a given condition of use of the nanomaterials that can generate the risk. Several approaches exist in the identification of the risks based on the information and data available (hazards and exposure) for carrying out the risk assessment. An overview of the different types of risk assessment is presented in Figure 7.

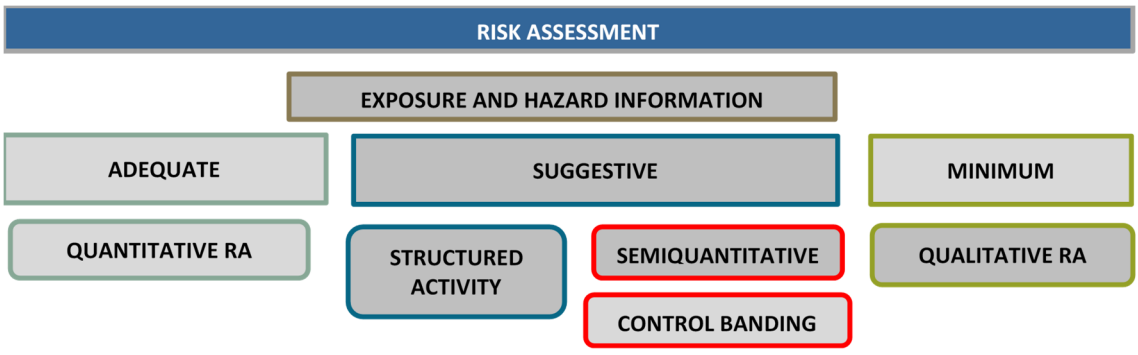



Figure 7. Overview of the risk assessment methods depending on the availability of information.

According to the figure above, in cases when very limited hazard or exposure data is available and the No-Effect levels cannot be established for certain effects (DNEL/PNEC), a quantitative risk assessment is not feasible and other qualitative methodologies such as control banding (CB) may apply.

A qualitative assessment differs from a quantitative assessment in that you cannot quantify the risk in the form of an RCR. Therefore, you must provide solid and consistent justification to support the conclusion that the operational conditions and risk management measures described in the exposure scenario are sufficient to avoid the likelihood of adverse effects. This often applies to irritants/corrosives, sensitisers, carcinogens, mutagens and reproductive toxicants.

There is no standardized methodology for performing a risk assessment to assess the potential risk associated with ENMs. The following sections describe some common methodologies used so far indicating their appropriateness. However, regardless of the methodology applied, the conclusion of the risk characterization must be justified.

### 3.5.1. Occupational Exposure estimation with modelling approaches

Occupational exposure to nanomaterials receives the most attention in the peer-reviewed literature, as the exposure during the synthesis of nanomaterials and manufacture of nano-enabled products would potentially be the highest one.

According to the European Chemicals Agency (ECHA) Guidance on requirements on information and chemical safety assessment, exposure of substances in the workplace refers to external exposure. Substances in the workplace may come into contact with the body and possibly enter into it by inhalation, by contacting and passing through the skin (dermal route) or swallowing (ingestion). The exposure can be defined as the amount of the substance ingested, the amount in contact with the skin and/or the amount inhaled (represented by the concentration of the substance in the breathing zone of a worker).

Most of the toxicity studies pertaining to nanoparticles focused on the respiratory tract exposures with few studies describing the gastrointestinal tract. The gastrointestinal tract exposures usually occur either unintentional from hand to mouth transfer of the ENM or materials that contain ENM. Considering occupational exposure, ingestion or oral exposure may occur in situations where there is exposure to aerosols and where contaminated skin or clothing may lead to exposure due to contact with the mouth region.

It could be minimized if good hygiene practices are followed (segregating working and eating facilities and adequate washing prior to eating, etc.). This route is generally considered less important, commonly arising from accidents or bad practices. Therefore, exposure by ingestion exposure is not usually considered and the assessment of workplace exposure is generally focused on inhalation and dermal exposure considered to be the main routes of exposure.

