

anses

French agency for food, environmental  
and occupational health & safety



# Assessment of the risks associated with nanomaterials

## Issues and update of current knowledge

Opinion of Anses  
Collective Expert Appraisal Report

April 2014

Scientific Edition



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The Director General

Maisons-Alfort, 15 April 2014

## **OPINION** **of the French Agency for Food, Environmental** **and Occupational Health & Safety**

### **on the assessment of risks associated with nanomaterials -** **issues and update of current knowledge**

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*ANSES undertakes independent and pluralistic scientific expert assessments.*

*ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.*

*It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.*

*It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).*

*Its opinions are made public. This Opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 15 April 2014 shall prevail*

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ANSES issued an internal request on 11 January 2012 in order to update current knowledge and review the "key" issues relating to the assessment of risks associated with nanomaterials to human health and the environment. This internal request followed several reports published by ANSES in 2006, 2008, 2010 and 2013 on the assessment of health risks and health issues associated with nanomaterials.

#### **1. BACKGROUND AND PURPOSE OF THE REQUEST**

The health and environmental implications related to the development and potential uses of manufactured nanomaterials are a significant social issue, both in France and at the international level. For this reason, on the occasion of the Fifth Ministerial Conference on Environment and Health organised by the World Health Organization's Regional Office for Europe, 53 health ministers from the member countries asked for the health and environmental issues related to nanomaterials and nanotechnologies to be listed as one of the key challenges in the 2010 Parma Declaration on Environment and Health. In this context, work on the development of new risk assessment methodologies, especially for people in the workplace, or on defining health and environmental safety tests, was undertaken by various institutions including the International Organisation for Standardization (ISO), the Organisation for Economic Cooperation and Development (OECD) and the European Commission. In France, this concern is mainly expressed by:

- the national "environmental health" and "occupational health" plans that have emphasised the need to conduct research and expert appraisal work to characterise potential hazards, exposures and risks to human health and the environment,
- the entry into force of the mandatory reporting of uses of substances with nanoparticle status as well as annual amounts produced, imported and distributed in France, in

accordance with Articles L. 523-1 to L. 523-8 of the French Environmental Code (“Grenelle II” Act of 12 July 2010).

In this context, ANSES has published several expert reports on the health issues associated with exposure to nanomaterials, for the general and working populations (in 2006, 2008 and 2010). These reports in particular highlighted the difficulties of assessing the risks associated with such exposure, and described the need for more knowledge and new tools in order to characterise the hazards and exposures to nanomaterials owing to their specific properties. More recently, the Agency published a report on a tool for assessing and managing the risks associated with occupational exposure to nanomaterials<sup>1</sup> (2010), as well as a 'state of the art' review of knowledge of the toxicity and ecotoxicity of carbon nanotubes (2012), and an assessment of the risks associated with an industrial development programme for carbon nanotubes (2013). Finally, since 2013, the Agency has been managing the scheme for mandatory reporting of nanoparticle substances for the Ministry of Ecology.

ANSES also contributes to various European and international projects on assessing the toxicity and ecotoxicity of nanomaterials. For example, the Agency coordinated the European joint action Nanogenotox, co-funded by the European Commission, whose results, published in 2013, highlighted the need to adapt OECD guidelines on genotoxicity testing of chemicals to the specific characteristics of nanomaterials.

Given the rapid advances in knowledge on this issue, the Agency issued an internal request in January 2012 to produce an updated summary of current knowledge and health and environmental issues related to exposure to manufactured nanomaterials.

## **2. ORGANISATION OF THE EXPERT APPRAISAL**

The expert appraisal was carried out in accordance with French standard NF X 50-110 “Quality in Expert Appraisals – General requirements of Competence for Expert Appraisals (May 2003)”.

Following a public call for applications, the Working Group (WG) on "Nanomaterials and health - food, environment, work" was set up in May 2012 and began working on 9 July 2012. The experts were recruited for their scientific and technical expertise in the fields of nanomaterial characterisation, toxicology, ecotoxicology, risk assessment and prevention, the history of science, philosophy, economics and regulation, especially of new technologies.

This collective expert appraisal fell within the area of expertise of the Expert Committee (CES) on "Assessment of the risks related to physical agents, new technologies and development areas". The methodological and scientific aspects of the WG's work were submitted to the CES for discussion on 26 October 2012, and 31 January, 24 September and 7 November 2013. The working group's report was endorsed by the CES on 17 December 2013.

ANSES analyses the vested interests declared by the experts before they are appointed and throughout their work, in order to prevent risks of conflicts of interest with respect to the points addressed in the expert appraisals. The experts' declarations of interests are made public on ANSES's website ([www.anses.fr](http://www.anses.fr)).

## **3. ANALYSIS AND CONCLUSIONS OF THE CES**

The Expert Committee (CES) on "Assessment of the risks related to physical agents, new technologies and development areas" endorsed the conclusions of the collective expert appraisal work described in the report and in this Opinion at its meeting of 17 December 2013 and informed the ANSES General Directorate accordingly.

<sup>1</sup> Control banding” report: <http://www.anses.fr/sites/default/files/documents/AP2008sa0407Ra.pdf>

■ **Manufactured nanomaterials: substances found on the market whose risks must be assessed**

Manufactured nanomaterials are found in a wide range of everyday products that are already being marketed (such as sunscreen, textiles, food, paints, etc.) and concern a large number of industrial sectors such as construction, automotive, packaging, chemicals, environment, agri-food, energy, cosmetics and health products. The presence of nanomaterials in these products raises questions, as well as controversy, about the state of available knowledge, the potential effects of these materials on health and the environment, the exposure of the general and working populations and, ultimately, about the risks associated with these substances.

■ **The contribution of previous studies on natural substances or substances unintentionally produced at the nanoscale**

Manufactured nanomaterials have physico-chemical characteristics that are often very different to the nanoscale substances found in the natural environment or produced unintentionally by various industrial or domestic processes. But this does not mean that the knowledge produced in the field of unintentional nanomaterials should be ignored (for example, on the ultrafine particles from air pollution or forest fires). In order to better characterise the risks associated with manufactured nanomaterials, it might actually prove very useful to draw inspiration from the experimental methodologies developed, especially those for atmospheric particles (characterisation, experimental models, realistic dose levels, etc.) and to take advantage of the numerous studies (epidemiological and experimental) conducted on these particles, which have, in some respects, similar behaviour to manufactured nanomaterials. Specialists in air pollution and those in manufactured nanomaterials should therefore share their methodological approaches. This should apply at an international level as well.

■ **Difficulties encountered in assessing the risks specifically associated with manufactured nanomaterials**

It has proved difficult to summarise knowledge of the toxicology and ecotoxicology of nanomaterials, for the following reasons:

- the research conducted generally highlights the fact that each case is unique: the toxicity and ecotoxicity behaviour in fact depends on various essential physico-chemical parameters (solubility, zeta potential, aggregation/agglomeration, size, shape, etc.). The change in these materials throughout their life cycle (change in the degree of oxidation, whether or not associated with dissolution and precipitation in a mineral form different from the original one, homo- and hetero-aggregation, adsorption, etc.) is an additional source of complexity that should not be neglected *a priori*;
- the uneven quality of the large number of scientific studies in the field of nanomaterials. The literature on toxic or ecotoxic aspects of nanomaterials must therefore be rigorously analysed by integrating all the available data. Assessing the risks associated with nanomaterials therefore requires adopting a multidisciplinary approach, which is essential to achieving a better understanding of the risks;
- although there is now an institutional definition of nanomaterials, recommended by the European Commission<sup>2</sup>, its scientific content is still being debated.

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<sup>2</sup> Communication from the Commission to the European Parliament, the Council and the European Economic and Social Committee. Second Regulatory Review on Nanomaterials, 3.10.2012

### ■ Current methodological progress

The methodology for assessing the risks associated with nanomaterials has advanced, in particular with regard to:

- more comprehensive physico-chemical characterisations of the manufactured nanomaterials tested, namely:
  - more physico-chemical parameters are now being measured;
  - for a given parameter, several measurement methods are sometimes available;
  - *in-situ* characterisation corresponding to the *in-vitro* environment;
- the development or adaptation of toxicological and ecotoxicological tests using more realistic exposure doses (in addition to acute exposure tests, exploration of chronic exposure tests, adaptation of the concentrations tested, development of studies in terrestrial and aquatic mesocosms);
- attempts at harmonisation and standardisation in physico-chemical characterisation and toxicological and ecotoxicological tests;
- more numerous publications in ecotoxicology;
- better documentation of exposure conditions in scientific articles;
- standardisation work underway on measuring exposure.

### ■ Changes in risk management and control

The legal obligation to report substances with nanoparticle status came into force in France on 1 January 2013. Other countries such as Belgium<sup>3</sup>, Italy and Denmark have followed suit (each in their own particular way) and mandatory reporting is now being considered by other countries such as Germany and the United Kingdom. The aim of the French legislation is to learn more about nanomaterials marketed in France, as well as the volumes in question and the uses to which they are put, and to have some traceability in the sectors in which they are used. The information reported to define the identity of nanomaterials (physico-chemical characterisation) will most certainly evolve, given predictable changes in characterisation methods. However, the implementation of the reporting scheme will initially help gain insight into the production and importation of nanomaterials in France, with the aim of improving understanding of potential exposure of populations and the environment to these substances.

Publications on the means of prevention associated with occupational exposure to nanomaterials and guides to best practices at work have been distributed since 2008.

Lastly, tools for risk assessment and/or graduated management, such as “control banding”<sup>4</sup>, (ANSES 2010) have also been developed.

### ■ Developments in discussions between stakeholders

Debates such as the Nanoforum<sup>5</sup> (2007-2009) and the national public debate (2009-2010), should in addition be mentioned as a way of organising discussion on manufactured nanomaterials.

### ■ Reiterating the observation about the lack of knowledge of the risks associated with nanomaterials

Despite the advances mentioned above, the fact remains that knowledge of toxicity, ecotoxicity and exposure to nanomaterials remains fragmented and it is still very difficult to assess the health risk associated with the use of a particular nanomaterial in a particular everyday product.

Persistent uncertainty remains as to:

<sup>3</sup> Draft royal decree on the marketing of manufactured nanoparticle substances (November 2013)

<sup>4</sup> <http://www.anses.fr/sites/default/files/documents/AP2008sa0407Ra.pdf>

<sup>5</sup> Nanoforum, organised by the French Conservatory of Arts and Trades (CNAM): <http://securite-sanitaire.cnam.fr/nanoforum/>

- the physico-chemical properties of the manufactured nanomaterials studied and how they may change depending on the environment;
- the methods and techniques available - or not - to characterise these properties (in physico-chemical and (eco)toxicological terms); there are still no reference protocols despite attempts by several national and international research programmes;
- knowledge about the exposure of populations and their environment to manufactured nanomaterials;
- the biological and (eco)toxicological effects of the manufactured nanomaterials studied.

#### ■ Recommendations of the Expert Committee (CES)

Considering the points described above and the recommendations of the Working Group as described in its expert report, the CES has made the following recommendations.

##### Regarding research, the CES recommends:

- that specialists in air pollution and in manufactured nanomaterials work together, to share their definitions and measurement or characterisation methods;
- that public scientific organisations and manufacturers in this sector continue to develop innovative concepts and effective methods (for sampling and characterisation) to improve assessment of the risks associated with manufactured nanomaterials. An accurate description is needed of exposure of workers and consumers to nanomaterials, as well as exposure of the public in general and the environment;
- continuing work to harmonise analysis protocols, firstly to systematise the detailed characterisation (physico-chemical parameters) of nanomaterials whose effects are to be studied, and secondly to characterise the nanomaterials *in situ*, in order to be able to compare the (eco)toxicological studies on these nanomaterials with each other. It therefore seems essential for scientific organisations such as national metrology institutes and standardisation committees to focus their efforts in order to improve the metrological traceability of physico-chemical characterisations of nanomaterials. This can be achieved in particular by developing new nanoscale reference materials, whether or not these have been certified, and by establishing standardised and validated procedures based on consensus to ensure reliable estimation of any measurement uncertainties;
- continuing research in toxicology, in order to adapt existing models and/or develop and validate new models, tests or methods for assessing nanomaterial toxicity (cell models that are more representative of the target organs, development of new toxicity tests, new methods of simulating exposure, high-throughput systems for increasing the speed of investigation, etc.), while working with realistic controlled doses in an attempt to define a comprehensive strategy for assessing nanomaterial toxicity. The availability of nanomaterials that can be used as positive and negative controls is necessary to enable these models, tests or methods to be validated;
- consolidating knowledge from specific studies on the affinity of proteins for nanomaterials (the protein *corona*) in order to determine a "signature";
- continuing efforts to learn more about each step of the life cycle, especially with the development of work in mesocosms.

##### Regarding risk assessment:

Given the considerable number of nanomaterials it would probably be too difficult to implement a risk assessment on a case-by-case basis. Such a systematic analysis is not feasible for managing the current situation in the short to medium term, considering the time needed and the extensive use of laboratory animals it would imply.

In order to reduce the number of cases, categories of nanomaterials should be established, even though their relevance with regard to the data currently available for risk assessment is still being debated.

The CES thus underlines the need for further work to:

- group together nanomaterials in categories according to their effects. The work, extending beyond the industrial sector that initiated the approach, should be continued to this end, especially by public research stakeholders;
- develop and assess the relevance of new alternative approaches to risk assessment (safer by design, QNAR models, decision tree based on the stages of the life cycle, etc.).

Regarding regulations, the CES recommends:

- making available information collected under the mandatory reporting scheme, that is not covered by industrial and commercial secrecy or military secrecy in accordance with Article L. 521-7 of the French Environmental Code;
- describing the contribution of data collected through the mandatory reporting scheme regarding the knowledge about the exposure to and traceability of nanomaterials and, where necessary, amending regulations in order to improve the scheme's effectiveness;
- concerning the establishment of similar reporting procedures in different countries, harmonising tools and methods for collecting information, with a view to gathering the information in a common data base that could be accessible to all;
- lowering the thresholds for registration of nanomaterials in the REACH Regulation. As it stands, REACH is only very partially applicable to nanomaterials, mainly because of the high thresholds in the amount produced, as stipulated by the procedure. This does not preclude other forms of regulation being implemented to take account of the specific characteristics of nanomaterials;
- developing safety data sheets specific to nanomaterials that would accompany the substances involved throughout the life cycle of the products;
- launching a debate on the relevance of labelling products containing nanomaterials (shape, related information, etc.);

Regarding public dialogue, the CES recommends:

- with respect to the risk governance process for nanomaterials, working towards transparency and greater participation by the groups concerned (citizens' associations, social partners, health professionals, etc.), especially regarding the suggestions made above.

#### **4. AGENCY CONCLUSIONS AND RECOMMENDATIONS**

The French Agency for Food, Environmental and Occupational Health & Safety reiterates the conclusions and recommendations of the CES on "Assessment of the risks related to physical agents, new technologies and development areas" outlined above. Moreover, taking particular account of all the work overseen by the Agency on the topic of nanomaterials, as detailed in the introduction to this Opinion (state of the art and assessment of the risks associated with carbon nanotubes, European joint action Nanogenotox, Opinion on the modification of the Annexes of the REACH Regulation, etc.), it complements them as follows.

Since the late 1990s, manufactured nanomaterials have no longer been confined to the field of research and the laboratory, and have been used in many industrial applications. They are now found in a wide range of everyday products (cosmetics, food products, construction products,

textiles, health products, sporting and leisure goods, etc.). This availability on the market has been accompanied by concern about the state of knowledge regarding the assessment of risks associated with these substances, both for the general public and those who may be exposed in the workplace, and for the environment.

The research published to date has shown that each nanomaterial has specific physico-chemical characteristics that may depend on its environment. In fact, their toxicity and ecotoxicity vary, not only between categories of nanomaterials, but even within these categories, as well as over their life cycle depending on their environment. Researchers are still unable, at the present time, to refer to an unequivocal, intersectoral, regulatory definition of nanomaterials.

At the same time, there have been advances in knowledge, in terms of better physico-chemical characterisation of nanomaterials, and adaptation and harmonisation of certain (eco)toxicological and other tests. In terms of hazard characterisation, since the last review of knowledge published by the Agency in 2010, various scientific publications have observed effects of some nanomaterials on certain living organisms. These effects demonstrated in experimental models concern:

- the persistence of nanomaterials in living organisms (animals or plants);
- growth retardation, abnormalities or defects in development or reproduction in model species reflecting the environmental compartments;
- the crossing of certain physiological barriers (blood-placental, testicular, intestinal, skin, alveolar-capillary);
- the genotoxic and carcinogenic effects of some nanomaterials;
- effects on the central nervous system in animals;
- immunosuppression phenomena;
- hypersensitivity and allergic reactions.

The questions relating to the dissemination of manufactured nanomaterials are also accompanied by doubts about the adequacy and relevance of the regulatory framework in force.

Certain sectoral regulations (cosmetics, novel foods, biocides) have adopted non-harmonised definitions of nanomaterials. However, they all currently struggle to take into account the specific issues of identity and physico-chemical and (eco)toxicological characterisation of nanomaterials. Concerning the REACH Regulation, the tonnages associated with the chemical registration procedure are in addition less suited to the problem of nanomaterials.

The entry into force of the mandatory reporting of nanoparticle substances, in accordance with Articles L. 523-1 to L. 523-8 of the French Environmental Code ("*Grenelle II*" Act of 12 July 2010), led to a first review of knowledge of the identity, uses and quantities of nanomaterials produced, imported and distributed in France<sup>6</sup>. This highlighted the presence of large tonnages of nanomaterials on the French market.

In this context, and taking into account the updating of knowledge by the Working Group on "Nanomaterials and health" and the CES on "Physical Agents", as well as the issues concerning the assessment of risks associated with nanomaterials, ANSES is making the following recommendations:

### **Research recommendations**

Regarding knowledge of the hazards, the Agency recommends implementing multidisciplinary research projects to develop knowledge of the characteristics and hazards of nanomaterials, mainly to promote development of appropriate safety tests for assessing the health risks of

<sup>6</sup> [http://www.developpement-durable.gouv.fr/IMG/pdf/Rapport\\_public\\_format\\_final\\_20131125.pdf](http://www.developpement-durable.gouv.fr/IMG/pdf/Rapport_public_format_final_20131125.pdf).

products containing nanomaterials (effective physico-chemical characterisation, detailed and reproducible protocols, contribution from the human and social sciences, etc.).

Regarding the routes of exposure, ANSES stresses that the oral route, which until now has been largely unexplored, should be the focus of specific research efforts.

ANSES also considers that, given the scientific results already published on the routes of absorption and translocation inducing a potential systemic risk, a biokinetic assessment is essential. Particular attention should be paid to potential target organs that are rich in cells of the reticuloendothelial system (RES) (macrophages and cells from reticular hematopoietic organs that play an important role in phagocytosis) such as the liver, spleen, bone marrow, lungs, etc. Finally, knowledge of the particular behaviour of a nanomaterial in a whole organism would also enable *in vitro* tests to be conducted, to investigate effects or mechanisms of action of nanomaterials.

The Agency considers that these biokinetics studies should be accompanied by research on the intrinsic physico-chemical characterisation of nanomaterials, as well as their behaviour within the test environment (physiological medium, natural environment, organ, etc.).

Regarding exposure to low doses, ANSES calls for the continued development of toxicological tests conducted with low doses, especially in the case of chronic exposure studies.