When no measured data is obtained according to section 3.4.2, modelling approaches are proposed in order to estimate the exposure and complete a general overview of the risk characterization along all the relevant life cycle stages. However, the determinants of exposure selected should be relevant for the considered route of exposure.

In the following section the Tier 1 tool ECETOC TRA is described while in section 3.5.2 higher level assessment tools are presented. However, it is worth to note that exposure prediction results based on these tools described in this guidance cannot be considered as “validated” and it is recommended to compare the results with measured data or even using more than one model in order to reduce the uncertainty associated in risk characterization.

#### i) ECETOC TRA tool for occupational exposure

ECETOC Targeted Risk Assessment is a first Tier tool that allows the user to perform worker and consumer assessment estimating inhalation and dermal exposures. ECETOC TRA is also provided in an integrated version that allows performing environmental assessment and it can be downloaded free of charge from *http://www.ecetoc.org/tra*.

Table 4. Strengths and limitations of the ECETOC TRA.

| STRENGTHS | LIMITATIONS |
|---|---|
| - Clear and user friendly structure; easy to understand and use | - The amount of product used is not taken into account |
| - Handling categories are directly linked to use descriptors (PROCs) | - Limited options for operational conditions and risk management measures |
| - Estimation of inhalation and dermal exposures | - The percentage of a substance in a mixture is not taken into account |
| - Duration of process is taken into account | - Personal protective equipment for dermal exposure is not taken into account |
| - Exposure scenarios are based on Estimation and Assessment of Substance Exposure model and experts | - The level of reduction using respiratory protective equipment are not specified in ECETOC TRA |
| - Risk Management Measures are taken into account in inhalation exposure | - Risk Management measures effects may sometimes be overestimated by ECETOC TRA, underestimating the dermal exposure compared to measured data |
| - Assessments can be saved for later use or modification | - Not applicable for non-minerals solids used at elevated temperature |

The basis of ECETOC TRA is a modified version of the EASE (Estimation and Assessment of Substance Exposure) exposure model version 2.0 developed by the UK Health and Safety Executive (HSE 2003). Strengths and limitations of the tool are summarized in Table 4.

The parameters needed as input data for the quantification of exposure within the ECETOC TRA for workers are:

- Molecular weight
- Physical state of the substance
- Vapour pressure (liquids or gases) or dustiness (solids)
- Process Category (PROC)
- Industrial or professional activity
- Indoor or outdoor activity
- Presence of Local Exhaust Ventilation
- Duration of the activity
- Respiratory protective equipment
- Concentration of the substance in the mixture

The user may develop the exposure assessment by correctly modifying input parameters that provides options for iteration. The parameters that are most likely to be easiest to implement and modify in a workplace are:

- Operational conditions:
  - Duration of the activity
  - Percentage of the substance used
- Risk Management measures:
  - Presence of Local Exhaust Ventilation
  - Use of Personal Protective Equipment

As overall conclusion, it can be stressed that basic concept of the ECETOC TRA model is adequate however scaling and calibration for nanoparticles exposure scenarios are needed. Moreover, exposures metric (mass concentration) is probably not appropriate hindering their application to ENMs as a main factor.

### 3.5.2. Higher Tier exposure assessment

When according to the Tier 1 assessment the level of protection is not adequate, a Tier 2 assessment is necessary. Higher Tier assessments are meant to be carried out by experience assessors who have more detailed information on the exposure scenario and on the specifications of the model to be able to carry out the assessment successfully. Several new approaches are under development by industry, two of these approaches most used so far are: Stoffenmanager exposure model and the Advanced REACH Tool (ART) for occupational exposure assessment.

#### ii) Stoffenmanager nano: a web-based tool for risk prioritization of ENMs

Control banding is a generic risk assessment methodology applied in the management of risks at workplace. An example of a control banding approach is the Stoffenmanager nano tool based on a band of hazards and exposures that are relevant in occupational settings. This semi-quantitative approach is helpful particularly in situations where information on hazards and exposure levels are limited.