Regarding the effects on the development of the nervous system, given the results of the review of neurotoxic effects on development and the crossing of physiological barriers, ANSES emphasises the need to conduct in-depth studies on the potential toxicity which might affect development of the nervous system and on the crossing of biological barriers.

Regarding the ecotoxicological effects, and despite the growing number of ecotoxicological studies published, the Agency recommends continuing research efforts especially on the transfer of nanomaterials to different parts of the food chain and on recycling products at the end of their life and then of waste.

These research recommendations should be taken into account especially in the context of the National Research Programme for Environmental and Occupational Health (PNR-EST).

In addition to this, given the existing gaps in knowledge, ANSES regrets that there are still only a small proportion of publications on the health effects of nanomaterials (less than 8%), as compared with the large number of publications on studies relating to their technological benefits. The Agency is therefore calling for the establishment of financial incentive mechanisms similar to those implemented in other areas (for example, electromagnetic fields) in order to address these deficiencies.

### **Recommendations on worker exposure to manufactured nanomaterials or products containing them**

ANSES recalls and reiterates the recommendations made previously (AFSSET, 2008), in particular the need:

- to declare manufactured nanomaterials as an "unknown level of hazard" and to handle them as cautiously as hazardous materials, i.e., by applying the safety procedures that are used to reduce exposure to hazardous materials. In this context, the Agency is calling for the use of nanomaterials in the workplace to be subject to risk assessments that specifically take into account the health issues related to these nanomaterials;
- to use the principles of "nano-safety": the STOP principle (Substitution, Technology, Organisation, Protection), notification of "nano-object" risks, archiving and traceability,

assessment of occupational exposures, atmospheric measurements, medical monitoring and training.

ANSES also recommends undertaking without delay feasibility studies on the regulatory classification of categories of manufactured nanomaterials for which there are sufficient data on their toxicological properties, for example under Regulation (EC) No. 1272/2008 known as the CLP Regulation<sup>7</sup>.

Accordingly, in 2006, the International Agency for Research on Cancer (IARC) classified titanium dioxide in the group of substances that are "possibly carcinogenic to humans" (2B), without specifying the nature of the titanium dioxide considered, nor its size, including its nanometric scale. The 'state of the art' literature review on the toxicity of carbon nanotubes published by the Agency in November 2012 identified a number of adverse effects to human health associated with exposure to these objects. An application for classification under the CLP Regulation should therefore be submitted for carbon nanotubes, as a first priority. Other nanomaterials such as silver, titanium dioxide, silica dioxide, zinc oxide, cerium oxide, aluminium oxide, gold, etc., are also sufficiently documented for their classification to be considered.

Finally, the Agency recalls the publication of qualitative risk assessment tools to provide guidance for controlling occupational risks (published by ANSES in 2010).

### **Recommendations on exposure to products containing manufactured nanomaterials**

Regarding the general public, the Agency is calling for increased traceability of consumer products containing nanomaterials, in order to better characterise individual exposure; this traceability cannot be achieved solely by the reporting scheme implemented at national level.

The Agency notes that the placing on the market of products likely to release nanomaterials that are toxic to the environment during their normal use or at the end of their life should be limited or regulated through life cycle analyses.

Regarding nano-products, once any hazards to human health or the environment have been identified, the Agency recommends weighing up the advantages and disadvantages to consumers or the community, and considering the appropriate conditions for the marketing of such products containing nanomaterials.

Furthermore, ANSES recalls its Opinion, published in April 2014, on the modification of the REACH Annexes with a view to taking nanoparticles into consideration. This Opinion relates to a draft regulatory text, submitted for public consultation by the European Commission and published on its website<sup>8</sup>.

Finally, the Agency underlines the existence of pragmatic, qualitative risk assessment tools developed to provide guidance for controlling risks to the general public (currently under development) and people who may be exposed in the workplace (published in 2010).

Marc Mortureux

<sup>7</sup> Classification, Labelling and Packaging of substances and mixtures.

<sup>8</sup> [http://ec.europa.eu/environment/consultations/nanomaterials\\_2013\\_en.htm](http://ec.europa.eu/environment/consultations/nanomaterials_2013_en.htm).

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# **Assessment of the risks associated with nanomaterials**

**Issues and update of current knowledge**

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**Internal Request No. 2012-SA-0273**

## **Collective Expert Appraisal REPORT**

**Expert Committee on**

**“Assessment of the risks related to physical agents, new technologies and  
development areas”**

**Working Group on**

**“Nanomaterials and health - food, environment, work”**

**April 2014**

## Key words

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Nanomaterials, risk assessment, exposure, hazard, ethics, society, regulation, research.

## Presentation of participants

**Foreword:** Outside experts, Expert Committee and WG members, or designated rapporteurs are all appointed in their personal capacity, *intuitu personae*, and do not represent their parent organisation.

### WORKING GROUP

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#### Chair

Mr Éric GAFFET – Director of the Jean Lamour Institute, Research Director at the UMR 7198 National Centre for Scientific Research (CNRS), University of Lorraine – Physical Chemistry, Characterisation

#### Vice-chair

Mr Fabrice NESSLANY – Head of Toxicology at the Institut Pasteur de Lille (IPL) – Toxicology, Genotoxicity

#### Members

Mr Jean-Yves BOTTERO – Research Director at the Centre for Research and Education in Environmental Geosciences (CEREGE) – Ecotoxicology

Mr Patrick CHASKIEL – Teacher-researcher at the University Paul Sabatier-Toulouse III – Economics, Communication Sciences

Mr Fernand DORIDOT – Teacher-researcher at the Catholic Institute of Arts and Trades (ICAM) in Lille – History and Philosophy of Science, Technology Governance

Ms Valérie FESSARD – Head of Toxicology of Contaminants Unit in the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) – *In vivo* toxicology

Mr Emmanuel FLAHAUT – Research Director at the National Centre for Scientific Research (CNRS) – Physical Chemistry, Ecotoxicology

Ms Stéphanie LACOUR – Research Fellow at the National Centre for Scientific Research (CNRS) – Regulation and Control of New Technologies

Mr Stéphane MALARD – Medical Advisor in Occupational Toxicology at the National Research and Safety Institute for the prevention of occupational accidents and diseases (INRS) – Occupational Medicine

Mr Charles MOTZKUS – Project Leader at the National Laboratory of Metrology and Testing (LNE) – Metrology, Physical Chemistry

Ms Catherine MOUNEYRAC – Director of the Institute of Biology and Applied Ecology, Catholic University of the West (UCO) – Ecotoxicology

Ms Myriam RICAUD – Expert at the National Research and Safety Institute for the prevention of occupational accidents and diseases (INRS) – Chemical Risk Prevention, Nanotechnologies

Mr Didier ROUXEL – Teacher-researcher at the University of Lorraine – Physical Chemistry, Surfaces and Interfaces

Mr Alan SANH – Assessor at the French National Agency for Medicines and Health Products Safety (ANSM) – Toxicology

Ms Anne VAN DER MEEREN – Researcher (CEA Arpajon) – Toxicology

## EXPERT COMMITTEE

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The work described in this report was monitored and adopted by the Expert Committee (CES) on "Assessment of the risks related to physical agents, new technologies and development areas".

### Chair

Martine HOURS – Physician-Epidemiologist, Research Director at the French Institute of Science and Technology for Transport, Development and Networks (Ifsttar).

### Members

Francine BÉHAR-COHEN – Ophthalmologist Practitioner, Research Director at the French National Institute for Health and Medical Research (Inserm).

Jean-Marc BERTHO – Researcher/Expert in Radiobiology at the Experimental Radiotoxicology Laboratory of the French Institute for Radiological Protection and Nuclear Safety (IRSN).

Jean-Pierre CÉSARINI – Retired (Director of the research laboratory on human skin tumours, A. de Rothschild Foundation and Inserm).

Frédéric COUTURIER – Engineer, Manager of the "Studies" department at the French National Frequency Agency (ANFR).

Jean-François DORÉ – Emeritus Research Director at the French National Institute for Health and Medical Research (Inserm).

Pierre DUCIMETIÈRE – Honorary Research Director at the French National Institute for Health and Medical Research (Inserm).

Aïcha EL KHATIB – Project Officer at the Paris Public Hospital System (AP-HP) – Avicenne Hospital.

Nicolas FELTIN – Project Manager at the National Laboratory of Metrology and Testing (LNE).

Emmanuel FLAHAUT – Research Director at the National Centre for Scientific Research (CNRS).

Eric GAFFET – Director of the Jean Lamour Institute, UMR 7198 CNRS – University of Lorraine.

Murielle LAFAYE – Engineer, Applications Coordinator at the French Space Agency (CNES).

Philippe LEPOUTRE – Acoustic Engineer, Manager of the Technical Unit of the Authority for Airport Nuisance Control (ACNUSA).

Christophe MARTINSONS – Doctor in Physics, Unit leader at the French Scientific and Technical Centre for Building (CSTB).

Catherine MOUNEYRAC – Director of the Institute of Biology and Applied Ecology and Professor of Aquatic Ecotoxicology at the Catholic University of the West (UCO).

Alain MUZET – Retired, National Centre for Scientific Research (CNRS), Physician, specialist in sleep and alertness.

Yves SICARD – Lecturer at the University Joseph Fourier, Scientific advisor to the French Alternative Energies and Atomic Energy Commission (CEA).

Alain SOYEZ – Laboratories Manager, Consulting Engineer, Nord-Picardie Pension and Occupational Health Fund (CARSAT).

Esko TOPPILA – Professor, Research Director at the Finnish Institute of Occupational Health.

Catherine YARDIN – Professor, Head of Department, Physician Biologist at the Dupuytren Hospital, Limoges University Hospital.

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**ANSES PARTICIPATION**

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**Scientific coordination**

Nathalie THIERIET – Scientific Project Manager – ANSES

**Scientific contribution**

Anthony CADÈNE – Scientific Project Manager – ANSES

Olivier MERCKEL – Head of Unit – ANSES

Benoit VERGRIETTE – Head of Unit – ANSES

**Administrative secretariat**

Sophia SADDOKI – ANSES

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**HEARINGS WITH EXTERNAL PARTIES**

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**Inserm – 12 September 2012**

Mr Claude LAMBRÉ – Honorary Research Director

**University of Paris Dauphine – 13 May 2013**

Ms Claire AUPLAT – Research Director

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## Abbreviations

AFM: Atomic force microscopy

AFNOR: French Standards Institute

AFSSAPS: French Health Products Safety Agency, now the French National Agency for Medicines and Health Products Safety (ANSM)

AFSSET: French Agency for Environmental and Occupational Health Safety

ANEC: European Association for the Co-ordination of Consumer Representation in Standardisation

APS: Aerodynamic Particle Sizer

Avicenn: Association for monitoring and civil information on the issues of nanosciences and nanotechnologies

BET: Brunauer, Emmett and Teller gas adsorption method

BEUC: European Consumer Organisation

BMBF: *Bundesministerium für Bildung und Forschung* – German Federal Ministry of Education and Research

BUND: *Bund für Umwelt und Naturschutz Deutschland* – Friends of the Earth Germany

CCNE: National Consultative Ethics Committee

CEN: European Committee for Standardization

CES: ANSES Expert Committee

CIEL: Center for International Environmental Law

CNAM: French Conservatory of Arts and Trades

CNDP: National Commission for Public Debate

CNRS: National Centre for Scientific Research

CNS: Central nervous system

COMETS: CNRS Ethics Committee

CRN: Certified reference nanomaterial

DGCIS: General Directorate for Competitiveness, Industry and Services

DLS: Dynamic Light Scattering

DNA: Deoxyribonucleic acid

DTA: Differential Thermal Analysis

EFSA: European Food Safety Authority

ELPI: Electrical Low-Pressure Impactor

FMPS: Fast Mobility Particle Sizer

FNE: France Nature Environnement

FP7: EU's Seventh Framework Programme for Research & Technological Development

GC: Gas Chromatography

GMO: Genetically modified organism

HPLC: High Performance Liquid Chromatography

HSS: Human and Social Sciences

ICP-MS: Inductively Coupled Plasma Mass Spectrometry

Ineris: National Institute for Industrial Environment and Risks

INRS: National Research and Safety Institute

InVS: French Institute for Public Health Surveillance

ISO: International Organization for Standardization  
NGO: Non-governmental organisation  
NNI: National Nanotechnology Initiative (United States)  
OECD: Organisation for Economic Cooperation and Development  
PAH: Polycyclic Aromatic Hydrocarbons  
PCB: polychlorinated biphenyl  
QNAR: Quantitative nanostructure-activity relationship  
QSAR: Quantitative structure-activity relationship  
R&D: Research & Development  
Raman: Raman Spectroscopy  
REACH: Registration, Evaluation, Authorization and restriction of CHemicals  
RIVM: *Rijksinstituut voor Volksgezondheid en Milieu* – National Institute for Public Health and the Environment (Netherlands)  
RN: Reference nanomaterial  
ROS: Reactive oxygen species  
SAXS and uSAXS: Small-angle X-ray scattering and ultrafine SAXS  
SDS: Safety data sheet  
SME: Small and medium enterprise  
SOP: Standard operating procedures  
SSA: Specific surface area  
STEP: Wastewater treatment plant  
STOP: Substitution, Technology, Organisation, Protection  
TEM: Transmission Electron Microscopy  
TGA: Thermogravimetric analysis  
TOF-SIMS: Time-of-Flight Secondary Ion Mass Spectrometry  
UIC: French Chemical Industries Union  
UV: Ultraviolet  
VOC: Volatile organic compounds  
VSSA: Volume Specific Surface Area  
WG: Working Group  
XRD: X-ray diffraction

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# 1 Background, purpose and procedure for handling the request

## 1.1 Context

The health and environmental issues related to the development and uses of manufactured nanomaterials are a significant social issue, and occupy an important place in the work of ANSES<sup>1</sup>. Since 2006, several expert reports published by the Agency have studied the health risks associated with dietary, environmental and occupational exposure. They stress the need for knowledge, monitoring and research on the associated hazards and on exposure to these materials, which often possess specific properties (Afsset 2006; Afsset 2008; Afsset 2010).

Alongside these expertise activities, the Agency has contributed to work on developing new risk assessment methodologies, especially aimed at professionals, and on defining health and environmental safety tests, both nationally and internationally (AFNOR, ISO, OECD, European Commission, etc.).

ANSES's expert work on manufactured nanomaterials includes permanent scientific monitoring, health and environmental risk assessment, methodological development work and definition of studies seeking to understand the exposure of specific populations to nanomaterials. The call for research projects in environmental and occupational health also enables ANSES to support research projects in the field of assessment of the health risks associated with nanomaterials.

To manage these different expertise activities in a consistent manner, on 9 July 2012, the Agency inaugurated a permanent Working Group (WG) on "Nanomaterials and health - food, environment, work" (intended to be renewed every 3 years), under the auspices of the Expert Committee (CES) on "Assessment of the risks related to physical agents, new technologies and development areas". The primary mission of this Working Group is to:

- produce a regular review of the state of knowledge on any potential health and environmental risks associated with manufactured nanomaterials, for all their uses;
- detect emerging signs of hazards and risks associated with manufactured nanomaterials, for all their uses;
- help with responding to requests for expertise made to the Agency;
- provide annual recommendations on research guidance intended in particular to help with the Agency's call for research projects on environment and occupational health.

The Agency also established a dialogue committee on "Nanomaterials and Health", as a forum for discussion, reflection and information on the scientific issues related to the potential health effects of nanomaterials and their assessment conducted by ANSES. Inaugurated on 21 November 2012, and seeking to ensure a balance between the different interest groups, it brings together representatives of associations and trade unions, federations of industry and institutions, as well as the experts of the permanent WG on "Nanomaterials and health".

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<sup>1</sup> On 1 July 2010, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) took over the missions of the French Food Safety Agency (AFSSA) and the French Agency for Environmental and Occupational Health Safety (AFSSET). It also inherited their achievements and values – scientific competence, independence in risk assessment, openness of expertise – with the intention of making them available for a broader and more multidisciplinary analysis of health issues.

## 1.2 Purpose of the expert appraisal

In a rapidly and constantly changing field, scientific and technical monitoring of the potential risks associated with exposure to manufactured nanomaterials is essential for conducting and updating expert appraisal work. As such, the main purpose of the permanent Working Group on "Nanomaterials and health - food, environment, work" is to produce a regular review of the state of knowledge and issues related to the hazards, exposures and health and environmental risks associated with nanomaterials, for all their uses, within the scope of ANSES's missions. The group's work is carried out in close coordination with the CESs with competence in regulated products at European (REACH, etc.) and national levels, especially with regard to the physico-chemical characteristics of nanomaterials, and with the CESs with competence in risks related to food, air, and water.

## 1.3 Procedure for handling the request: resources used and organisation

ANSES entrusted this expert appraisal to the Working Group on "Nanomaterials and health - food, environment, work", reporting to the Expert Committee (CES) on "Assessment of the risks related to physical agents, new technologies and development areas".

The report produced by the Working Group takes account of observations and additional information supplied by the members of the CES throughout the appraisal. This expert appraisal was therefore conducted by a group of experts with complementary skills. It was carried out in accordance with the French Standard NF X 50-110 "Quality in Expertise Activities – General requirements of Competence for Expert Appraisals (May 2003)".

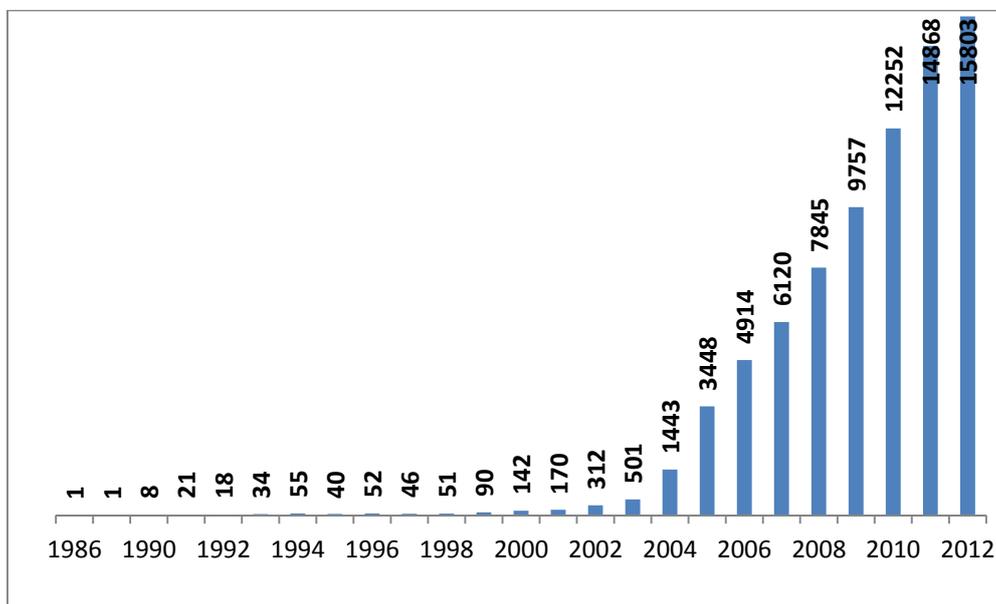
## 2 Introduction

The health risks associated with the development, use and environmental release of manufactured nanomaterials occupy an important place in the current literature on emerging risks (World Economic Forum, Marsh *et al.* 2011).

Following on from previous work conducted by the Agency on this subject, this document proposes to set out the state of scientific knowledge and current issues related to the hazards, exposures and health and environmental risks associated with manufactured nanomaterials, as well as the socio-economic and regulatory environments.