Stoffenmanager Nano is based on a published scientific conceptual model of exposure (Marquart 2007, Tielemans 2007a) and assesses the risks of nanomaterials in the workplace in a qualitative manner in order to priorize the different exposure scenarios. The results help in the assessment of risks associated with the use and handling of nanomaterials. The tool is freely available as a nano module within the web-based generic Stoffenmanager risk-banding tool (www.stoffenmanager.nl or directly via *http://nano.stoffenmanager.nl*).

According to ISO[3] definition for nanoparticles, primary particles exceeding the nanorange (defined as a size range between 1 and 100 nm in at least one dimension) are outside the applicability domain. Therefore, Stoffenmanager Nano applies to MNOs with a primary size between 1 and 100 nm (including agglomerates and aggregates that could retain nano-specific properties) and/or to products with a specific surface area of $\geq (1/\rho)\ 60\ m^2\ g^{-1}$. The Stoffenmanager tool needs more information than Tier 1 models, but it is more flexible and the results are more accurate and probably less conservative. Strengths and limitations of the tool are summarized in Table 5.

Table 5. Strengths and limitations of the Stoffenmanager nano tool.

| STRENGTHS | LIMITATIONS | RECOMMENDATIONS |
|---|---|---|
| - Clear and user friendly structure; easy to understand and use | - Gases, fibres, solid objects (articles in REACH) other than wood or stone and "hot work techniques" like welding or waste burning cannot be used to assess exposure | - The most conservative option of the dustiness is recommended to be used in case of doubt |
| - Based on handling categories that are required in the title of exposure scenarios under REACH | - Handling categories are not directly linked to use descriptors (PROCs). However, PROCs can be transposed to Stoffenmanager handling categories. | - Run the model with several combinations of input parameters and select the most conservative option from the handling categories that are possibly relevant. |
| - Several choices for operational conditions and Risk Management measures compared to simpler models | - Dustiness category is not always obvious. | |
| - The output is based on statistical analyses of the relation between deterministic scores and around 1000 real exposure measurements | - No direct quantitative influence of parameters such as use rate or ventilation rate | |
| - Assessments can be saved for later use or modification | - Changes in the calibration in the tool over time are not visible to the user | |

[3] International Organization for Standardization Nanotechnologies, 2008b.

| STRENGTHS | LIMITATIONS | RECOMMENDATIONS |
|---|---|---|
| - Several control strategies with different Risk Management Measures can be selected and the effect of these strategies on the exposure estimate can be calculated | - Some parameters used to determine exposure are difficult to apply in the context of REACH, for example room volumes. | |

According to a general framework, Stoffenmanager Nano tool consists of a hazard and exposure bands combined in a risk matrix. Both issues are discussed below.

***Hazard banding approach***

Unlike the generic Stoffenmanager, alternative physicochemical parameters (such as particle diameter and length, morphology, (water) solubility, agglomeration, bioavailability, (surface) reactivity, etc) are needed as input for the hazard banding approach for Stoffenmanger Nano due to toxicological data of MNOs are generally lacking. However, as this information is not very accessible, a more pragmatic stepwise approach is applied according to Figure 8.
High water solubility is generally considered as low priority since nano-specific properties are expected to be lost when particles are in solution. This does not mean that these MNOs cannot be toxic, however not specifically related to their nano-size.

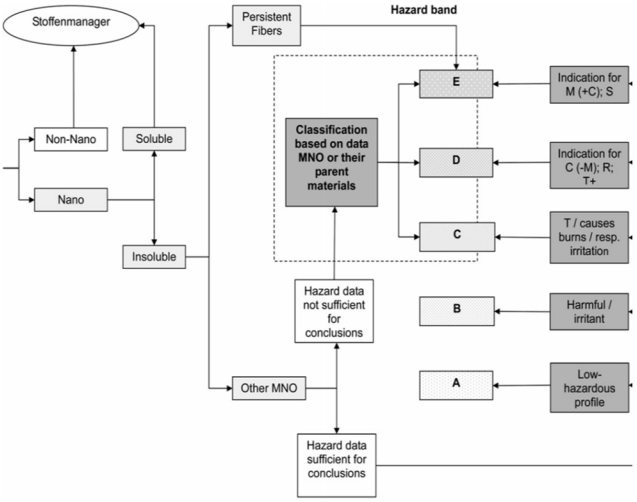



Figure 8. Schematic illustration of the stepwise approach for hazard banding (B. Van Duuren-Stuurman et al).

In case where water solubility is not known, the MNO is considered non-soluble. The next step in the hazard banding is the distinction of persistent nanofibers which are classified in the highest hazard category (E) due to the uncertainty associated to the relevant threshold and the severity of the potential health effect. MNOs are treated as nanofibers only when there is an indication for fiber-like properties either in the size range or nomenclature, otherwise MNOs will be considered as non-fibers when no information is available.In most cases, where toxicological data are not available for a hazard assessment, hazard banding in Stoffenmanager nano is either based on the limited data available on the MNO or on the hazardous potential of its parental material.

A list of the most widely used MNOs has been published by Rijksinstituut voor Volksgezondheid en Milieu (2010) and assigned to relatively high hazard bands (C through E), according to the Table 6.

Some MNOs for which specific concern has been raised, have been assigned to the highest hazard band (E). Other MNOs on the list widely used are assigned to a hazard band based on the hazard of the parental material. For MNOs, partly characterized, hazard band D or C is proposed dependent on the particle size. Hazard band C applies when the primary particle diameter is > 50 nm and hazard band D when the primary particle diameter is ≤ 50 nm. The attribution to either C or D is rather conservative compared to that applied for the generic Stoffenmanager.

MNOs are assigned to hazard band E when their parental material is classified for carcinogenicity, mutagenicity, reproduction toxicity, sensitization or the parental material is not specified based on a precautionary principle. In all other cases, hazard band D is applied.

Table 6. Classification of MNOs in hazard bands based on insufficient toxicological data.

| Type of MNO | Hazard band | Based on |
|---|---|---|
| C60 (fullerenes) | D | Particle-specific data |
| Carbon black | >50 nm: C; ≤50 nm: D | Parent material and (limited) particle-specific data |
| Ag (nano silver) | >50 nm: C; ≤50 nm: D | Parent material and (limited) particle-specific data |
| Fe (iron) | >50 nm: C; ≤50 nm: D | Parent material and (limited) particle-specific data |
| Au (gold) | >50 nm: C; ≤50 nm: D | Parent material and (limited) particle-specific data |
| Pb (lead) | E | EPA Carc. B2; probable human carcinogen |
| La (lanthanide) | >50 nm: C; ≤50 nm: D | Parent material and (limited) particle-specific data |
| TiN (titanium nitride) | >50 nm: C; ≤50 nm: D | Parent material and (limited) particle-specific data |
| $TiO_2$ (titanium dioxide) | >50 nm: C; ≤50 nm: D | Parent material and (limited) particle-specific data |
| $CeO_2$ (cerium oxide) | >50 nm: C; ≤50 nm: D | Parent material and (limited) particle-specific data |
| ZnO (zinc oxide) | >50 nm: C; ≤50 nm: D | Parent material and (limited) particle-specific data |
| $SiO_2$ (silica or silicon dioxide) | Unknown: E; crystalline/quartz: E; amorph, <50 nm: D; amorph, >50 nm: C | Particle-specific data; crystalline silica/quartz has been associated with carcinogenicity (IARC) |
| $Al_2O_3$ (aluminum oxide) | >50 nm: C; ≤50 nm: D | Parent material and (limited) particle-specific data |
| FeO (iron oxides) | >50 nm: C; ≤50 nm: D | Parent material and (limited) particle-specific data |
| $Sb_2O_5$ (antimony oxide) | E | Parent material classified as Carc Cat 3; R40 |
| $SnO_2$ (tin oxide) | >50 nm: C; ≤50 nm: D | Parent material and (limited) particle-specific data |
| CoO (cobalt oxide) | E | Parent material labeled R43 |
| Nanoclay | >50 nm: C; ≤50 nm: D | Parent material and (limited) particle-specific data |
| Nano-polystyrene | >30 nm: C; ≤50 nm: D | Parent material and (limited) particle-specific data |
| Other MNOs | MNOs containing several parent materials: most critical hazard band; parent material unknown: E; parent material classified for C, M, R, or S: E; not classified for C, M, R, or S: D | |

EPA = Environmental protection agency; Carc. = carcinogen; IARC = International Agency for Research on Cancer.