This summary report adopts a deliberately concise format. It is supplemented by annexes documenting the main points covered. It draws primarily on the available academic literature, although other types of work have occasionally been taken into account, with reservations regarding the quality of information that this implies (taking grey literature into account: scientific articles from journals without peer review, association reports, texts published on the internet, etc.). Given the increasing number of publications on nanomaterials identified in bibliographic databases specialising in medicine and the life sciences, and the diversity of scientific publications dealing with the topic of nanomaterials (see Figure 1), the experts of the Working Group selected the sources of scientific information that seemed to them the most representative and relevant for conducting this summary.

The Working Group focused specifically on nanomaterials manufactured intentionally, and not on all the nanomaterials naturally present in the atmosphere or produced unintentionally. In this document, the abbreviated term "nanomaterial" will mainly be used instead of "manufactured nanomaterial".

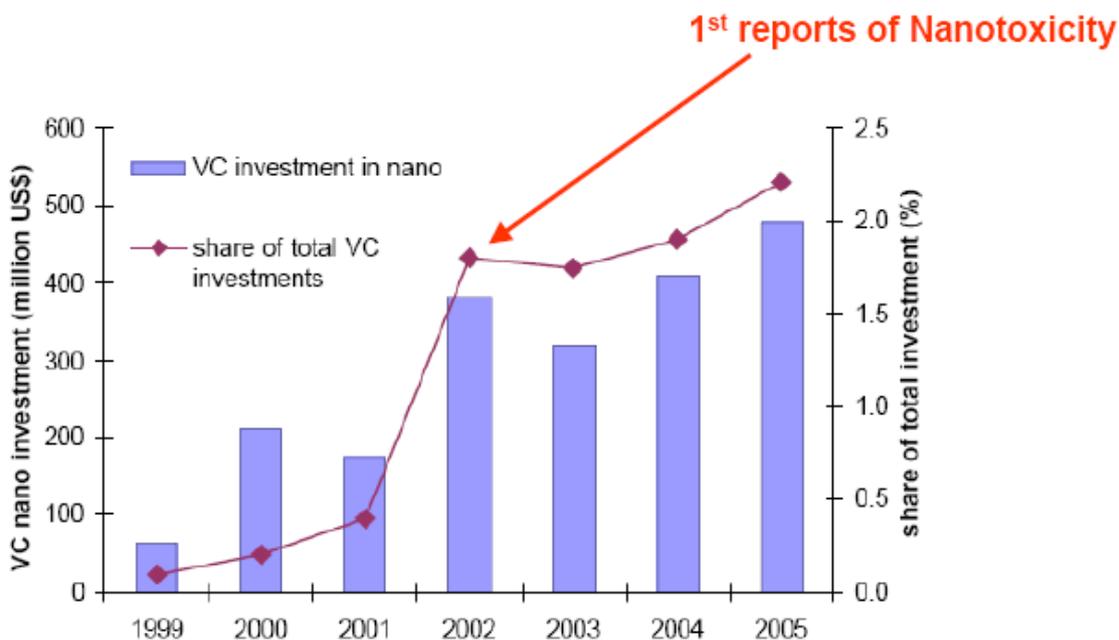


**Figure 1: Growth in the number of scientific publications (key word: "nanomaterial" on the PubMed website specialised in medicine and life sciences)**

The definition of the term "nanomaterial" poses, in itself, many questions and raises controversy. The proposed definitions are still the subject of many discussions by scientific, regulatory and institutional bodies, as well as within civil society, and according to the Working Group, they are not entirely satisfactory as they stand. Current definitions do not all take into account certain important

parameters such as solubilisation (transforming a substance into a solution or colloidal suspension) or the average size of agglomerates and aggregates. And yet knowledge of these parameters is important to help us understand the manufactured nanomaterials' mechanisms of action on living things. The Working Group chose not to favour a particular definition of nanomaterials from among all those previously published, which are provided in Annex 2.

Manufactured nanomaterials and the associated risks raise economic and political issues at the international level, as was pointed out twice in reports from the World Economic Forum in Davos (World Economic Forum 2009; World Economic Forum, Marsh *et al.* 2011)<sup>2</sup>. For example, Figure 2 illustrates the impact of awareness about the potential health and environmental risks on investments by venture capital companies in the "nano" field.



Source: Hullmand *et al.* (Hullmann 2006)

**Figure 2: Venture capital (VC) funding for "nano" activities in the entire world, as capital invested and as a proportion of investments**

In order to assess a risk related to the use of an object (product), it is necessary firstly to characterise the intrinsic hazard – i.e., the toxicity and ecotoxicity of the object (product) – and secondly to determine the human and environmental exposure to this object (product).

Given the very large number of nanomaterials with differing properties, not only compared to materials at the macroscale but also from one nanomaterial to another, and the extreme variability of expected or existing applications for these different nanomaterials, it would be inappropriate to assess the risks while considering "nanomaterials" as a single substance.

Consequently, the assessment of the risks associated with manufactured nanomaterials remains, at present, mainly qualitative in nature and favours a case-by-case approach. However, the lack of

<sup>2</sup> Extract from the *World Economic Forum's Global Risks Report, Marsh et al. 2011*: "A final outlier is threats from new technologies – unintended consequences for human, animal or plant life from the release of agents into the biosphere created by genetic engineering, synthetic biology or nanotechnology. Stakeholders rated this threat as of low impact and likelihood. However while experts interviewed concurred that numerous regulatory authorities in this area lower the risk's likelihood, it was being underestimated in terms of impact. (...)"

scientific certainty about the risks, far from paralysing their assessment, should instead stimulate the use of innovative procedures in this process, supported by the available scientific knowledge.

This summary review presents an overview of the economic and regulatory environment of manufactured nanomaterials, the main scientific knowledge about their health effects, and the different risk assessment methodologies available. It also proposes areas for improvement for assessing the health and environmental risks, and approaches to be considered for managing these risks. The Working Group decided to consider the following subjects as outside the scope of the expert appraisal:

- assessment of the risks associated with all manufactured nanomaterials and their uses;
- natural or unintentionally produced nanomaterials, as well as all nanotechnologies;
- the risks associated with medical devices, human medicines and cosmetics containing nanomaterials were not specifically studied;
- a "benefit/risk balance" type calculation, which would, in any event, be too poorly documented at the present time.

In addition, the contribution of the human and social sciences (HSS) in this review is intended to shed light on some of the conditions making it difficult to hold a constructive debate on "nanomaterials", or even any debate at all<sup>3</sup>, related to the complexity of the field in question, the history of the topic of environmental health risks, and the social and industrial issues that lie ahead. The role of the HSS is not therefore to construct an expert appraisal of the social acceptability of nanomaterials, but on the contrary to test the development of nanosciences, nanotechnologies and nanomaterials using methodologies and robust knowledge from research conducted in the various fields of the human and social sciences.

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<sup>3</sup> Such public debates have been an integral part of the French and European legislative process since the mid-1990s, and are, in some areas, even mandatory in application of the Barnier Act. On 25 June 1998, the Fourth Ministerial Conference "Environment for Europe" in Aarhus, Denmark, therefore approved the "Convention on Access to Information, Public Participation in Decision-making and Access to Justice in Environmental Matters", known as the Aarhus Convention, according to which (Article I) *"In order to contribute to the protection of the right of every person of present and future generations to live in an environment adequate to his or her health and well-being, each Party shall guarantee the rights of access to information, public participation in decision-making, and access to justice in environmental matters in accordance with the provisions of this Convention"*. France ratified the Aarhus Convention on 8 July 2002. It entered into force in France on 6 October 2002, through Act No. 2002-285 of 28 February 2002 authorising approval of the Aarhus Convention and Decree No. 2002-1187 of 12 September 2002 promulgating the Aarhus Convention.

### 3 Socio-economic and regulatory environment

#### 3.1 Uses and state of the market for manufactured nanomaterials

The collected information presented below should be considered in light of the limited number of existing sources, providing data that are also very heterogeneous.

##### 3.1.1 Nanomaterials: uses and claimed benefits

The uses and applications of nanomaterials are diverse and cover many industrial areas, which are currently difficult to determine precisely. Difficulties in accessing sources and the variability of the methodologies used by the available inventories, whether public or private, complicate the estimation of data reliability. Among the initiatives by non-governmental organisations (NGOs) that have attempted to address the lack of official inventories of nanoproducts placed on the market, we can mention in particular those of the European Consumer Organisation (BEUC) and the European Association for the Coordination of Consumer Representation in Standardisation (ANEC), initiated in 2010 (Anec and Beuc 2010; Anec and Beuc 2012)

The various examples of current applications or claimed uses include:

- in computing and electronics: silicon/carbon nanotube systems for processors, nanosilver as an antibacterial agent for computer keyboards and mice;
- in medicine: targeted transport of active substances (vectorisation), medical imaging, antibacterial operating tables;
- in cosmetics and hygiene products: sunscreens with UV filters, toothpaste containing abrasive silicon dioxide nanoparticles, hairdryers or adhesive plasters containing nanosilver as an antibacterial agent;
- in food: silicon dioxide nanoparticles used as an anti-caking agent in foods such as salt, "smart" packaging;
- in construction: paints and varnishes, self-cleaning windows;
- in sport and leisure: tennis rackets containing carbon nanotubes for strength, soft toys containing nanosilver as an antibacterial agent;
- in the area of security and defence: explosives, special coatings for objects, etc.

Table 1 gives an indication of the possible areas of application depending on the nature of the nanomaterials, as well as examples of finished products, found in the different inventories available.

**Table 1: Areas of application by type of nanomaterials**

Nanomaterials	Areas of application	Examples of finished products *
Nano-oxides	Structural composites – Anti-UV components – Mechanical-chemical polishing of substrates for microelectronics – Photocatalytic applications – Construction	Food additives, paints, cosmetics, inks, tyres
Nanometallic materials	Antimicrobial and/or catalysis sectors – Conductive layers in screens, sensors or energetic materials	Medical dressings, food wrap films, coatings (refrigerator), work surfaces, self-cleaning windows or walls, clothing, food contact materials, ingestible food packaging
Carbon blacks	Transport, Construction, Printing	Tyres, inks, paints

Nanoporous materials	Aerogels for thermal insulation in the fields of electronics, optics and catalysis – Biomedical field for applications such as vectorisation or implants	Water filtration membranes, paints, adhesives, fertilisers
Carbon nanotubes	Electrically-conducting nanocomposites – Structural materials – Nanoelectronics, biomedical	Tennis rackets, flexible screens, vehicle bumpers, headlamps, batteries, tyres
Bulk nanomaterials	Transport, construction, sports equipment	Hard coatings – Structural components for the aerospace and automotive industries, pipes for the oil and gas industries, the sports sector or the anti-corrosion sector
Dendrimers	Medical field – Cosmetics field	Administration of drugs, rapid detection
Quantum dots	Optoelectronic applications (screens)	Photovoltaic cells, inks and paints for applications such as anti-counterfeiting marking
Fullerenes	Sport (nanocomposites) and cosmetics sectors	Mascaras, beauty creams, golf balls
Nanowires	Electronics, optoelectronics, photovoltaic	Applications in the conductive layers of screens or solar cells and electronic devices

\* This list of finished products is derived from the non-exhaustive inventory compiled by ANSES for its 2010 report (Afsset 2010) on nanomaterials, as well as the draft inventory published by the Belgian government in 2013 (FPS 2013).

In the agri-food sector, a recent study by the National Institute for Public Health and the Environment of the Netherlands (jointly involving RIVM, Rikilt and Philips) confirmed the widespread use of silica nanoparticles ( $\text{SiO}_2$ ) as additives in food products for applications such as anti-caking or drying, or as a viscosity modifier (in sauces, seasoning, food powders, etc.) (Dekkers, Krystek *et al.* 2011). More recently, the presence of manufactured nanoparticles of titanium dioxide ( $\text{TiO}_2$ ) was detected in a wide variety of food products (Weir, Westerhoff *et al.* 2012).

### 3.1.2 Overview of the French market

Since 2007, various surveys have been carried out in France with the aim of mapping the stakeholders involved with nanomaterials (producers, integrators, researchers, users) in order to learn more about exposure of workers, residents and the general population (Table 2).

Table 2: Results of three surveys into the nanomaterials sector in France

Source	Year	Selection Criteria	Establishments concerned	Total number of contacts	Total number of responses	Number of "nano" players	Stakeholders
AFSSET (Afsset 2008)	2008	Registration on <a href="http://www.nanomateriaux.org">www.nanomateriaux.org</a> participation, symposia, conferences	Industries and research laboratories	219	99	33	13 laboratories 26 establishments, no distinction between production and use
INRS (Honnert and Grzebyk 2011; Honnert and Mater 2012; Honnert and Vincent 2007)	2007	Production of a bibliography, consultation of databases (customs, INSEE), participation in conferences and site visits in consultation with the Nanomaterials and Nanotechnology Commission of the National Technical Committee for the chemicals, plastics and rubber industries (CTNE).	Industrial production of nanopowders, excluding public and private research laboratories	1047	465	20	Producers of carbon black, amorphous silica, alumina, rare earth minerals, titanium dioxide, clays, etc.
	2011	Production of a bibliography: scientific and technical articles, patents, symposia summaries, memos from professional federations, etc.	Use of codes for five industry sectors: dyes and pigments, inorganic chemicals, basic plastics, paints, varnishes, inks and mastics, manufacture of plastic plates, sheets, tubes and profiles.			88	11 producers 75 users (industrial establishment using materials containing nanomaterials) 2 producers & users
	2012	Construction companies using nanoscale titanium dioxide	22			Producers of cements, windows, paints, varnishes, sealants, etc.	
DGCIS (DGCIS 2012)	2012	More than 50 scientific, economic, industrial and institutional documentary sources were used by D&Consultants	Ten selected industrial sectors: Construction, Transport, Healthcare industry (excluding medicine), Luxury (cosmetics and textiles), Agro-industry-energy, Consumer goods and household equipment industries, Information technologies and services, Defence, Eco-industry	350	260	130-180	40-50 producers 30-40 integrators/processors of nanomaterials 60-90 users of nanomaterials (end use)

In June 2012, the General Directorate for Competitiveness, Industry and Services (DGCIS) of the Ministry of Productive Recovery published a complete version of the study entitled *Les réalités industrielles dans le domaine des nanomatériaux en France [Industrial Realities in the field of Nanomaterials in France]* (DGCIS 2012). This study presents the value chain from the production of nanomaterials through to the various markets of application. It thus provides more information on the economic aspects (turnover, quantities, etc.) than the workforce involved in the nanomaterials sectors. The study identified 40 to 50 French producers, of which 80% are SMEs. Annual production of nanomaterials has reached a total of 135,000 tonnes, of which 90% are nanoparticles (mainly titanium dioxide, silica dioxide and cerium dioxide). Nanofibres and nanotubes meanwhile account for several dozen tonnes per year.

It should be noted that the results from annual production at the national level mentioned in this study (DGCIS 2012) differ from those derived from studies published in 2007 by the National Research and Safety Institute (INRS (Honnert and Vincent 2007) and mentioned in the Agency's report (Afsset 2008). These give the following production figures:

- 485,000 tonnes of silica by approximately 1300 operators;
- 469,000 tonnes of alumina by nearly 1000 operators;
- 300,000 tonnes of calcium carbonate;
- 240,000 tonnes of carbon black by 280 operators;
- 250,000 tonnes of titanium dioxide in submicron form and 10,000 tonnes in nanoparticle form involving 270 operators;
- 10 tonnes of carbon nanotubes per year corresponding to the French production capacity involving one operator.

On 1 January 2013 the mandatory reporting of uses of substances with nanoparticle status and of annual quantities produced, imported and distributed in France came into force, in accordance with Articles L. 523-1 to L. 523-8 of the French Environmental Code.

A public report on this scheme was published on the website of the Ministry of Sustainable Development (Ministère du Développement durable 2013). The main results from the 2013 annual scheme, concerning nanoparticle substances produced, imported and distributed in 2012, are as follows:

- 670 French entities submitted at least one report;
- of the French players submitting a declaration: 22% were importers, 6% were producers, 68% were distributors, and 4% were classified as "other";
- 280,000 tonnes of nanoparticle substances produced and 220,000 tonnes of nanoparticle substances imported into France in 2012 were reported, a total of 500,000 tonnes of substances with nanoparticle status placed on the French market in 2012.

Table 3 presents the updated types of nanomaterials produced and/or imported in France in 2013 in the largest quantities (above 100 t).

**Table 3: Categories of substances produced and/or imported in larger quantities in 2013 (above 100 tonnes)**

Generic name	Quantity-based intervals
carbon black	> 100 000 t
silicon dioxide	> 100 000 t
calcium carbonate	10 000 t à 100 000 t
titanium dioxide	10 000 t à 100 000 t
aluminium oxide	1 000 t à 10 000 t
boehmite (al(oh)o)	1 000 t à 10 000 t

calcium 4-[(5-chloro-4-methyl-2-sulphonatophenyl)azo]-3-hydroxy-2-naphthoate	1 000 t à 10 000 t
mélange réactionnel de dioxyde de cérium et de dioxyde de zirconium	1 000 t à 10 000 t
polyvinyl chloride	1 000 t à 10 000 t
silicic acid, magnesium salt	1 000 t à 10 000 t
2-propenoic acid, 2-methyl-methyl ester, polymer with 1,3-butadiene, butyl 2-propenoate and ethenylbenzene	100 t à 1 000 t
2,2'-[(3,3'-dichloro[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[n-(2,4-dimethylphenyl)-3-oxobutyramide]	100 t à 1 000 t
3-hydroxy-n-(o-tolyl)-4-[(2,4,5-trichlorophenyl)azo]naphthalene-2-carboxamide	100 t à 1 000 t
3,6-bis(4-chlorophenyl)-1h,2h,4h,5h-pyrrolo[3,4-c]pyrrole-1,4-dione	100 t à 1 000 t
3,6-bis-biphenyl-4-yl-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione	100 t à 1 000 t
4,4'-diamino[1,1'-bianthracene]-9,9',10,10'-tetraone	100 t à 1 000 t
diiron trioxide	100 t à 1 000 t
iron hydroxide oxide yellow	100 t à 1 000 t
isostearate d'oxyde de fer	100 t à 1 000 t
Kaolin	100 t à 1 000 t
silicic acid, aluminum magnesium sodium salt	100 t à 1 000 t
silicic acid, aluminum sodium salt	100 t à 1 000 t

This raises legitimate questions about the lack of reporting of certain manufactured nanomaterials, despite them being known and identified by the scientific community, such as nanosilver and even carbon nanotubes. An improvement to the reporting scheme seems necessary to clearly identify the manufactured nanomaterials produced, distributed and imported into the country.

### 3.1.3 Situation on the international market

#### ➤ Germany

The following information comes from a survey conducted by the DGCIS (DGCIS 2011) which compared France and Germany. Germany is one of the leading nations in Europe in the field of nanotechnologies (research and development, industrialisation, marketing, etc.), and ranks third in the world behind the United States and Japan, according to the company Lux Research (Lux Research 2006). In the 1990s, the federal government made a commitment to nanotechnologies, and heavily subsidised their development. It also sought early on to identify the companies involved in this sector.

A directory of nanotechnology companies was therefore established (BMBF) and in 2008 it recorded 740 German companies, of which approximately 80% were SMEs. The number of nanotechnology companies rose to 985 in 2011. Half of these companies are in industrial sectors. Medical, control and measurement devices, the chemical industry and construction of machinery and equipment are at the forefront. A significant proportion of companies (38%) fall within the service sector (R&D and business services), where they sell know-how (consulting, engineering, R&D services).