### *Exposure banding approach*

This model describes a stepwise transfer of an MNO from the source via various transmission compartments to the worker. Nine modifying factors are incorporated in the model related to source emission, transmission and immission. The modifying factors are as follows according to the Figure 9.

The Stoffenmanager nano exposure model is used to categorize scenarios (see Equation 1) in relative exposure bands which are derived by multiplication of relative multipliers (on a logarithmic scale) for the various modifying factors using the same exposure algorithm as used for the generic Stoffenmanager (Marquart et al., 2008).

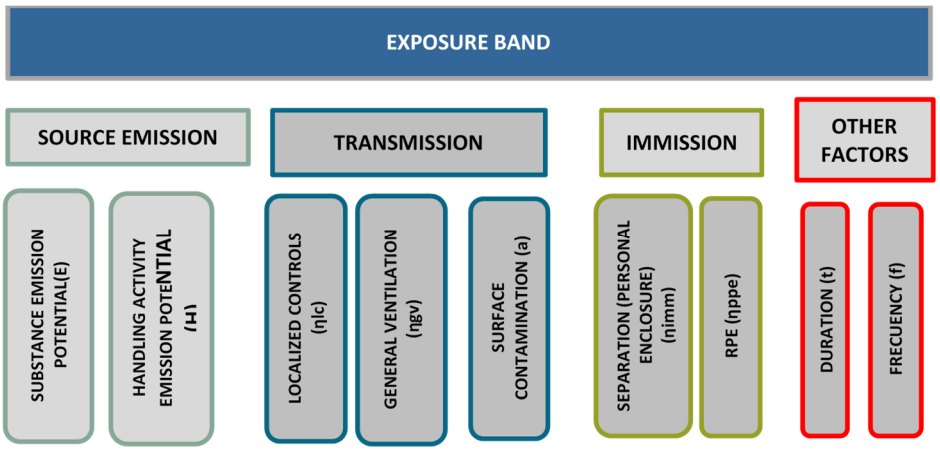



Figure 9. Exposure determinants involved in calculating exposure band.

Equation 1. Exposure algorithm used to categorize scenarios in relative exposure bands.

$$B = \left[\left(C_{\mathrm{nf}}\right) + \left(C_{\mathrm{ff}}\right) + \left(C_{\mathrm{ds}}\right)\right] \cdot \eta_{\mathrm{imm}} \cdot \eta_{\mathrm{ppe}} \cdot t_{\mathrm{h}} \cdot f_{\mathrm{h}}$$

$$C_{\mathrm{nf}} = E \cdot H \cdot \eta_{\mathrm{lc\_nf}} \cdot \eta_{\mathrm{gv\_nf}},$$

$$C_{\mathrm{ff}} = E \cdot H \cdot \eta_{\mathrm{lc\_ff}} \cdot \eta_{\mathrm{gv\_ff}},$$

$$C_{\mathrm{ds}} = E \cdot a,$$

The algorithm distinguishes near-field emissions (1m of the worker) from far-field emissions because a source of emission that is relatively far from the worker has a lower influence on the worker´s personal exposure than a source very close to the worker. The parameters needed as input data for the quantification of exposure within the Stoffenmanager are:

- Physical state of the substance
- Activities involving articles (solid objects) that may cause emission of dust
- Vapour pressure (liquids) or dustiness (solid articles, firm granules or flakes, granules or flakes, coarse dust, fine dust, extremely dusty products)
- Quantity of the substance used, frequency and duration
- Type of dust emitted from solid objects (currently only stone or wood)
- Percentage of the substance in the product
- Level of dilution (liquid products) (undiluted equal to 100%)
- Handling category
- Local controls (including local exhaust ventilation (LEV) and containment)
- Distance of the worker from the source (near-field or far-field)
- Presence of secondary emission sources:
- Workers using the same substance simultaneously
- A period of drying or hardening after the activity (with prolonged emission of vapours)
- Room volume
- General ventilation
- Emission control measures
- Personal protective equipment
- Work area is regularly cleaned and the machinery and equipment inspected and kept in good order.

Once the exposure-related questions have been answered by the user for each of the modifying factors described above, the final qualitative exposure score is assigned to an exposure band (1-4) using the described exposure algorithm.

Table 7. Stoffenmanager nano exposure bands (table adapted from marquart et al., 2008)

| EXPOSURE BAND | RANGE STOFFENMANAGER NANO SCORES |
|---|---|
| 1 | **< 0.002** |
| 2 | **0.002 – 0.2** |
| 3 | **0.2 - 20** |
| 4 | **> 20** |

Finally, the results from the hazard and exposure banding are combined in a risk matrix which gives the risk priority band (1: high priority risk, 2: medium priority risk and 3 low priority) according to the figure below. With increasing concern for hazard and/or exposure, the risk band will also increase priority (see Table 7 and Figure 10).

| Hazard band / Exposure band | A | B | C | D | E |
|---|---|---|---|---|---|
| **1** | 3 | 3 | 3 | 2 | 1 |
| **2** | 3 | 3 | 2 | 2 | 1 |
| **3** | 3 | 2 | 2 | 1 | 1 |
| **4** | 2 | 1 | 1 | 1 | 1 |

Figure 10. Priority bands in the stoffenmanager. hazard: a= lowest hazard and e=highest hazard, exposure: 1= lowest exposure and 4=highest exposure (adapted from marquart et al., 2008).

As a final conclusion, high hazardous substances, such as persistent fibers, or MNOs with an unknown hazardous profile are given a high priority risk regardless of the exposure band based on a conservative approach. In the same way and in view of the overall uncertainty associated, high exposure bands will automatically lead to high priority risk for all hazard categories except category A.

### iii) Advanced REACH Tool (ART) for occupational exposure assessment

The ART tool incorporates both a mechanistic model and an empirical part with information from an exposure database. Both parts are combined using a Bayesian statistical process in order to produce a variety of realistic and reasonable worst-case exposure estimates for specific scenarios.

The model consists of determinants which describe exposure scenarios on the substance, activity and further contextual level (such as room size, Local Exhaust Ventilation, etc.). It takes into account several operational conditions and risk management measures throughout the whole exposure pathway from source to worker.

Strenghts and limitations of the tool are summarized in the table below.

Table 8. Strengths and limitations of the art tool.

| STRENGTHS | LIMITATIONS | WAYS TO COMPENSATE FOR LIMITATIONS |
|---|---|---|
| - Suitable for liquids and solids that are used in processes or formed during processes such as fracturing of solid objects, abrasive blasting, impaction on and handling of contaminated objects. | - Not suitable for substances formed through reaction processes (e.g. exhaust fumes, rubber fumes) or gases or fibres | - Data gaps for inputs could be set by joint registrants depending on the industry sector or substance category |
| - Several choices for operational conditions and Risk Management measures | - More information requirements are needed compared to Tier 1 models | - Full shift exposure levels for short term activities can be calculated |
| - Calibration with extensive measured data | - ART cannot estimate dermal exposures | |
| - Choice of several percentiles of the resulting exposure distribution | - Changes in the calibration in the tool over time are not visible to the user | |
| - The mechanistic model results are combined with measured data in a Bayesian statistical process | - Difficulty converting the determinants driving the exposure estimates into operational conditions and risk management measures | |
| - Indication of the uncertainty of the mechanistic model result | | |