A survey (Luther 2009) of the 740 German companies was conducted in 2008 in order to assess the degree of their involvement in nanotechnologies. Thirty per cent of companies reported that nanotechnologies accounted for over 60% of their turnover and 20% of companies indicated that nanotechnologies accounted for between 30% and 60% of their turnover. In total, this public report (Luther 2009) estimated that in Germany, the companies involved in the nanomaterials sector probably employ about 60,000 employees and generate turnover of around 30 billion euros.

#### ➤ Switzerland

In Switzerland, a study (Schmid, Danuser *et al.* 2010) showed that approximately 580 companies manufactured or used nanomaterials in 2010, which represented 0.6% of manufacturing firms. About 21% of producer or user companies were from the chemicals sector. The automotive and electronics industries were also well represented.

#### ➤ Belgium

The number of companies bringing products to market containing nanomaterials in all sectors, assessed by a study of the Federal Public Service for health and the environment, was estimated at between 35,000 and 45,000. This represents about 15 to 20% of companies in Belgium (FPS 2013).

If the entire supply chain is taken into consideration, the number of unique products is as follows: 2000 to 5000 substances, 80,000 to 160,000 preparations, and 800,000 to 1,300,000 articles containing nanomaterials.

A product is considered unique if, regardless of its position in the supply chain, it is placed on the Belgian market with its own identifier (i.e. paints of different colours are regarded as individual products).

#### ➤ Italy

Concerning the inventory of industrial operators potentially exposed to nanomaterials, a single study, on Italy, was identified (Boccuni, Rondinone *et al.* 2008; Mantovani and D'Andrea 2004). It stated that there are nearly 1300 operators in the field of R&D and nearly 10,000 in the area of ultrafine powder production processes. Over 340,000 people work in areas that use powders, meaning that a total of over 350,000 operators are potentially exposed in Italy.

The great variability in the figures presented above illustrates the difficulty of conducting an accurate and reliable analysis of the market for manufactured nanomaterials. The information collected depends on how the surveys are conducted, the industry sector covered by the survey authors and the degree of cooperation from industry.

In future, knowledge about the state of the French market should be improved by the mandatory reporting (see Annex 5), since 1 January 2013, and in accordance with Articles L. 523-1 to L. 523-8 of the French Environmental Code, of uses of substances with nanoparticle status as well as annual quantities produced, imported and distributed in France, which applies to all manufacturers, distributors or importers. The first results from the 2013 campaign show that on the deadline of 30 June 2013, more than 930 respondents, including more than 90 foreign suppliers, had submitted more than 3400 reports.

Following this pioneering French approach, similar initiatives are planned in Italy, currently being implemented in Belgium and Denmark, and attracting interest from other countries such as Germany and the United Kingdom.

## 3.2 Ethics and civil society

### 3.2.1 Overview of the ethical issues associated with manufactured nanomaterials

#### ➤ Definitions and general points

Expressed very generally, ethics is the field of thought concerned with notions of good and bad, right and wrong, and with individual or collective values, virtues, principles and standards. These notions have been given several definitions throughout history, resulting in the emergence of different ethical traditions. *Meta-ethics* is sometimes described as a study of the meaning and status of moral terms, distinguished from *normative ethics* that takes as its purpose the determination of the states of good or bad things, as well as the determination of actions that, from a moral point of view, it would be good or bad to take. The development of manufactured nanomaterials, and that of nanotechnologies more generally, as a field of activity, is therefore concerned by normative ethics.

Ethical thinking began considering the case of nanomaterials and nanotechnologies in the United States from the early 2000s. Workshops on the "Social Implications of Nanotechnologies" were organised by the NNI (National Nanotechnology Initiative) with the underlying idea of preparing society for the inevitable advent of "converging technologies" (Laurent 2010). This then raised the issue of new health risks potentially posed by nanomaterials owing to their specific characteristics. It was accompanied, within these workshops, by questions about the applications of nanotechnologies in electronics and medicine, such as the threats to individual freedom attached to increasingly miniaturised tracking devices, or how humans could be transformed as a result of this claimed "convergence". These concerns were reflected, among other things, by the gradual recruitment of ethicists and sociologists in nanotechnology development programmes, particularly in the United States. This then led to the development of a completely separate academic field<sup>4</sup>, nanoethics, which has now achieved an international profile and, in particular, a specialised journal *NanoEthics*© (*NanoEthics*). Meanwhile, a number of official bodies, both in France and abroad (United Kingdom, Canada), have devoted reports to the ethical aspects of the development of nanomaterials and nanotechnologies. Annex 4 of this document presents the main issues discussed by the ethics of nanotechnologies in the broadest sense, as well as a summary of the contributions of the main official reports.

This ethical thinking devoted to "nanotechnologies" is notable for having brought together concerns that were initially separate and typically expressed by different players. Issues about health and respect for the environment attached to the development of new types of materials were combined with political issues such as widespread surveillance, and more philosophical issues such as the future of mankind under the effect of "human self-improvement" permitted by new technologies.

Within the ethics literature on nanotechnologies, the theme of manufactured nanomaterials, often considered solely in terms of the "risk", sometimes acts as the "zero degree" of ethical questioning (for example the work of R. Larrère (Larrère 2009)). In any case it seems clear that beyond the development of nanomaterials, each new step taken in the development of nanotechnologies will bring new questions with it, and in particular that the convergence of scientific disciplines, if it occurs, will in return give rise to a convergence of the ethical concerns traditionally associated with each of these disciplines. Problems dealt with so far, such as knowing what a physician must

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<sup>4</sup> The specific nature of the academic field is discussed later in this section.

disclose to a patient, will be renewed and made more complex with the development, through nanotechnologies, of instruments capable of ultra-early diagnosis. Alternatively, the possibility of introducing nano-robots into the human body that are capable of dealing autonomously with pathogenic cells will give rise to new problems of legal and moral liability, etc.

The fact remains that the development and use of manufactured nanomaterials in itself is enough to raise genuine issues of ethics, expressed and relayed by different types of players.

1) The development of manufactured nanomaterials first raises questions about the moral principles of sincerity and transparency. Although there was a time when the prefix "nano" could work as a sales argument, it now tends to be deliberately omitted by sellers of products containing nanomaterials, particularly in sensitive areas such as food. This practice contributes to a sense of disempowerment often expressed by civil society, and is particularly exacerbated by the lack of knowledge about the risks associated with these products. More systematic labelling, widely called for by NGOs, could provide an answer to this question. However, this would have other drawbacks, such as replacing the collective responsibility of society, faced with the potential risks, with individual responsibility – whether or not to consume a particular product – despite lacking the knowledge to exercise it.

2) In line with the precautionary principle, the idea is expressed in civil society that the development of nanomaterials should be implemented with caution<sup>5</sup>, and the general state of the planet is often put forward to encourage even more restraint. Thus, their placing on the market and probable release into the environment bring to mind other examples of products with uncertain risks<sup>6</sup>. Added to this is a questioning of the concept of risks and benefits, which, according to some authors (Brignon 2010), reaches its limits with manufactured nanomaterials. While this method is traditionally used to compare the advantages and disadvantages of a technology, uncertainty about the risks of nanomaterials reaches in some eyes a degree such that the huge risks could never be balanced by any benefits, however great. The advertised benefits are also sometimes subject to significant moral criticism. Some denounce the lack of realism (and even hypocrisy) in the promises attached to nanomaterials, in terms of reducing a product's environmental impact or increasing its "sustainability" (Friends of the Earth 2010; Ipen and EEB 2009). The existing applications are also sometimes morally challenged and regularly accused of being "frivolous" (the case of anti-odour socks, discussed at the national public debate, is a good example). From this point of view, the debate on nanomaterials shows the tension between two conceptions of "responsible" development, according to whether it is regarded as something whose risks are controlled from one end of the product life cycle to the other, or as something whose long-term consequences (health, environment, society, etc.) have been carefully and collectively compared against its expected benefits.

3) The ethical debate around the development of manufactured nanomaterials often also mentions the risk of "*nanodivide*", or the increase in the gap between rich countries – able to fully enjoy the benefits of the new materials – and poor countries that would be unable to benefit (The Royal Society 2004). This concern is exacerbated by the claim that nanomaterials would meet some of the needs of developing countries (such as

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<sup>5</sup> This rhetoric is supported by many players from the voluntary sector, as well as environmentalists, consumer groups, trade unions, etc. It was expressed in many published works by players at the national public debate on nanotechnologies in 2009-2010 (Official website of the national debate on nanotechnologies: the initial URL <http://www.debatpublic-nano.org> has now expired, but its archives are still available here: <http://cpdp.debatpublic.fr/cdpd-nano>). The remaining sections of this report give a more detailed analysis of the different positions of the players in the debate.

<sup>6</sup> The examples of PCBs, pesticides, phthalates, etc. in particular are mentioned repeatedly (e.g. in contributions by players from the voluntary sector at the national public debate).

decontamination of water using nanoparticle filters), but in a *de facto* market largely dominated by Western patents, making access to nanoproducts, in economic terms, almost impossible to the poorest. In agriculture, the use of nanomaterials as pesticides raises fears about pressure on natural species and soil depletion, and at another level impoverishment of farmers. This brings us to considerations that are more political than ethical, which are by no means unique to nanomaterials, although in this case, due to the intensity of their effects, they are likely to be renewed and expanded.

➤ **Outline of the recommendations from official bodies**

Since the early 2000s, the ethical and societal issues associated with the development of nanotechnologies have become a subject of global concern. In France and abroad, a number of official organisations have dedicated studies and reports to this topic, which are briefly summarised below; in Table 4 for France and Table 5 at the international level.

**Table 4: French studies and reports on ethics and nanomaterials**

Year	Authors	Recommendations*
2004	General Mining Council and General Information Technology Council on the topic of nanotechnologies (Dupuy and Roure 2004)	13 recommendations, including the creation of interministerial coordination in synergy with all stakeholders
2006	COMETS <sup>7</sup> / CNRS	8 recommendations
2007	National Consultative Ethics Committee (CCNE) (Comité Consultatif National d'Ethique 2007)	The recommendations cover: <ul style="list-style-type: none"> <li>- availability of information;</li> <li>- nanometrology;</li> <li>- the imbalance induced by the acceleration in placing nanoproducts on the market<sup>8</sup>, which may compromise the ability to make essential choices;</li> <li>- the need for multidisciplinary research;</li> <li>- the need to prioritise all the protective measures required for workers in contact with nanomaterials;</li> <li>- the need for containment in the places where nanomaterials are studied and produced.</li> </ul>

\* The full recommendations can be found in Annex 4 of this document

**Table 5: Other Canadian studies and reports on ethics and nanomaterials**

Year	Authors	Publication
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<sup>7</sup> COMETS is a consultative body of 12 members, researchers or engineers in a broad variety of disciplines, reporting to the CNRS board. Established in 1994, it examines the ethical aspects raised by the practice of research, makes recommendations and raises staff awareness.

<sup>8</sup> A nano-product is a product that contains manufactured nanomaterials.

Year	Authors	Publication
2006	Québec, Commission on the Ethics of Science and Technology (Commission or CEST1)	An Position Statement, " <i>Ethics and Nanotechnologies: a basis for action</i> " (Trottier and Duquet 2006)
2011	Québec, Commission on the Ethics of Science and Technology (Commission or CEST1)	An Opinion on " <i>Ethical Issues of Nanotechnologies in the agri-food sector</i> " (Beaudry 2011)

### 3.2.2 Identifying points of agreement and disagreement

The points most often discussed in the field of nanotechnologies can be classified according to three themes: social surveillance (e.g. traceability), living things (nanobiotechnologies) and food-environment-health risks (nanomaterials). While these questions do not all relate to the same issues, they intersect and complicate the problems posed by each area of nanotechnology. Given the scope of the expert appraisal defined by the Working Group, only the food-environment-health risks will be considered in this report. The questions raised by nanotechnology applications, including nanomaterials, have been the subject of numerous public debates since the early 2000s, some still ongoing (Bullich 2009). Some of these have been organised by public institutions, such as the Nanoforum held by the French Conservatory of Arts and Trades (CNAM 2007-2009), the Grenelle environmental round-table discussions (2007, health-environment workshop), the National Commission for Public Debate (CNDP, 2009-2010) or the Cité des Sciences (January-February 2006 and then June 2007). These institutional initiatives have been supplemented by numerous public meetings organised by civil society, for example: Nanomonde (Fondation Sciences Citoyennes 2006), Nanoviv (Vivagora 2006) and Avicenn (Sciences et Démocratie 2013). In this perspective, the large number of debates can be interpreted in different ways.

Firstly, it reflects the rise of concern about "nanomaterials" in the public arena and the fact that this is now being taken into account by institutions and companies. In response to this, a number of expert committees and commissions have been set up at European (European Commission 2012) and national level (such as for example within ANSES or Ineris).

Secondly, the succession of debates, often repeating the same questions, reveals the difficulty in finding a solution that is broadly shared by all stakeholders. It is thus possible to identify points of agreement and disagreement, which in the latter case can extend to radical opposition to nanotechnologies including nanomaterials (ranging from Pièces et main d'œuvre t (PMO 2008) to Friends of the Earth (Friends of the Earth 2010)).

The organisation of numerous debates on the same subjects therefore leaves open the possibility that their findings may be adopted according to the meaning preferred by the "decision maker". The practice is the same, for example, with the expert reports or outlook reports intended for the public authorities, which act as a source of arguments which they can draw upon to justify or refine their decisions.

More generally, the proliferation of institutional debates is both a response to pressure exerted by associations and trade unions, and an attempt to escape from a situation marked by confrontation with no visible way out. Since the issues of pollution and nuclear power in the 1970s, followed by GMOs in the 1980s and 1990s, public opinion's expectations with regard to the authorities have risen. In this sense, the theme of nanotechnologies, and more specifically nanomaterials, are a continuation of this trend: the reports on nanomaterials submitted by companies have often faced difficulties (they are often not verified) and the concerns of a worried public.

#### ➤ The reference to the precautionary principle

This is a reference common to all stakeholders. This consensus is based largely on the refusal to allow a repeat of the disastrous example of asbestos, especially due to a similarity, whether proven or not, between the toxicological effects of asbestos and those of certain carbon nanotubes.

The French Constitution has remained the same since 1958, despite being amended several times, including on 1 March 2005 with the introduction of the Charter for the Environment, whose Article 5 defines the terms of use of the precautionary principle: "When the occurrence of any damage, albeit unpredictable in the current state of scientific knowledge, may seriously and irreversibly harm the environment, public authorities shall, with due respect for the principle of precaution and the areas within their jurisdiction, ensure the implementation of procedures for risk assessment and the adoption of temporary measures commensurate with the risk involved in order to preclude the occurrence of such damage".

The use of the precautionary principle appears in most of the memoranda from the parties called to appear at the debate of the National Commission for Public Debate (CNDP), as well as in government undertakings (interministerial communication of 27 October 2011, published on 13 February 2012), following the debate organised by the CNDP.

However, views differ as to how it should be implemented:

a) associations (Friends of the Earth, Citizen Science Foundation) and industry federations (National Chemical Industries Federation, CGT (Fédération Nationale des Industries 2009)) have voted for partial or total moratoriums on nanomaterials or nanotechnology products, based on the precautionary principle. However, given the difficulties in defining the concept of nanomaterials, the operational nature of these requested moratoriums seems wrongly established, especially since the pervasive nature of nanomaterials in everyday products would make such a measure difficult to implement (Birraux and Le Deaut 2012);

b) stakeholders such as the French Chemical Industries Union (UIC) or the France Nature Environnement (FNE) federation recommend limiting (under protection) or avoiding exposure to nanomaterials. At this stage, however, there is a shortage of research and studies on management practices and risk control in workshops or laboratories. Among the 'field' studies available (Dedessus-Le-Moustier and Drais 2012), it can be noted that:

- most of the 50 safety data sheets (SDS) assessed in an Australian study provided insufficient information on the health and safety risks associated with nanomaterials contained in products (Safe Work Australia 2010);
- relevant physico-chemical characterisation is essential for assessing the specific risks of manufactured nanomaterials. However, in practice, little information on these characteristics is available and communicated throughout the supply chain (TNO 2012).

#### ➤ Taking into account the entire life cycle of nanomaterials

The Working Group considers that it is essential to take into account the life cycle of nanomaterials when assessing the risks associated with their use. Considering the full life cycle of nanoproducts means identifying potential risks from product design through to disposal or recycling, and including consumption. This approach raises the problem of traceability and control of risks during the different stages of product processing. This problem is unusual in the case of nanomaterials insofar as each case is specific and the toxicity and ecotoxicity of nanomaterials can change during the life cycle (association, aggregation, surface contamination, etc.). Furthermore, this approach does not take into account the chemical complexity of objects derived, through physical or chemical alteration, from products containing nanomaterials (i.e. cement, wall coatings, cosmetics, etc.) and their evolution over time. For example, in the case of a sunscreen containing nanoparticles of titanium dioxide ( $\text{TiO}_2$ ) in an aqueous medium, the presence of aluminium oxide ( $\text{Al}_2\text{O}_3$ ) on the surface of the  $\text{TiO}_2$  nanoparticles greatly limits the production by  $\text{TiO}_2$  of reactive

oxygen species (ROS), thereby accelerating skin aging (Auffan, Pedeutour *et al.* 2010; Jassby, Farner Budarz *et al.* 2012)<sup>9</sup>.

The transmission of SDSs throughout the supply chain should enable the product to be tracked during its industrial processing stages, i.e., during a part of its life cycle. In addition to the intrinsic advantages of SDSs, their content should be specifically adapted to nanomaterials and sufficiently understandable to enable the safe and controlled use of nanomaterials by companies and consumers.

#### ➤ The need for regulations applicable to nanomaterials

All of the stakeholders (industry, trade unions, associations, etc.) broadly agree on the need for regulations to control manufactured nanomaterials. Indeed, current regulations at French and European level taking into account the specific characteristics of nanomaterials are still limited in number and scope.

Most regulations related to chemicals are based on the OECD guidelines, in particular for characterising them and assessing their (eco)toxicity. These guidelines, however, require adaptations to take account of the specific characteristics of nanomaterials, as indicated in document ENV/JMMONO(2009)21 by the OECD. Pending these adaptations, companies continue to employ the guidelines related to chemicals, by default as they claim, even though they may be scientifically questionable.

Meanwhile, the European Commission favours considering manufactured nanomaterials as conventional chemicals, using existing regulations, including REACH, which is the regulatory spearhead<sup>10</sup>. However, NGOs (such as for instance CIEL, ClientEarth and BUND at the European level, or FNE in France<sup>11</sup>) as well as the European Trade Union Confederation consider that this regulation is not suited for taking into account all the specificities of nanomaterials; they are therefore demanding new regulations, for example based on the model of REACH<sup>12</sup>.

### 3.3 Regulations applicable to manufactured nanomaterials

The overview given in this report focuses on regulations specific to nanomaterials as applicable in France. It therefore includes elements of European Union law and French law, but does not mention the more or less restrictive rules that have been adopted in other countries. Moreover, it is worth recalling, as an introduction, that nanomaterials have not emerged in a world devoid of standards. Most of the standards that apply to these new materials actually predated them. This is also the case with rules on the protection of workers in particular, or the REACH Regulation. These pre-existing legal standards will only be mentioned in the context of this summary to the extent that they have been adapted specifically to nanomaterials.

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<sup>9</sup> The exposure of the surface of the TiO<sub>2</sub> (degradation of surface coatings) will therefore depend on the kinetics of dissolution of aluminium oxides or oxy-hydroxides that can also lead to adsorption of molecules and heteroaggregation which will further limit the production of ROS.

<sup>10</sup> "The Commission will therefore, based on available information on technical progress, including the REACH Implementation Projects on Nanomaterials and experience with the current registrations, in the upcoming REACH review assess relevant regulatory options, in particular possible amendments of REACH annexes, to ensure clarity on how nanomaterials are addressed and safety demonstrated in registrations." (Second Regulatory Review on Nanomaterials, 3 October 2012)

<sup>11</sup> CIEL: Center for International Environmental Law; BUND: Friends of the Earth Germany; FNE: France Nature Environnement.

<sup>12</sup> "Nanomaterials have distinct properties and all available scientific evidence needs to be taken into account by the Commission", Stakeholders' Response to the Communication on the Second Regulatory Review on Nanomaterials, 23 October 2012.

Two specific traits can be highlighted in the way nanomaterials have been gradually taken into consideration by the French regulatory framework.

While it may appear that the rules applicable to nanomaterials have been "toughened" over time, the reality is quite different. Faced with a problem of delimiting their scope of action, the authorities have indeed intervened to establish legal rules, but without offering a clear framework (see **Erreur ! Source du renvoi introuvable.**). The field of nanomaterials is marked mainly by standards inflation and a lack of consistency between its stakeholders (see **Erreur ! Source du renvoi introuvable.**). Despite the fragmentary efforts undertaken to adapt existing regulatory frameworks to this diverse and potentially infinite group comprising nanomaterials, the lack of practical social and economic assessments of their deployment continues to be felt.

### 3.3.1 A gradual "toughening" of the specific regulatory framework for nanomaterials

The form of the standards dedicated to nanomaterials has gradually evolved since the mid-2000s, from non-binding standards – European Commission communications, in particular – to the adoption of legal standards in the strict sense of the term, especially since 2009. This development does not mean that the enactment of standards falling within the scope of "soft law"<sup>13</sup> has been phased out. On the contrary, we now see a proliferation of initiatives relating to technical standards (ISO TC 229, CEN TC 352, AFNOR X457) (Auplat 2012; Auplat 2013)<sup>14</sup> or more or less institutionalised charters and guides to good practice. For example, in 2009, the UIC published a *Guide de bonnes pratiques Nanomatériaux et HSE [Guide to good practice for nanomaterials and health, safety & environment]* (Cellule Innovation de l'U. I. C. 2009), while the German company BASF published a dedicated code of conduct which went as far as to recommend the organisation of greater transparency in communication (BASF 2013). Finally, in 2008 the European Commission published a recommendation on a code of good conduct for responsible research in nanosciences and nanotechnologies<sup>15</sup>. This recommendation includes items relating to the assessment and management of risks associated with nanomaterials. Without clarifying the scope of standards with respect to nanomaterials, these initial changes nevertheless illustrate the importance that the "nanomaterials" issue has gradually taken on in the public arena.

### 3.3.2 Fragmentary establishment of standards

Although nanomaterials did not emerge into a world devoid of standards, several options were possible for adapting the existing regulatory system in France and Europe to the characteristics of these emerging objects. Noting their generic nature and their scientific, technical, innovative and perhaps even economic potential, authorities could have decided to develop a dedicated set of rules. The opposite choice was in fact made at European level, by incorporating a few provisions specific to nanomaterials in the body of legislation being renewed for the most part, without challenging the classifications and branches of law already in place within the legal system (see Annex). The two regulatory reviews published by the European Commission, respectively in 2008<sup>16</sup> and 2012<sup>17</sup>, are unequivocal in this regard.

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<sup>13</sup> A collection of texts with uncertain significance in terms of standards: statements of principle, resolutions by international organisations, as well as codes of conduct, charters of good practices, etc. By extension, the concept covers all texts whose legal character was not determined by reference to a binding constraint or sanction, including voluntary standards emanating from national and international organisations.

<sup>14</sup> The Working Group interviewed Claire Auplat following the publication of her work on the regulation and role of ISO TC229.

<sup>15</sup> Commission Recommendation on a code of conduct for responsible nanosciences and nanotechnologies research C(2008) 424 final - Brussels, 07/02/2008.

<sup>16</sup> Communication from the Commission to the Council, the European Parliament and the European Economic and Social Committee COM(2008) 366 final - Brussels, 17.6.2008 Regulatory aspects of nanomaterials.

Without going so far as to build a coherent set of rules taking into consideration all the issues raised by the life cycle and placing on the market of nanomaterials, France nevertheless adopted a stronger position, in the two *Grenelle* environmental Acts and their implementing legislation. There is now a reporting requirement in France, for all those who "*manufacture, import or distribute substances with nanoparticle status, in a pure state or contained in mixtures, without being bound, or in materials intended to release such substances in normal or reasonably foreseeable conditions of use*". The next summary of the Working Group will provide an opportunity to come back to this legal mechanism which, while original, no doubt has room for improvement from a legal standpoint<sup>18</sup>.

In order to identify nanomaterials that are already present in Europe (and are therefore potential sources of exposure), the Commission meanwhile chose to respond positively to the wishes expressed by the European Parliament and the Council. On 18 October 2011, it adopted a definition of nanomaterials. This trend is the same as the one followed in France, through the establishment by the *Grenelle* Acts and their implementing decrees of the reporting requirement for substances with nanoparticle status.

The choice of how to define nanomaterials and the difficulties that are still attached to the exercise are indicative of a less liberal functioning of standards than that adopted by the United States. The characteristic features of the development of the regulatory framework dedicated to nanomaterials are at least partly explained by the difficulties encountered by the public authorities in building a realistic and relevant standards-based image of the objects they have been confronted with in recent years. However, it seems that there is no intention of changing strategy, at least in the short term. The standards being established are aimed at nanomaterials, understood as a whole.

### 3.3.3 REACh and nanomaterials

The European REACh Regulation (EC No. 1907/2006), which came into force on 1 June 2007, provides for the registration, evaluation, authorisation and restriction of chemical substances. REACh makes industry responsible for assessing and managing the risks posed by chemicals and providing appropriate safety information to their users. Therefore, registration dossiers must include data on the hazards and risks of the chemicals being registered.

The REACh Regulation does not contain specific provisions on nanomaterials: they are likened to conventional chemicals. As a result, the registration requirement does not take into account the specific characteristics of nanomaterials. Although manufacturers, importers and downstream users must already ensure the safe use of each substance (irrespective of the form) under REACh, nanomaterials pose new challenges for regulators such as the European Commission and the European Chemicals Agency (ECHA).

In 2009, the European Commission initiated two projects: the "REACh Implementation Project on Nanomaterials" (RIPoN) aiming to provide the key points for the implementation of REACh to cover nanomaterials, in particular concerning the information required and the chemical risk assessment (RIPoN2 – training required, RIPoN3 – chemical risk assessment). A third report from the RIPoN project on the identity of substances was unable to reach a consensus on the recommendations.

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<sup>17</sup> Communication from the Commission to the European Parliament, the Council and the European Economic and Social Committee, COM(2012) 572, Brussels, 02.10.2012, Second regulatory review on nanomaterials.

<sup>18</sup> *Legislative drafting is a "science" (applied science) of legislation that seeks to determine the best methods for preparing, drafting, enacting and implementing standards* based on the work of the Centre for legislative study, technique and assessment (CETEL) - Faculty of Law - University of Geneva, available at the following address: <http://webdroit.unige.ch/cours/general/def/legistique.html> (last viewing on 9 March 2007)

Therefore the Commission, in collaboration with CARACAL<sup>19</sup>, is continuing its discussions on the registration of nanomaterials in REACH.

In 2011, the European Commission issued a special recommendation on the definition of nanomaterials. This recommendation should be used in the different European regulations, including REACH.

The European Commission responded to the report on the review of the REACH Regulation (EC) No 1907/2006 (Commission Européenne 2013) by announcing that it would undertake an impact assessment on the revision of the annexes of the Regulation in order to adapt them to nanomaterials. To this effect, it has initiated a consultation with Member States via the CASG Nano (a subgroup of CARACAL in charge of nanomaterials) and a wider stakeholder consultation *via* a public consultation posted on the Commission's website. The Commission proposal to revise the annexes will go ahead on the basis of this impact assessment and should be published in spring 2014 (the public consultation ended on 13 September 2013). The Commission's proposal will then be submitted to the Member States, via the REACH Committee, for a decision by the comitology procedure<sup>20</sup>.

For its part, in October 2012, ECHA set up a Working Group on nanomaterials with the aim of discussing the scientific and technical aspects relevant to REACH and CLP processes, and making recommendations on strategic issues. This is an informal advisory group consisting of experts from Member States, the European Commission, ECHA and accredited stakeholders, with a mandate to "give informal advice on any scientific and technical question concerning the implementation of the REACH and CLP Regulations in the field of nanomaterials".

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<sup>19</sup> CARACAL is composed of representatives of Member States competent authorities for REACH and CLP, representatives from competent authorities of EEA-EFTA countries as well as a number of observers from non-EU countries, international organisations and stakeholders,

[http://ec.europa.eu/enterprise/sectors/chemicals/reach/caracal/index\\_en.htm](http://ec.europa.eu/enterprise/sectors/chemicals/reach/caracal/index_en.htm)

<sup>20</sup> The "Comitology" procedure can be defined as the process of adopting measures for implementing legal acts, providing that these measures are adopted by the Commission assisted by a committee of experts from Member States.

## 4 Review of the scientific knowledge of the risks associated with nanomaterials

As stated previously, a quantitative assessment of the risks associated with a chemical implies proven knowledge of the hazards to health and the environment, and of the levels and durations of exposure. This section proposes a review of the available knowledge on characterisation of the hazards and exposures related to the uses of nanomaterials.

### 4.1 Assessment of exposure

Occupational exposure situations are most frequently found among employees required to manufacture or use nanomaterials in companies and research laboratories. But so far, very few data have been published on the scenarios, levels, frequencies and durations of exposure, or on the individual and collective protective measures. In addition, the measurement strategies and tools to be applied for quantifying exposure to nanomaterials have not been consolidated and therefore not yet gained consensus at national and international level. Some of the measurement techniques and analysis protocols remain complex and expensive, and are not widely used. Furthermore, most of the strategies used in work station studies essentially aim to identify and characterise the emission by a process, rather than the exposure of the employee required to work on this process.

Despite the absence of suitable measurement instruments or methods, it is still possible to perform a qualitative assessment of exposure to nanomaterials (Anses 2011a). To do this, each situation liable to induce exposure is rigorously analysed.

Various data relating to exposure are collected:

- the state of the nanomaterial(s) being handled: powder, liquid suspension, gel, embedded in a matrix, etc.;
- the operations carried out: weighing nanopowders, transferring paint containing nanomaterials, cutting plastic containing nanomaterials, etc.;
- the propensity of nanomaterials to be found in the air or on surfaces, i.e. to form aerosols or droplets;
- the quantities handled;
- the duration and frequency of operations;
- the routes of exposure of workers or consumers: inhalation, ingestion and/or dermal contact;
- individual susceptibility;
- the measures of prevention and protection (aiming to reduce exposure) that may be implemented.

However, it should be noted that significant work on exposure by inhalation has been conducted at national and international levels in recent years. This includes the following, on aerosol metrology devoted to exposure to nanomaterials:

- developments in testing methods to assess the performance of real-time aerosol sampling and measurement devices (Jacoby, Bau *et al.* 2011; NANODEVICE);
- developments of measuring devices (Fierz, Houle *et al.* 2011; Meier, Clark *et al.* 2013) and sample aerosols;

- campaigns on inter-comparison of various measurement instruments (Asbach, Kaminski *et al.* 2009; Kaminski, Kuhlbusch *et al.* 2013; Leskinen, Joutsensaari *et al.* 2012);
- developments in tools to help interpret measurement data;
- etc.

#### Concerning occupational exposure in particular:

- proposed criteria for measuring exposure, strategies for qualitative and quantitative exposure assessment (Beurskens-Comuth, Verbist *et al.* 2011; Brouwer, Duuren-Stuurman *et al.* 2009; Collectif 2011; Methner, Hodson *et al.* 2010b; Ramachandran, Ostraat *et al.* 2011; Witschger, Le Bihan *et al.* 2012), some of which are dedicated to specific nanomaterials, such as CNTs or TiO<sub>2</sub> (Niosh 2011; Niosh 2013);
- work on the definition of exposure scenarios (Fleury, Bomfim *et al.* 2013; Hristozov, Gottardo *et al.* 2014; Kuhlbusch, Asbach *et al.* 2011; Nowack, David *et al.* 2013);
- some fifteen publications since 2010 reporting field data but also providing information on protective measures implemented in the field, or on aerosols that should be considered in future experimental toxicology studies (Dahm, Evans *et al.* 2013; Methner, Hodson *et al.* 2010a; Zimmermann, Derrough *et al.* 2012);
- publications on the emissivity of different types of nanomaterials under different stresses (Burdett, Bard *et al.* 2013; Dahmann and Monz 2011; Evans, Turkevich *et al.* 2013; Witschger, Le Bihan *et al.* 2012);
- work on the development of exposure databases (Fransman, Pelzer *et al.* 2012).

Some of this work has been conducted as part of projects such as Nanosh (Nanosh), Nanodevice (NANODEVICE) and Nanogenotox (Nanogenotox 2010). There has also been work on the generation and characterisation of aerosols for the purpose of inhalation studies; this is being conducted by teams with joint expertise in "aerosols" and "toxicology" (i.e. as is being planned for NANoREG).

Concerning exposure of the general population, two situations should be considered: direct exposure to products containing nanomaterials, and indirect exposure related to the environment. A previous report by ANSES (ANSES 2010) highlighted the lack of traceability of nanomaterials, and only by conducting a non-exhaustive inventory has it been possible to determine the possible presence of nanomaterials in consumer products. Nevertheless, the information supplied by producers or distributors is not by any means sufficient for characterising and quantifying exposure to nanomaterials via these products. Since this report was published, research carried out by different organisations has been able to obtain some data on food (Bouwmeester, Dekkers *et al.* 2007) and on the environment (e.g. on catalytic exhausts and cerium oxide (HEI 2001)). Lastly, if exposure of the general population is to be properly assessed, exposure of users of the product must be considered, taking into account all the stages of its life cycle.

Some NGOs, just like public and private laboratories (institutes, health and even industrial agencies), are now able to perform measurements themselves (e.g. FOE Australia), making the control of exposure measurements a new issue (As You Saw 2013; Friends of the Earth ; Friends of the Earth).

Some institutes such as NIOSH (Niosh 2011; Niosh 2013), IFA (IFA) and the BSI (BSI 2007) already propose indicative exposure limit values for nanomaterials. These provisional values are based on incomplete toxicological data or extrapolation from the values established for better known particles. These organisations state that compliance with these values does not guarantee that an individual will not develop a disease, but rather that they are an aid to decision making. In 2011, NIOSH established two indicative exposure limit values for titanium dioxide: 2.4 mg/m<sup>3</sup> for fine titanium dioxide and 0.3 mg/m<sup>3</sup> for ultrafine titanium dioxide (particle diameter below 100 nm). The development of knowledge on occupational exposure and on the hazards associated with

carbon nanotubes has led NIOSH to reduce by a factor of 7 the exposure limit value previously estimated in 2010 (Anses 2011b; Anses 2012). Thus, in 2013, it proposed an occupational exposure limit value for carbon nanotubes of 1 µg/m<sup>3</sup>.

## 4.2 Identification and hazard characterisation of nanomaterials

Given the vast number of nanomaterials, whose characteristics vary not only from those found at the macroscopic scale, but also from one nanomaterial to another, or even from one form to another, it is not possible to consider "the" hazard associated with these materials as a whole.

Consequently, identification and hazard characterisation of nanomaterials require:

- a census that is as comprehensive as possible and regularly updated, of the nanomaterials or products containing nanomaterials found on the market;
- taking into account the identification and hazard characterisation of ultrafine particles, on which there is more background knowledge and which provide greater insight on any potential hazard of nanomaterials. For example, ultrafine particles from atmospheric pollution can lead to specific health effects (rhinitis, asthma, bronchitis and cardiovascular disorders in vulnerable individuals) that could also apply to manufactured nanoparticles;
- in the case of a nanomaterial, conducting a review of the scientific literature on toxicity and ecotoxicity, which might usefully be supplemented by research on the parent material(s), i.e. the same material (the same chemical nature and crystal structure) at the micro- or macroscopic level. Indeed, it is generally accepted that the corresponding nanomaterials have at least the same toxicity, or are even more hazardous, than any parent materials, even though exceptions to this rule have been reported in the literature. This review should take into account the results of studies both in cell models (*in vitro*) and in animals (*in vivo*), or even in humans (especially for the parent material);
- with regard to occupational activity in a company or a research laboratory for a professional user, labelling information should be consulted and existing regulations enforced. For example, the safety data sheet (SDS) usually provided by the supplier can yield information on the nanomaterial handled, especially regarding toxicology and regulatory aspects. And there are indeed SDSs specific to nanomaterials containing information relating to the specific surface area, particle size, etc.

The scientific and technological advances in nanotoxicology (knowledge of the biological and physico-chemical properties of nanomaterials) are particularly evident. However, many questions still remain about the potential risks to human health and the environment:

- What are the physico-chemical properties that determine the toxicity and ecotoxicity of nanomaterials and their behaviour in the body?
- Can nanomaterials be genotoxic or carcinogenic, can they modify immune responses, induce organ-specific toxicity, or be toxic for reproduction?
- Is there a "nanospecific" effect?

The feasibility of epidemiological studies<sup>21</sup> in health is a major issue. To date, only one project for monitoring workers in contact with nanomaterials is known in France. After reviewing the various

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<sup>21</sup> Identification and characterisation of a hazard related to a product or type of products can also draw upon epidemiological studies. The starting point is then the observation of an exceptional prevalence, or one that differs from the average in the general population, of one or more diseases, in an attempt to identify the probable cause of the condition and establish a link between the handling of one or more products and the disease. However, such methods assume that the frequency of a disease can easily be linked to a group of people exposed to a suspect agent. Nanomaterials pose a problem in this regard, because of their relative novelty.

epidemiological surveillance protocols that could realistically be used to study the long-term effects of exposure to nanomaterials (Boutou-Kempf, Marchand *et al.* 2011), the French Institute of Health Surveillance (InVS) proposed setting up a two-part monitoring scheme, firstly, a prospective cohort study (which would only concern a few nanomaterials regarded as priority) and secondly, repeated cross-sectional surveys (which would investigate all nanomaterials).

#### 4.2.1 Physico-chemical characterisation

There are many physico-chemical parameters determining the properties and reactivity of nanomaterials that seem relevant to toxicology (Oberdörster, Elder *et al.* 2009) and ecotoxicology (Stone, Nowack *et al.* 2010).