The parameters needed as input data for the quantification of exposure within the ART tool are:

- Duration
- Type of material used (powdered, granular or pelletised material; solid objects, liquids)
- For powdered, granular or pelletised material:
  - Dustiness
  - Moisture content
- For solid objects:
  - Material of the solid object
  - Moisture content
- For liquids:
  - Temperature
  - Vapour pressure
  - Boiling point
  - Viscosity
  - Activity coefficient
- For all materials: weight fraction

- Primary near-field emission source. If yes, secondary far-field sources also need to be assessed

- Activity class (subclasses are sometimes also defined)
- Spray direction
- Drop height (e.g in transfer)
- Scale of the activity: use rate or surface area

- The following information on RMM for primary sources (near-field and far-field):

- Supression techniques
- Containment without extraction

- Local exhaust ventilation
- Measures to limit surface contamination and fugitive emissions: enclosure of process, effective housekeeping, general housekeeping
- Conditions and measures of dispersion:
    - For indoors: room size and ventilation rate
    - For outdoors: placement of source relative to buildings and of workers relative to source

- The following information on RMM for only primary far-field sources:

- Emission source segregated from the worker (also for secondary far-field sources)
- Worker separated from the emission source by a personal enclosure

As output data, ART provides the following results:

• **Full-Shift exposure** (recommended for REACH): ART calculates an overall distribution for full-shift exposures. In this case, the 90$^{th}$ percentile provides the exposure level, which has a 10% probability of being exceeded by the exposure of a randomly selected worker on a randomly selected day.

• **Long-Term average exposure**: ART calculates the distribution of workers' long-term average (mean) exposure (e.g. over a period of months). In this case, the 90$^{th}$ percentile provides the long-term mean exposure level, which has a 10% probability of being exceeded by the long-term exposure of a randomly selected worker.

As a general conclusion, it can be stressed that due to exposure and hazard assessment are an essential part in the risk assessment analysis, it is recommended that exposure assessment data would be refined in order to obtain more reliable values for risk characterization. Moreover, the refinement of the hazard assessment is also recommended to be carried out in order to derive a higher safe limit value (DNELs and PNECs).

The worst case scenario assuming a conservative approach based on a precautionary principle maybe likely to overestimate the risks in the workplace. This may be due to the following factors:

✓ Hazard: The most conservative DNELs/PNECs values may be used
✓ Less data, more uncertainty associated
✓ NPs Exposure metric: High uncertainty when expressing number of particles measurements in mass units (mg/m$^3$) in order to compare with safe limit values.

Main limitations and uncertainties detected are due to:

- Uncertainties arising from the spatial variation
- Fluctuations in the levels of background which could not be monitored
- NPs Exposure metrics: High uncertainty when expressing number of particles measurements in mass units
- Differences in measurement techniques employed by each instrument
- Own software limitations
- ECHA guidelines on Characterisation of dose [concentration] – response for human health and environment, not specifically adapted for nanomaterials
- Hazard: very low (conservative) DNELs and PNECs

### 3.5.3. Environmental risk assessment methodology

The estimation of risk according to the ratio PEC/PNEC is an established method in the process to authorize new chemical substances in the EU described in details in the Technical Guidance Document on Risk Assessment (ECB, 2003). The assessment is based on representative measured data and/or model calculations. The exposure is measured by the PEC whereas the PNEC estimates the expected toxicity. In principle, human beings as well as ecosystems in the aquatic, terrestrial and air compartment are to be protected (ECB, 2003). Additionally the Technical Guidance Document on Risk Assessment (ECB, 2003) states: "If it is not possible to conduct a quantitative risk assessment, either because the PEC or the PNEC or both cannot be derived, a qualitative evaluation is carried out of the risk that an adverse effect may occur".

# 4. Best practice recommendations for risk assessment of nanomaterials

The present guideline contains detailed information to support the risk assessment of ENMs in the context of REACH, covering the whole product value chain.