##### ➤ SCENIHR opinion on the definition of a "nanomaterial"

In 2010, the European Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) issued its opinion on the essential elements of a definition of the term "nanomaterial" (Scenihr 2010). The experts worked on three points: the types of physico-chemical properties specific to nanomaterials, the size thresholds above which the specific properties of nanomaterials are likely to occur, and the methodology for physico-chemical characterisation of nanomaterials. The conclusions of the SCENIHR suggest the following:

- Although the physico-chemical properties of the materials vary depending on their size, there is no scientific justification for an upper and/or lower size limit to define all nanomaterials.
- No single methodology (or group of tests) can be applied to all nanomaterials.
- Size is the universal element that is included in all proposed definitions thus far: it is the most appropriate measurand. An understanding of the size distribution of a nanomaterial is essential, and the number size distribution is the most relevant. For dry powders, the SCENIHR also proposes using the volume specific surface area (VSSA) expressed in  $\text{m}^2/\text{cm}^3$  to identify nanomaterials: this is obtained by multiplying the specific surface area by mass ( $\text{m}^2/\text{g}$ ) by the mass density of the material (in  $\text{g}/\text{cm}^3$ ).

This shows the European expert committee's desire, in accordance with the precautionary principle, to propose a methodology which, although not denying the difficulties inherent in the uncertain nature of risks associated with nanomaterials, enables advances in knowledge about them, by adopting an appropriate, reproducible criterion for minimum characterisation.

##### ➤ OECD dossier on health and environmental safety of manufactured nanomaterials

In 2006, a Working Party on Manufactured Nanomaterials was established within the Organisation for Economic Cooperation and Development (OECD). In 2007, this Working Party implemented a programme to conduct safety tests on several nanomaterials. The results of these tests were compiled in a dossier resembling a standard chemical dossier, except that the required physico-chemical criteria had been adapted to the specific characteristics of nanomaterials. Sixteen parameters were selected and are described in a paper by the OECD (ENV/JM/MONO(2008)13/REV). Following on from this work, the OECD updated the electronic version of the data compilation to adapt it to nanomaterials, by adding 13 of these parameters (the other 3 were already in the database) to the IUCLID 5.5 database<sup>22</sup>.

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<sup>22</sup> IUCLID: International Uniform Chemical Information Database. IUCLID is a software application for entry, storage and exchange of data on the intrinsic properties and hazards of chemicals.

### ➤ Draft Standard ISO/TR 13014:2012<sup>23</sup>

In 2012, a draft standard on the physico-chemical characterisation of manufactured nano-objects subject to toxicological testing was published by the ISO (ISO/TR 13014:2012). This draft describes the relevant physico-chemical properties of nano-objects needed for risk assessment:

- particle size/particle size distribution;
- aggregation/agglomeration state;
- form;
- specific surface area;
- chemical composition, purity with the level of impurity;
- surface chemistry;
- surface charge;
- solubility and dispersibility.

This draft standard stresses the importance of not relying on the commercial characteristics stated by the suppliers, and the need to characterise the impurities that may be the main cause of adverse effects. It also recommends an independent characterisation of the physico-chemical properties of the material in the state prior to toxicity testing. This characterisation can be conducted at several levels: "as received", meaning when it first comes out of the packaging, "as administered" meaning the material prepared for its introduction into the *in vitro* or *in vivo* test systems, and finally "after administration", referring to the material after it has been inserted into the toxicology test system. These tests may also allow comparison of data generated by different laboratories. In addition, this standard proposes a plan for developing a test report. For each physico-chemical parameter mentioned above, this document provides a "description" for identifying the parameter, a "clarification" providing additional information on the parameter, a "relevance" describing the toxicological significance of the parameter according to the state of knowledge, and finally a "measurand" whose measured value is used to quantitatively assess the physico-chemical parameter. A diagram illustrating the use of physico-chemical characterisation in toxicology tests is provided in Annex A of the standard and a list of measurement methods and standards associated with each parameter is provided in Annex B.

Other specific behaviour of nanomaterials can also be determining factors, such as the kinetic constant for solubilising metal ions in the case of metallic nanomaterials. In this case, the effects can be compared to the ionised metallic form (Valdiglesias, Costa *et al.* 2013). Conversely, the potential (re)formation of nanomaterials in animals treated with metal ions from an intermediate dissolution or microscopic fragments has also been reported (Trabelsi, Azzouz *et al.* 2013; van der Zande, Vandebriel *et al.* 2012; Walczak, Fokkink *et al.* 2013).

### ➤ The European Nanogenotox<sup>24</sup> project

A European project called "Nanogenotox", launched in 2010 and completed in 2013 (Nanogenotox 2010), sought to characterise the physico-chemical properties of different manufactured nanomaterials (titanium dioxide, silicon dioxide and carbon nanotubes) as comprehensively and

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<sup>23</sup> Annex 13 lists all the standards published by ISO TC 229 until March 2014.

<sup>24</sup> Nanogenotox is a European joint action. One of its objectives was to develop a robust, sensitive and specific methodology for characterising the genotoxic hazard by studying the *in vitro* and *in vivo* genotoxicity and toxicokinetics of 14 nanoparticles (SiO<sub>2</sub>, TiO<sub>2</sub> and CNTs) that could be used to determine the genotoxic risk associated with exposure to nanomaterials. <http://www.nanogenotox.eu/>. Annex 14 lists all the technical reports associated with the project.

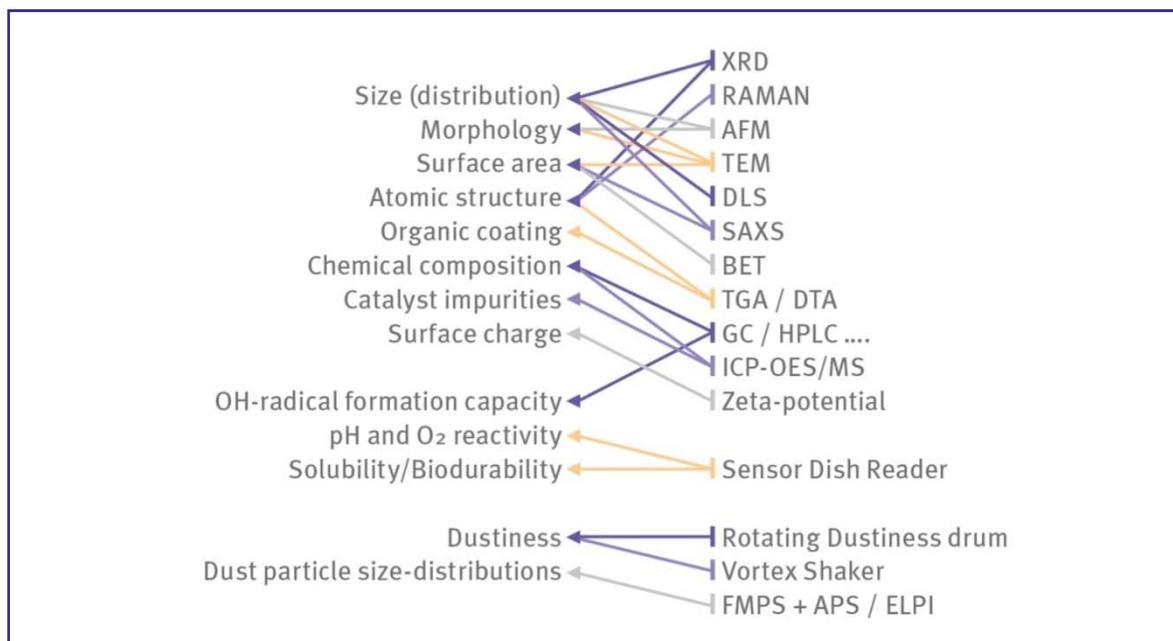
relevantly as possible, in order to develop a robust and reliable method of testing the genotoxic potential of these nanomaterials. The validated procedures are to be proposed for a project co-funded by the European Union under the 7<sup>th</sup> Framework Programme for Research & Technological Development (FP7), which is primarily aiming to develop standardised procedures for regulatory purposes (NANoREG, "a common European approach to the regulatory testing of manufactured nanomaterials").

The physico-chemical parameters measured were:

- average size (or distribution) of primary and secondary particles (aggregates);
- morphology of particles and fibres;
- atomic structure;
- chemical composition;
- contaminants;
- catalysts and the associated organic matter;
- surface charge (zeta potential according to pH).

Hydrochemical reactivity<sup>25</sup> and short-term solubility were also characterised. Similarly, the resuspension power of nanomaterials in powder form and the number size distributions of emitted particles were studied with two different dustiness tests (vortex shaker and rotating drum).

Standard operating procedures (SOP) were developed within this project. At the same time, many complementary methods (XRD, TEM, AFM, DLS and SAXS) were used to determine the size of the primary particles or other physico-chemical parameters, as shown in Figure 3. The results demonstrated the complementary nature of the measurement methods; in particular, several techniques can be used to measure the same parameter and, inversely, one technique can be used to characterise several parameters.



Source: Nanogenotox 2013 (Nanogenotox 2010)

**Figure 3: Summary list of physico-chemical parameters and measurement techniques used in Nanogenotox<sup>26</sup>.**

<sup>25</sup> Hydrochemistry studies the chemical processes that influence the distribution and circulation of chemical compounds in water.

Two different techniques were selected for measuring the size distribution of particles suspended in a liquid: DLS and SAXS. In this project, these two methods were identified as being applicable for both the SiO<sub>2</sub> and TiO<sub>2</sub> nanomaterials tested. However, doubts were raised about the use of these measurement methods for carbon nanotubes. In general, using SAXS to measure aggregates in dispersion media, prepared according to the dispersion protocol used for the toxicity tests, gave mean aggregate sizes lower than those obtained by DLS.

The specific surface area (SSA) measurements were taken using BET and SAXS, but also TEM tomography based on a 3D morphological analysis. The results showed that there seems to be a linear correlation between the data obtained by BET and those obtained by SAXS. Regarding the TEM tomography technique, despite the interesting data obtained, this method was shown to be difficult to implement at the present time.

In this project, a procedure was developed to disperse nanomaterials for *in vitro* and *in vivo* toxicity testing. This dispersion protocol uses serum albumin, identified as being suitable for stabilising particles (stabiliser) at low concentrations, in order to limit any adverse toxicological effects. The details of this protocol are available on the project website (see Annex 15, Deliverable 3 (Nanogenotox 2010)).

This dispersion protocol is stated as being applicable to all nanomaterials tested in the Nanogenotox project. However, it is unable to obtain a suspension composed exclusively of primary particles of nanomaterials; aggregates and agglomerates remain. This protocol provides stable dispersion for an hour for all the categories of nanomaterials studied, including carbon nanotubes.

#### 4.2.2 Assessment of nanomaterial toxicity

The routes of exposure to nanomaterials considered for humans are inhalation (main route for workers), ingestion (predominant route for the general population) and dermal contact. While the pulmonary route has been the subject of many research projects, the other two routes have been investigated far less in toxicology, although the presence of some nanomaterials has been quantified in foods and results on migration or release in foods from food contact materials or textiles were recently reported in publications.

Although they are still insufficient, studies seeking to precisely define the biodistribution and toxicity of nanomaterials and identify their physico-chemical parameters have increased in number over recent years. Some of these studies have demonstrated that the route of exposure and the animal species are parameters liable to modify the biokinetics and toxicological effects, in the same way as for conventional chemicals. Similarly, any surface modification of nanomaterials, such as that occurring in the presence of biological fluids during toxicological tests, is a key parameter.

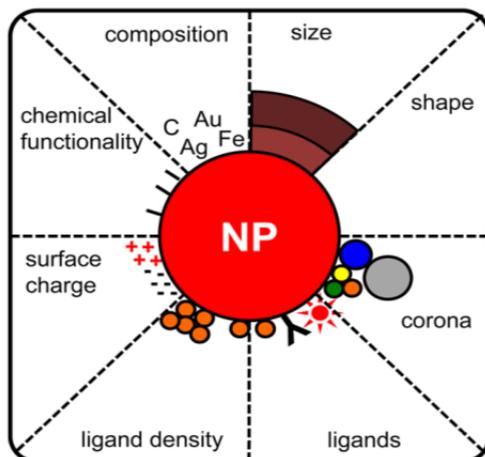
##### ➤ Biokinetic studies

Biokinetic studies have shown that the persistence of nanomaterials in the body can vary depending on physico-chemical properties that may influence control over crossing physiological barriers (see Figure 4). Similarly, the composition of the corona (crown of proteins and lipids) that envelopes the nanomaterials, even partially, could play a role in the crossing of barriers as well as in controlling cellular uptake or even exocytosis (externalising the nanomaterials) (Bashir M, Verma

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<sup>26</sup> Abbreviations: XRD: *X-ray diffraction*; Raman: *Raman Spectroscopy*; TEM: *Transmission Electron Microscopy*; AFM: *Atomic Force Microscopy*; DLS: *Dynamic Light Scattering*; SAXS and uSAXS: *Small Angle X-ray Scattering and ultrafine SAXS*; BET: *Brunauer, Emmett and Teller gas adsorption*; TGA: *Thermo gravimetric analysis*; DTA: *Differential Thermal Analysis*; GC: *Gas Chromatography*; HPLC: *High Performance Liquid Chromatography*; ICP-MS: *Inductively Coupled Plasma Mass Spectrometry*; FMPS: *Fast Mobility Particle Sizer*; APS: *Aerodynamic Particle Sizer*; ELPI: *Electrical Low-Pressure Impactor*.

*et al.* 2012; Cedervall, Lynch *et al.* 2007; Lundqvist, Stigler *et al.* 2008; Monopoli, Åberg *et al.* 2012; Shrivastava, Nuffer *et al.* 2012; Zhang, Burnum *et al.* 2011).

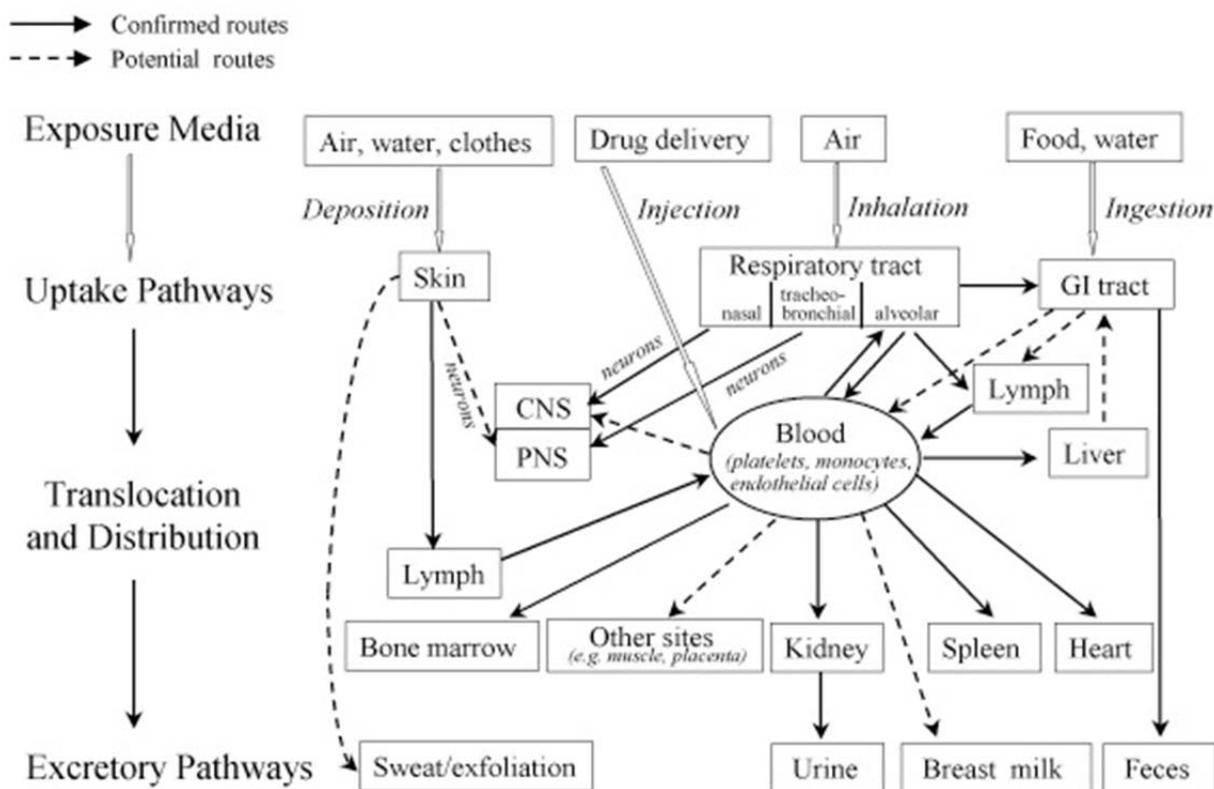


Source: Rauch (Rauch, Kolch *et al.* 2013)

**Figure 4: Physico-chemical parameters influencing cellular uptake of nanomaterials**

Thus, a growing number of studies is taking into account not only the intrinsic properties of the nanomaterials, but also those they acquire depending on the environment in which they are found (Fraga, Faria *et al.* 2013; Leite-Silva, Le Lamer *et al.* 2013; Li, Wang *et al.* 2013; McClements and Xiao 2012; Napierska, Thomassen *et al.* 2012; Prasad, Wallace *et al.* 2013; Sabbioni, Fortaner *et al.* 2014; Troncoso, Aguilera *et al.* 2012; Walkey and Chan 2012). For example, the role of surfactant in the lungs or mucus in various mucous membranes, in interactions with nanomaterials, as well as interactions with enzymes or microbial flora in tissues upon contact, are topics that have been discussed in recent publications seeking to better describe internal exposure and the factors modulating the effects of this exposure (das Neves, Rocha *et al.* 2012; Gasser, Wick *et al.* 2012; Mura, Hillaireau *et al.* 2011; Mwilu, El Badawy *et al.* 2013; Schleh, Kreyling *et al.* 2013; Schuster, Suk *et al.* 2013; Troncoso, Aguilera *et al.* 2012).

Two types of physiological barriers should be distinguished (see Figure 5): those controlling passage from the organ first exposed to the blood or lymph (alveolar-capillary, skin, intestine), and those controlling the flow of blood to the systemic organs (blood-brain, placenta, testis). The mechanisms governing the crossing of the various barriers may be distinct.



Source: (Oberdörster, Maynard *et al.* 2005)

**Figure 5: Biokinetics of nanoparticles**

While many pathways of uptake and translocation (migration of particles from their site of deposition) have been demonstrated, thus indicating a potential systemic risk (Nel, Xia *et al.* 2013; Singh, Manshian *et al.* 2009), others are still hypothetical, for example, from the bloodstream to the central nervous system (CNS) or the placenta, from the liver to the gastrointestinal tract, etc. (Oberdörster, Maynard *et al.* 2005). However, recent papers have shown precisely the capacity of TiO<sub>2</sub> nanoparticles or modelled polystyrene nanoparticles to cross the placental barrier, preventing normal embryogenesis (Hougaard, Jackson *et al.* 2010; Shimizu, Tainaka *et al.* 2009; Wick, Malek *et al.* 2010; Yamashita, Yoshioka *et al.* 2011), or to cross the blood-testis barrier (Yoshida, Hiyoshi *et al.* 2010). These results from animal studies are associated with high doses ( $\geq 0.1$  mg). However, they do raise the question of the toxicity of nanomaterials for reproduction. Overall, the molecular transport mechanisms responsible for the transport of nanomaterials to the systemic organs have not yet been elucidated (Kulvietis, Zalgeviciene *et al.* 2011).

Regarding the passage of nanomaterials to the brain, three pathways have been proposed: axonal transport, passage via the olfactory bulbs, or passage via the blood-brain barrier, after alteration of its properties by the nanomaterials themselves or in the case of disease (Simko and Mattsson 2010).

Ultimately, biokinetic assessment should provide information on internal exposure and residual concentrations of nanomaterials in the organs first exposed and the systemic organs, and also help

determine the phenomena of sequestration and translocation (including storage, retention and clearance<sup>27</sup> in the secondary organs). Particular attention should be paid to potential target organs that are rich in cells of the mononuclear phagocyte system (macrophages and cells from reticular haematopoietic organs that play an important role in phagocytosis) such as the liver, spleen, bone marrow, lungs, etc.

Knowledge of the particular behaviour of a nanomaterial in a whole organism would also enable *in vitro* tests to be conducted, to investigate the effects and/or mechanisms of action of nanomaterials. Indeed, the cells specific to or representative of a target organ, as well as the maximum concentrations determined for the tested systems, can then be selected from the results of the preliminary biokinetic assessment.

### Comments

One of the initial difficulties in analysing biokinetics is detecting the nanomaterial in the biological material, and taking into account the influence of any changes involved in its detection. In particular, if the nanomaterials are labelled (direct or indirect, fluorescent or radioactive), which facilitates their detection *in situ*, it is essential that this should not significantly alter the behaviour of the nanomaterial once administered. In addition to the detection and measurement of the tissue load on a given organ, it is essential to determine the form and/or size in which the nanomaterial is found. It should be noted that this determination can be difficult and may require specific equipment. Quantitative detection methods that can be used for nanomaterials associated with biological material should be provided. For example, nanomaterial internalisation studies have been facilitated through the use of new technologies such as time-of-flight secondary ion mass spectrometry (TOF-SIMS) or confocal microscopy coupled to Raman spectroscopy (Drescher, Giesen *et al.* 2012; Freese, Uboldi *et al.* 2012; Ingle, Dervishi *et al.* 2013; Malfatti, Palko *et al.* 2012; Sun, Chen *et al.* 2013).

Nanomaterials can be absorbed by the body and migrate to other organs, leading to a potential systemic risk. The crossing of certain biological barriers by nanomaterials has been verified (alveolar-capillary barrier, for example), while others still need to be confirmed (crossing of the placental (TiO<sub>2</sub>), blood-testis and blood-brain barriers). The ability of nanomaterials to overcome these barriers and their persistence in the body vary according to their physico-chemical characteristics, which influence not only their intrinsic properties but also their ability to interact with the environment in which they are found.

### ➤ Toxic effects

In general, the toxicological assessment of nanomaterials should be carried out in such a way as to address the majority of potential adverse effects, especially genotoxicity, carcinogenicity, immunotoxicity, reproductive toxicity and neurotoxicity. However, the strategy to be implemented to elucidate their toxicity should be adapted to the field of use and/or exposure levels.

In order to validate the toxicity tests under the experimental conditions of each laboratory, it is imperative that reference assessment methods for nanomaterials be developed.

It is also essential to ensure that the use of excessively high concentrations *in vitro* or dose levels *in vivo* does not lead to an incorrect interpretation of the results for the study of different criteria (genotoxicity, etc.). Few studies are available on the reversibility of effects that might demonstrate the reversible, persistent or delayed nature of the toxicity, for a post-treatment period of appropriate duration. This additional information can prove very useful when interpreting toxicological data and in practice, when they are used in the context of risk assessment. Thus,

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<sup>27</sup> Clearance is the ability of a tissue, organ or organism to eliminate a given substance from a body fluid (blood, lymph, etc.).

examinations carried out after a period of recovery may demonstrate a delayed effect or the regression or even cancellation of the effects. The lack of research on the reversibility of effects may lead to different biases in the interpretation and conclusion of toxicological studies. Indeed, with the onset of major clinical signs (morbidity and/or fatality) during this recovery period, reversibility testing may also indicate that the tested doses were too high, thus calling into question the relevance of the initial effects observed.

Lastly, the nanomaterials themselves should not interfere with the systems used to assess their toxicity. For example, with cytotoxicity assays in the presence of nanomaterials with oxidising potential, it is difficult to use the MTT marker<sup>28</sup>, which is sensitive to oxidation. Indeed, under these conditions, there is a possible risk of overestimating cell survival (Lupu and Popescu 2013).

### In vitro studies

For *in vitro* tests, it appears necessary to use suitable cell models that are able to internalise nanomaterials and appropriate for mimicking conditions of human exposure. Thus, the cell model used must be representative of the target organ(s): first organs exposed and/or organs exposed after translocation determined during biokinetic tests. In every case, it is preferable to use cells of human origin (Honma and Hayashi 2011) and to have a certain amount of information such as their endocytosis and exocytosis ability, their repair and apoptosis capacity, their ability to deal with reactive oxygen species, etc.

Besides the selection of the cell line, the experimental culture conditions can also be modified and improved to obtain systems that better resemble *in vivo* exposure. Thus, toxic and kinetic effects were recently assessed with more realistic exposure systems such as lung cells cultured at the air-liquid interface (Aufderheide, Halter *et al.* 2013; Mertes, Praplan *et al.* 2013; Mrakovcic, Absenger *et al.* 2013) or with more appropriate cell models, especially co-culture systems (Hackenberg, Zimmermann *et al.* 2011; Klein, Hennen *et al.* 2011; Loo, Grigsby *et al.* 2012; Napierska, Thomassen *et al.* 2012).

Furthermore, regardless of the test system used, interactions of nanomaterials with the experimental system, such as affinity for proteins, for certain nutrients, growth factors, etc., must be taken into account.

### In vitro genotoxicity

Regarding the genotoxic potential, three modes of action can be considered with regard to nanomaterials: direct interaction with DNA, interaction with the mitotic apparatus, or production of free radicals, whether or not resulting from an inflammatory process (Gonzalez, Lison *et al.* 2008; Sargent, Shvedova *et al.* 2009).

Some standard models of mutagenesis and genotoxicity do not seem fully suited to the study of nanomaterials, for the following reasons:

- possible interactions of suspensions of nanomaterials with the test systems used, especially with the different types of culture media supplemented with sera and therefore rich in protein, agar, etc.;
- the high doses recommended by the OECD regulatory guidelines are unrealistic;
- interference with cytotoxicity tests used to determine the range of concentrations;
- possible genotoxic side effects (Savolainen, Alenius *et al.* 2010; Warheit, Borm *et al.* 2007).

For example, the bacteria used in the Ames test (OECD guideline 471) have a cell wall that remains impassable for most nanomaterials. This test is therefore unsuited to the assessment of

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<sup>28</sup> MTT is 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) bromide.

mutagenesis of nanomaterials, due to a high risk of false negatives (Balasubramanyam, Sailaja *et al.* 2010; Landsiedel, Kapp *et al.* 2009; Singh, Manshian *et al.* 2009).

In contrast, the regulatory gene mutation tests for chemicals in mammalian cells (OECD 476) using murine cells (L5178Y, CHO, V79) have certain shortcomings (detoxification enzymes, p53, etc.) that may overestimate the observed effects and lead to an incorrect assessment.

However, given that some nanomaterials are able to induce structural (clastogenicity) and numerical (aneuploidy) chromosome aberrations, just like carbon nanotubes (Muller, Decordier *et al.* 2008), the *in vitro* micronucleus test (OECD 487) seems well suited for demonstrating these two types of effects, after first ensuring that the nanomaterials have indeed been internalised by the cell models used.

### In vivo studies

As far as possible, *in vivo* studies should be performed using the most realistic mode of administration, i.e., closest to that related to the route of human exposure considered. While the toxic effects resulting from pulmonary exposure have been investigated more than others (in particular to contribute to occupational risk assessment), methods of animal exposure have evolved to more realistic systems than conventional intratracheal administration, with aerosol production and exposure that is either "nose only" or of the whole animal (Creutzenberg, Bellmann *et al.* 2012; Geraets, Oomen *et al.* 2012; Jeon, Yu *et al.* 2012).

Similarly, chronic exposure to low doses should be prioritised. An increase has also been noted in publications reporting subchronic studies *in vivo* and *in vitro* (Adachi, Yamada *et al.* 2013; Hackenberg, Zimmermann *et al.* 2011; Mrakovcic, Absenger *et al.* 2013; Sang, Zheng *et al.* 2012; Seok, Cho *et al.* 2013; Shahare and Yashpal 2013; Sun, Tan *et al.* 2012). Indeed, the administration of massive doses in toxicity studies may induce non-specific toxic effects of the nanomaterial that are difficult to extrapolate to human exposure. For example, many studies have used single administration (intracavitary or pulmonary) with doses exceeding the lung overload threshold, causing cytotoxicity and then inflammation. The relevance of these observed effects is highly questionable with regard to the much lower levels of actual human exposure. Muller and Oberdörster (Muller, Decordier *et al.* 2008; Oberdörster, Elder *et al.* 2009) confirm that overly excessive dose levels should be avoided for *in vivo* studies. In his publication, Oberdörster (Oberdörster, Elder *et al.* 2009) rightly criticises the relevance of the effects on the central nervous system observed by Wang J *et al.* (Wang, Liu *et al.* 2008) following repeated bolus<sup>29</sup> treatments at the rate of 7.5 mg of TiO<sub>2</sub> administered intranasally in mice, corresponding to excessive doses in humans of 17.5 g.

### In vivo genotoxicity

The regulatory genotoxicity tests initially recommended (OECD 474 and OECD 475) target haematopoietic cells. However, bone marrow is not the tissue most exposed to nanomaterials, and the relevance of these tests on this tissue is therefore questionable. In contrast, it would be more relevant to conduct genotoxicity tests, such as the comet assay (test of primary DNA damage) or the micronucleus test, on target organs exposed first (e.g. intestine, colon, pneumocytes (Muller, Decordier *et al.* 2008)) or exposed after translocation (e.g. liver). These two approaches for detecting the genotoxicity of nanomaterials were used in the framework of the European joint action Nanogenotox (see Annex 14). In addition, the European Food Safety Authority (EFSA) recently published recommendations on the use of *in vivo* comet assays in alkaline conditions (Efsa 2012) and the OECD has begun drafting its guideline which should be made public in 2014.

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<sup>29</sup> The bolus corresponds to the one-time administration of a single dose of the drug or product, in this case single intranasal administration of TiO<sub>2</sub>.

Meanwhile, inflammation, which can induce secondary genotoxicity *in vivo* (the case with TiO<sub>2</sub>, (Trouiller, Reliene *et al.* 2009)) and cause carcinogenesis processes (Kundu and Surh 2008), should also be investigated, for instance by assaying for the presence of pro-inflammatory mediators and/or markers, counting macrophages, etc. This information may prove necessary *a priori* for the choice of doses to be tested and/or *a posteriori* for discussing the results on a mechanistic level.

There are still few available *in vivo* studies on toxicity to the nervous system or reproduction. Regarding carcinogenesis, very few publications are available, but they report effects with cobalt and nickel nanoparticles, and carbon nanotubes (Hansen, Clermont *et al.* 2006; Poland, Duffin *et al.* 2008; Sakamoto, Nakae *et al.* 2009; Takagi, Hirose *et al.* 2008).

Genotoxic effects of several nanomaterials have been demonstrated *in vitro* (CNT, ZnO) and *in vivo* (CNT, TiO<sub>2</sub>). This genotoxicity can be direct, via the interaction of nanomaterials with DNA or the mitotic apparatus, or related to the production of free radicals that may or may not result from an inflammatory process. Although few studies are available on the subject, carcinogenic effects have also been demonstrated in animals exposed to nanomaterials such as carbon nanotubes or cobalt and nickel nanoparticles. However, studies at low doses and in exposure conditions similar to human exposure are still too rarely performed and should be prioritised. There are still few available *in vivo* studies on toxicity to the nervous system or reproduction.

### Toxicity to the immune system

Given their structure and their unique properties, nanomaterials can potentially interact specifically with the immune system and be capable of modifying immune responses (Afssaps 2011).

Size is a particularly important parameter in the recognition of nanomaterials by immune cells (dendritic cells or macrophages especially). It is often mentioned that the effectiveness of phagocytosis is reduced for nanomaterials compared to larger particles. However, there are other internalisation routes. Thus, the recognition of nanomaterials by "trapping" receptors (on certain cells of the immune system) can induce the release of cytokines that can cause a pulmonary inflammatory response. In particulate form, the material can have adjuvant properties that can lead to an exacerbation or change in the type of immune response to a given antigen (Th1 vs. Th2 response) and can then induce hypersensitivity or allergic reactions (Afssaps 2011).

The absorption of nanomaterials or their recognition by human dendritic cells can also lead to immunosuppression phenomena (Afssaps 2011). Finally, nanomaterials are theoretically capable of modifying antigens themselves, leading to autoimmune manifestations. However, if nanomaterials are able to interact with the immune system, in a beneficial or harmful way, little is known about the mechanistic details of these interactions.

Assessment of immunomodulation or of the immunotoxic potential of nanomaterials is therefore recommended, especially for pulmonary exposure, but the lack of appropriate guidelines adds to the problem's complexity (Hussain, Vanoirbeek *et al.* 2012). Among the possible biomarkers of immunotoxicity, cytokines that mediate and regulate the immune response may be used, provided they are specific (Elsabahy and Wooley 2013).

Given their structure and their unique properties, nanomaterials can potentially interact specifically with the immune system and be capable of modifying immune responses. Adjuvant effects, an inflammatory reaction or on the contrary immunosuppressive phenomena have been observed with some nanomaterials.

### Toxicity for development of the nervous system

The known presence of elevated inflammatory responses, increased levels of oxidative stress, impairment of neuronal function and changes in cell morphology in adult animals suggests that exposure to nanomaterials may cause toxicity for the development of the nervous system, especially due to the greater vulnerability of the developing brain. A recent review examined the current published results on different neurotoxic effects of nanomaterials on development, in order to identify gaps for future risk assessments (Powers, Bale *et al.* 2013). Fewer than 10 animal studies have assessed developmental neurotoxicity, and yet limited evidence suggests that *in utero* and postnatal exposure to nanomaterials is possible, with results indicating changes in synaptic plasticity, gene expression and neurobehaviour. Although the available data are not robust enough to reach conclusions on the neurobehavioral risks following exposure to nanomaterials, they indicate that a thorough study of the potential toxicity for development of the nervous system is justified.

Limited evidence suggests that *in utero* and postnatal exposure to nanomaterials is possible, with results indicating changes in synaptic plasticity, gene expression and neurobehaviour. More robust studies are however required to enable an assessment of the neurobehavioral risks following exposure to nanomaterials.

### ➤ New approaches

Comprehensive "omics" approaches (genomics, proteomics, metabolomics) have also been used recently to determine the mechanisms of action involved and effect signatures (Cui, Liu *et al.* 2012; Dong, Choi *et al.* 2013; Gui, Sang *et al.* 2013; Li, Ze *et al.* 2013; Schnackenberg, Sun *et al.* 2012). With conventional chemical compounds, these methods tend to be used for further study (elucidation of a mechanism of action) or in high-throughput tests ("screening"), but can in no way replace the regulatory methods used routinely for chemical substances.

Concerning nanomaterials, rapid and innovative *in vitro* toxicity tests are currently being carried out, usually as part of high-throughput "screening" platforms (Nel, Xia *et al.* 2013). The toxicity criteria investigated include markers of cell viability and proliferation, metabolism, genotoxicity (e.g.:  $\gamma$ H2AX, (Sergent, Paget *et al.* 2012)), internalisation of nanomaterials, etc. (Fruhvirth, Fernandes *et al.* 2011).

Work to develop digital tools able to predict biological events induced by nanomaterials "from their structure and their physico-chemical properties" (Fourches, Pu *et al.* 2010; Fourches, Pu *et al.* 2011) inspired by uses of QSAR<sup>30</sup> models have recently been used (Epa, Burden *et al.* 2012; Riego-Sintes 2012; Winkler, Mombelli *et al.* 2013; Xia, Monteiro-Riviere *et al.* 2011; Yanamala, Kagan *et al.* 2013). However, the predictive *in silico* approach using QNAR ("Quantitative Nanostructure-Activity Relationship") models is not yet regarded as a sufficiently reliable methodology because of the great diversity of structures and the lack of *in vivo* and *in vitro* data on which algorithms can rely (Winkler, Mombelli *et al.* 2013).

### Conclusion

It appears from the analysis of *in vivo* and *in vitro* studies on the toxicity of nanomaterials that they are able to penetrate the body and be distributed in various organs with varying retention times. Although toxic effects have been demonstrated during exposure to some nanomaterials

<sup>30</sup> The use of "Quantitative structure-activity relationship" models (QSAR models) has developed considerably, primarily in the pharmaceutical industry. These models seek to correlate a molecular structure with specific chemical functions to a well-defined effect such as the biological activity or chemical reactivity of a molecule or protein.

(genotoxicity, carcinogenicity induced by carbon nanotubes, nickel or cobalt), other possible effects need to be confirmed.

Although several of the available studies can be criticised because of the high dose levels, unrealistic modes of administration of nanomaterials compared to human exposure, or cell models used that are sometimes not appropriate, the identified toxic effects warrant more in-depth studies. Particular attention should be paid to the experimental models used and the experimental design. In addition, development of reference nanomaterials is crucial in order to validate the toxicity tests under the experimental conditions of each laboratory.

### 4.2.3 Ecotoxicity

Nanomaterials are likely to affect different physical (air, water, soil, sediment) or biological compartments of the environment. The number of studies investigating the ecotoxicity of nanomaterials has increased significantly over the past five years. These studies have mainly been conducted in the aquatic environment (Wong, Leung *et al.* 2013), primarily in freshwater. Only a few studies have been conducted in seawater. Due to the complexity of the techniques applied to characterise nanomaterials in soil and sediment, few studies are currently available on this subject. Yet this work has great ecological relevance with regard to the aggregation and agglomeration properties and the sedimentation of nanomaterials in the atmosphere and in aquatic environments. Transfer studies of nanomaterials found in different parts of the food chain that could cause contamination at the highest levels (including humans) are only in the initial stages (Ferry, Craig *et al.* 2009; Lowry, Espinasse *et al.* 2012). The few studies on this subject have been conducted with short food chains (predator-prey model) (Wong, Leung *et al.* 2013; Zhu, Wang *et al.* 2010). Recently, a reduction in diversity and changes in the structure of soil bacteria communities exposed to gold and titanium dioxide nanoparticles was observed (Nogueira, Lopes *et al.* 2012). In all these studies, biomagnification of nanomaterials remains uncertain.

Routes of exposure (direct contact with the skin or gills, or ingestion) of organisms to nanomaterials differ according to the species considered (e.g. algae, molluscs, crustaceans, fish, soil organisms). Adsorption of nanomaterials on the surface of microorganisms such as bacteria and generation of reactive oxygen substances can damage cell membranes, thereby facilitating entry into the cell of the nanoparticles responsible for toxicity. Aquatic vertebrates, such as fish, can be exposed to nanomaterials by direct ingestion or by the entry of nanomaterials through the epithelial cells of the skin and gills. Soil organisms such as worms are also exposed by direct contact and/or ingestion of soil particles.

In general, the most frequently reported sub-lethal toxic effects include oxidative stress and genotoxicity at the sub-individual level, and stunted growth, anomalies and/or defects in development or reproduction at the individual level (Wong, Leung *et al.* 2013).

However, many uncertainties remain and can give rise to different interpretations.