Provided below is a summary of some of the key recommendations. These are by no means exhaustive and should be considered in the context of the further information outlined in compendium of guidelines developed in the framework of the LIFE REACHnano project.

According to the outcomes of the LIFE REACHnano project and the current EU legislative framework, the following general actions are recommended:

- Gather information on the specific physicochemical, toxicological and ecotoxicological properties of the ENMs produced and/or used. This requires the collection and evaluation of all available and relevant information that may support the identification of hazardous properties of the ENMs.

- Identify sources of release and evaluate the likelihood of exposure in the workplace on the basis of the specific operative conditions of the company. Aspects such as the duration (min/h) of the task, frequency (days - weeks) and amount of material handled (ng or mg), among others, should be adequately defined;

- Compare the levels of exposure estimated/measured with the threshold levels for each effect. Risks are regarded as controlled under REACH when the exposure levels to the substance are below the threshold levels considered as safe, both for humans and for the environment. For effects with no threshold levels, emissions and exposures have to be minimised or avoided for risks to be considered to be controlled.

In the context of REACH regulation, if risks are under control, the CSA ends here. If risks are not under control, the CSA has to be refined, either by obtaining more data on the properties of the substance, changing the conditions of manufacturing or use, or making more precise exposure estimations. The process is iterative and will continue until the risks are shown to be under control. The conditions of manufacturing and use under which the risks are under control constitute what is called the final exposure scenario.

# 5. References

### List of Figures


- Figure 1. Risk Assessment framework under REACH
- Figure 2. Hazard Assessment approach established by REACH
- Figure 3. Exposure Assessment approach established by REACH
- Figure 4. Conceptual representation of the steps to develop QSAR models
- Figure 5. MFA environmental model example
- Figure 6. Exposure assessment using direct-reading instruments
- Figure 7. Overview of the risk assessment methods depending on the availability of information
- Figure 8. Schematic illustration of the stepwise approach for hazard banding (B. Van Duuren-Stuurman et al)
- Figure 9. Exposure determinants involved in calculating exposure band
- Figure 10. Priority bands in the stoffenmanager. hazard: a= lowest hazard and e=highest hazard, exposure: 1= lowest exposure and 4=highest exposure (adapted from marquart et al., 2008).

## List of tables


- Table 1. Toxicological endpoints and in vitro test available for risk assessment under REACH
- Table 2. Ecotoxicological relevant endpoints and in vitro test available for risk assessment under REACH
- Table 3. Main modeling approached available
- Table 4. Strenghts and limitations of the ecetoc tra
- Table 5. Strenghts and limitations of the Stoffenmanager nano tool
- Table 6. Classification of MNOs in hazard bands based on insufficient toxicological data
- Table 7. Stoffenmanager nano exposure bands (table adapted from marquart et al., 2008)
- Table 8. Strenghts and limitations of the art tool


## Equations


Equation 2. Exposure algorithm used to categorize scenarios in relative exposure bands

## Further Information


Packaging, Transport and logistics research center

Contact: Carlos Fito

email: *cfito@itene.com*

Location: SPAIN  Website URL: http://www.itene.com

LEITAT Technological Centre

Contact: Natalia Fuentes

email: *nfuentes@leitat.org*

Location: SPAIN  Website URL: http://www.leitat.org

Nanotechnology Industries Association

Contact: David Carlander

email: *david.carlander@nanotechia.org*

Location: BELGIUM  Website URL: http://nanotechia.org/

INVASSAT. Instituto valenciano de seguridad y salud en el trabajo

Contact: Esteban Santamaría

email: *santamaria_est@gva.es*

Location: SPAIN  Website URL: http://www.invassat.gva.es/


---

*http://www.lifereachnano.eu - http://tools.lifereachnano.eu*


Contents Manager
ITENE - Packaging, Transport and logistics research center
Albert Einstein, 1. 46980 Paterna. Valencia (Spain)
E-mail: *cfito@itene.com*



**REACHnano is partly funded by the European Commission Life+ with grant agreement LIFE11 ENV/ES/549**