Contradictory results may be partly attributed to methodological considerations. In particular, there are no standardised protocols for conducting ecotoxicity tests with nanomaterials. It has already been mentioned that a standard protocol valid for all nanomaterials is unfeasible. The variability of the results is also related to a combination of the variability of the biological models and that of the origin (synthesis method, post-processing) of the nanoparticles. A recent review (Handy, van den Brink *et al.* 2012) described the different conditions to be complied with for conducting ecotoxicity tests with nanomaterials. Some publications (see (Stone, Nowack *et al.* 2010) specify the different physico-chemical characteristics of nanomaterials that must be measured, mainly after synthesis (raw state), or in the initial dispersion medium. However, few studies have provided a complete characterisation of the size distribution, agglomeration state, surface chemistry and charge of the

nanomaterials in the exposure environment, and the change in speciation<sup>31</sup> in the recipient environments, especially at the cellular, organ or organism level. And yet many changes can occur in these parameters when nanomaterials are transferred from their dispersion medium (deionised water in most cases) into the test environment (e.g. sediment, fresh water, sea water) and into recipient organisms. Changes in these parameters depending on abiotic (e.g. salinity, oxygen content, temperature) and biotic factors (e.g. natural organic matter, interactions with bacteria) can modify the biokinetics, bioavailability and excretion, as well as the toxicity with regard to organisms, through mechanisms that are a long way from being elucidated. For example, the presence of sulphide ions in the medium will transform  $\text{Ag}^{\circ}$  and  $\text{Ag}^{+}$  into  $\text{Ag}_2\text{S}$ , giving it little or no toxicity at the predictable environmental concentrations (Levard, Hotze *et al.* 2012).

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<sup>31</sup> Speciation is the distribution of an element between its different physico-chemical forms in a given environment.

## 5 Risk assessment methods

### 5.1 Limitations of conventional methods for assessing health and environmental risks

Regardless of the field concerned (work, consumption, environment, food), a quantitative health risk assessment is typically based on identifying the hazards, defining dose-response relationships, identifying and assessing exposure and finally, characterising the risks resulting from exposure to a hazard.

In the case of nanomaterials, despite the progress made in recent years, the level of knowledge of exposure (exposure scenarios, metrological data) and the hazards (toxicity, ecotoxicity) is still insufficient for following this type of approach in its entirety.

The hazards of nanomaterials are particularly difficult to identify and characterise, mainly because of:

- the diversity of existing nanomaterials;
- the lack of data on the effects in humans (epidemiological studies in particular);
- the lack of both adapted and standardised study protocols;
- the often insufficient physico-chemical characterisation of the materials studied, which frequently means results cannot be compared between different studies;
- the many parameters liable to influence the biological effects of nanomaterials;
- uncertainties concerning the crossing of certain biological barriers;
- complexity and still incomplete understanding of the mechanisms involved in the toxicity of nanomaterials;
- the lack of validated predictive toxicity models as an alternative to animal experimentation.

It does not however seem reasonable to implement a hazard characterisation for each nanomaterial, on a case-by-case basis, even if it is justified in view of some of the above points, because of the time it would require (large number of nanomaterials and gaps in knowledge) and the issues (ethical, economic, etc.) raised by the use of laboratory animals on a very large scale.

The representativeness of the nanomaterials studied and the relevance of the doses used in experimental studies compared to actual exposure also raise questions. Indeed:

- effects observed in (eco)toxicity tests are difficult to interpret because the doses are sometimes too high (e.g. death of a rat by suffocation as a result of inhalation and not because of the toxicity of the nanomaterial);
- moreover, (eco)toxic effects may be observed at low doses, whereas at higher doses, no effect is observed.

Identifying and assessing exposure also face a number of difficulties, especially due to:

- the difficulty in identifying nanomaterials and products containing them, likely to release them during their life cycle (aging, machining, etc.);
- the difficulty in adapting methods for sampling and exposure characterisation to routine use;
- the lack of data on exposure scenarios;

- the lack of consensus on the measurement method to use and the physico-chemical parameters to be taken into consideration (chemical composition, mass, surface, number, etc.);
- the difficulty in distinguishing nanomaterials from ultrafine particles already found in the atmosphere (ambient noise);
- the lack of a validated biomarker of exposure.

For all these reasons, the conventional approach to risk assessment shows its limits in the case of nanomaterials. This justifies, at least temporarily, the use of alternative methods (mainly qualitative approaches).

## 5.2 Alternative health risk assessment methods for nanomaterials

As indicated above, and given the still limited knowledge on the toxicity and exposure levels of nanomaterials, it is therefore not possible to apply quantitative risk assessment methods. Other methods are consequently proposed. Qualitative risk assessment methods attempt to prioritise the risks in order to provide the risk manager with options for preventive action. They are based on ratings of the hazard and exposure factors, defined according to classes, with results being estimated according to risk levels.

Several alternative risk assessment approaches are currently available.

These approaches have been designed to meet different purposes (e.g. assist in prevention of occupational risks, prioritisation of risks for nanoproducts, etc.). They apply to specific objects (e.g. nanomaterials, nanoproducts, nanoparticles only, etc.) and are aimed at different targets (e.g., consumers, general public, workers, etc.). The operating principle and the logic of implementation therefore differ greatly from one approach to another.

Among the assessment or management support methods analysed, the advantages and disadvantages of those deemed most relevant by the Working Group were estimated and have been summarised in Table 6.

**Table 6: Summary of advantages and disadvantages of selected risk assessment methods and a management tool adapted to nanomaterials**

Assessment method	Advantages	Disadvantages
<p><b><u>Lux Research – 2005</u></b> (Lux Research 2005) <i>A prudent approach to Nanotech Environmental, Health, and Safety Risks</i></p>	<ul style="list-style-type: none"> <li>• Synthetic view, communication.</li> <li>• Applicable to nanoproducts.</li> </ul>	<ul style="list-style-type: none"> <li>• Major methodological problems in estimating toxic potential (ratings set by "families" of nanomaterials, does not take into account the life cycle).</li> <li>• Little explanation of the rating system.</li> <li>• No option to update the parameters used.</li> <li>• No assessment of toxicity or risk at each stage of the life cycle.</li> <li>• No assessment of uncertainties.</li> </ul>
<p><b><u>DuPont - 2007</u></b> (DuPont 2007) <i>Nano Risk Framework</i></p>	<ul style="list-style-type: none"> <li>• Identification of the relevant parameters in number and quality for a risk assessment over the life cycle.</li> <li>• Summary support matrix of available data.</li> <li>• Consideration of costs and time needed for the assessment.</li> </ul>	<ul style="list-style-type: none"> <li>• Reference work and not a structured assessment method.</li> <li>• No consideration of uncertainties.</li> </ul>

Assessment method	Advantages	Disadvantages
<p><b>Paik - 2008</b> (Paik, Zalk <i>et al.</i> 2008) <i>Control banding tool for risk level assessment and control of nanoparticle exposures</i></p>	<ul style="list-style-type: none"> <li>Simple (rating of parameters) and structured approach.</li> <li>Hazard level: integration of robust data (toxicological data on the nanomaterial) and other alternative data (data on the "parent" material and physico-chemical properties of the nanomaterial).</li> <li>The unavailability of data is not an obstacle (¾ increase).</li> </ul>	<ul style="list-style-type: none"> <li>Different purposes: tool intended for controlling risks in the occupational environment and more suited to the issue of nanomaterials than nanoproducts.</li> <li>Proportion of user subjectivity introduced in assigning ratings to various parameters.</li> <li>Criteria are insufficient for the exposure band (no consideration of the nanomaterial matrix or the processes).</li> <li>No distinction between exposure routes/environmental compartments.</li> <li>No assessment of uncertainties.</li> </ul>
<p><b>FOPH-FOEN - 2011</b> (FOPH-FOEN 2013) <i>Precautionary matrix for Synthetic Nanomaterials</i></p>	<ul style="list-style-type: none"> <li>Structured risk rating method (questionnaire with closed responses).</li> <li>Method of rating uncertainties.</li> <li>Complementary consideration of "worst case" data.</li> <li>Method already tested and updated accordingly.</li> <li>The unavailability of data is not an obstacle (max increase).</li> </ul>	<ul style="list-style-type: none"> <li>Different purposes: tool more suited to the issue of nanomaterials than nanoproducts and does not allow ranking (only 2 levels of risk).</li> <li>Insufficient number of criteria, especially for the hazard level (no toxicological data).</li> <li>No distinction between exposure routes/environmental compartments.</li> <li>Doubts over relevance of the proposed thresholds.</li> </ul>
<p><b>ANSES - 2011</b> (Anses 2011a) <i>Graduated health risk management (control banding)</i></p>	<ul style="list-style-type: none"> <li>Simple and structured approach.</li> <li>Hazard level: integration of robust data (toxicological data on the nanomaterial) and other alternative data (data on the "parent" material and physico-chemical properties of the nanomaterial).</li> </ul>	<ul style="list-style-type: none"> <li>Different purposes: tool intended for controlling risks in the occupational environment and more suited to the issue of nanomaterials than nanoproducts.</li> <li>Proportion of user subjectivity introduced via the physico-chemical parameters (solubility and reactivity of the nanomaterial).</li> <li>Criteria are insufficient for the exposure band (no consideration of the nanomaterial matrix or the processes).</li> <li>No distinction between exposure routes/environmental compartments.</li> <li>No assessment of uncertainties.</li> </ul>
<p><b>Hansen - 2011</b> (Hansen, Baun <i>et al.</i> 2011) <i>NanoRiskCat</i></p>	<ul style="list-style-type: none"> <li>Convergent objectives: specific method for assessing nanoproducts.</li> <li>Simple and structured approach.</li> <li>Summary possible and traceability of results.</li> <li>Link with REACH categories.</li> <li>The unavailability of data is not an obstacle (the level of hazard/exposure is incremented).</li> </ul>	<ul style="list-style-type: none"> <li>Expression of levels of potential hazard/exposure, not risk.</li> <li>No distinction between exposure routes/environmental compartments.</li> <li>Doubts over appropriate REACH categories.</li> <li>No assessment of uncertainties.</li> </ul>

It should be noted that the approach proposed by ANSES, shown in Table 6, although based on the process of hazard characterisation and exposure assessment, also offers recommendations for prevention. This therefore places this tool at the boundary with risk management.

Furthermore, ANSES issued an internal request in 2011 with a view to developing a pragmatic method for assessing levels of risk to health and the environment for everyday products containing nanomaterials. This work, which is still ongoing, seeks to produce a semi-quantitative method for assessing:

- levels of exposure and hazard associated with their use;
- an interpretation of these results in terms of levels of health risks;
- confidence levels to be assigned to each of these results.

The Working Group responsible for this work is therefore currently developing an intermediate approach based on the strengths of each of the above methods analysed, and is seeking a practical solution to the critical points identified.

## 6 Reduction of exposure to nanomaterials

The health risks associated with products containing manufactured nanomaterials are still currently very difficult to assess. In addition, consumer information on the presence of manufactured nanomaterials in these products is very limited.

Concerning certain professional users (producers for example), better knowledge of the exposure to and physico-chemical characteristics of nanomaterials enables the risk to be qualitatively assessed, and exposure reduction strategies to be developed in consequence.

Strategies to reduce exposure and good working practices to be applied in the companies and laboratories concerned should be designed and implemented on a case-by-case basis for the time being. They aim to reduce employee exposure to the lowest possible level and are mainly based on limiting occupational exposure (Ricaud and Witschger 2012): level of exposure, duration of exposure, number of employees exposed, etc.

In practical terms, this involves defining and implementing safe working practices that are adapted according to the results of the risk assessment. They will need to evolve gradually along with the publication of validated information on the hazards of nanomaterials to health and safety. These practices resemble the recommendations for any activity entailing exposure to hazardous chemicals. They are of particular importance because of the great capacity of nanomaterials for persistence and diffusion (aerosolisation and dispersion) in the workplace atmosphere.

In this context, particular attention should be paid to nanomaterials for which few toxicological data are available or for which initial research has demonstrated toxic effects, especially in animals.

The main themes of the exposure reduction approach are based on the hierarchical principle of implementing means of prevention, namely the STOP principle (Substitution, Technology, Organisation, Protection) already presented in the report published by the Agency in 2008 (Afsset 2008). These measures primarily include the following:

### Substitution

- modifying the process or activity in a way that no longer produces or uses the nanomaterials, or replacing the nanomaterials by non-nanoscale substances with no known health effects;
- optimising or modifying certain processes or procedures that are especially vulnerable to exposure: manufacturing or using nanomaterials in liquid form; discontinuing critical operations such as transfer, weighing, sampling, etc.; limiting the quantities of nanomaterials handled; etc.

### Technology (or collective measures)

- optimising the process to reduce dust levels as far as possible, thereby limiting exposure: prioritise closed systems, mechanised processes and automated operations;
- capturing nanomaterials emitted at the source: in the laboratory, install ventilated enclosures (glove boxes, fume hoods or laminar flow devices) and in the workshop, set up a local exhaust ventilation system (suction ring, suction table, suction splashboard, etc.),
- cleaning floors and work surfaces regularly (with wet cloths or a vacuum cleaner fitted with a very high efficiency filter, exceeding class H13) (Ricaud, Chazelet *et al.* 2011);
- filtering the air before discharging it to the outdoors: using very high efficiency "absolute" air filters exceeding class H13 according to the NF EN 1822-1 Standard.

## Organisation

- setting boundaries for the work area, erecting signs, and restricting this area solely to employees directly concerned;
- collecting and processing waste: package waste in sealed, closed and labelled bags, then send it to a Class 1 waste disposal centre, an incinerator or a cement kiln.

## Protection (personal protective measures)

- using personal protective equipment if collective protective measures prove insufficient: wear a respiratory protective device with a filter (class P3 filter) or insulator and, depending on the duration of the work, a suit with a disposable hood or Type 5 chemical protective overalls and waterproof gloves;
- in addition, as part of a process to continuously improve the performance of the means implemented, it will be necessary to:
  - train and inform the exposed employees about the potential risks and the preventive measures, according to the current state of knowledge;
  - ensure the traceability of operator exposure, i.e. note and retain all relevant information on their exposure;
  - analyse and exploit data on incidents and accidents;
  - establish medical monitoring for workers potentially exposed.

## 7 Areas for improvement in assessing the health and environmental risks associated with manufactured nanomaterials

The variety of approaches to risk assessment mentioned in the previous sections illustrates the difficulties raised by the issue of uncertain risks. Some alternative methods of assessment (inspired for instance by control banding) overlap risk assessment and risk management (CPP 2010). Is this blurring of boundaries between assessment and management specific to nanomaterials or, more broadly, is it correlated with the growing consideration of uncertain risks? In any event, this development has been addressed by sociological studies that should be taken into account. These studies suggest that, in the presence of uncertain risks, focusing too closely on the scientific aspects of the uncertainties encountered can lead to inappropriate management of the risks being considered (Borraz 2008). In this sense, it seems important to emphasise that ANSES, alongside the expert group on nanomaterials, has established a Dialogue Committee on "Nanomaterials and Health". This committee provides a forum for exchange regarding the expertise activities conducted by the Agency with the interest groups represented. It should thus provide the Agency with insight into all the types of uncertainty attached to the development of nanomaterials, providing further understanding of the problem of assessing uncertain risks.

### 7.1 Analysis of uncertainties

The identification and characterisation of uncertainties in risk assessment is covered extensively in the literature, and this is particularly the case with nanomaterials. Different definitions and approaches have been proposed, mainly depending on what is considered to be the nature of the uncertainties and their quantitative and/or qualitative dimensions. Some authors (Wickson, Gillund *et al.* 2010) point out that the lack of knowledge (likely to be overcome), which creates difficulties in applying a conventional risk assessment approach (CPP 2010), is not the only uncertain element, but is combined with uncertainties of an epistemic (related to knowledge) and ontological nature (relating to the very essence of nanotechnologies), requiring renewed and broadened assessment and governance methods (Senjen and Hansen 2011), when applying precautionary strategies (SRU 2011).

According to the classification proposed by Wickson *et al.* (Wickson, Gillund *et al.* 2010):

- quantitative forms of uncertainty (taken in the broadest sense) cover the concepts of *risk* (calculable probability of occurrence of a known adverse effect) and *uncertainty* (probability that is difficult to calculate due to a lack of information or knowledge of an adverse effect);
- qualitative forms of uncertainty fall into three distinct registers:
  - *indeterminacy* (the partial and conditional nature of knowledge mobilised during reductionist approaches does not completely rule out the risk of "surprises");
  - *ambiguity* (the various forms of production and interpretation of knowledge each provide their own values and can lead to divergent and even contradictory conclusions);
  - *ignorance* (the adverse effect is not even known and therefore cannot be considered let alone calculated).

For Ren and Roco (IRGC 2006), the classification of risks (and related uncertainties) focuses on four situations covering successive generations of nanotechnologies<sup>32</sup> and can be organised according to two categories:

- firstly, the *simple* risks and *complex* risks, whose probabilities can potentially be calculated, subject to acquisition of a minimum of knowledge on the hazards and exposure, and some societal and socio-economic insight;
- secondly, the *uncertain* risks and *ambiguous* risks that warrant more specific assessment and governance methods. Manufactured nanomaterials would fall into the first category, according to these authors.

Apart from the differences of approach regarding the risk governance modes to be promoted, there is consensus about the knowledge gap. The inadequacy of conventional risk assessment methods is recognised (Grieger, Hansen *et al.* 2013), as well as the need to characterise the uncertainties in the hope of gradually overcoming certain deficiencies. For example, it has been estimated that just assessing the toxicity of nanomaterials currently on the market in the United States could, according to various scenarios, cost (R&D) between 250 million and 1.2 billion dollars, and take between 30 and 50 years (Choi, Ramachandran *et al.* 2009).

An analysis of the scientific uncertainties and lack of knowledge about the potential risks to the environment, health and safety associated with nanomaterials (Grieger, Hansen *et al.* 2009) emphasises the many limitations to conducting quantitative risk assessments and the premature nature of any results that may ensue. Whether regarding their location, level or nature, the uncertainties are numerous. Shortcomings in the following areas of knowledge should be overcome as a priority, notwithstanding the time and resources needed to achieve this:

- assessment and development of detection equipment and measuring instruments;
- standardised tests and procedures, with a view to conducting a full physico-chemical characterisation of nanomaterials, in both biotic and abiotic systems;
- development of knowledge on toxicity, ecotoxicity and monitoring of exposure, bearing in mind that the lack of standardised methods for characterising nanomaterials hinders comparisons and the drawing of conclusions.

The bioaccumulative and persistent potential of nanomaterials in organisms should also be taken into account, as well as their fate and behaviour in the environment, including any changes or transformations they may undergo (Klaine, Koelmans *et al.* 2012).

Hazard identification is an important step in risk assessment. Despite the multiple sources of uncertainty connected with this and subsequent steps, and given the magnitude of the task to be accomplished, some authors nevertheless propose an approach based on assessing the weight of evidence (Hristozov, Zabeo *et al.* 2012) to compare and prioritise nanomaterials<sup>33</sup>. In response to the lack of standardised tests available and the contradictory conclusions that can be drawn from the different research and experimental protocols on toxicity, this approach suggests a multicriteria quantitative analytical method taking into account the quality and relevance of data.

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<sup>32</sup> Passive nanostructures (since 2000), active nanostructures and nanodevices (since 2005), integrated nanosystems (since 2010) and heterogeneous molecular nanosystems (expected in 2015).

<sup>33</sup> More generally, discussions on the assessment, both quantitative and qualitative, of existing databases and scientific knowledge on which experts rely to formulate their conclusions during risk assessment work have been listed in a SCENIHR memorandum - Scenihr (2012) Memorandum on the use of the scientific literature for human health risk assessment purposes – weighing of evidence and expression of uncertainty. European Commission, Brussels.

## 7.2 Outlook for HSS research on the issue of health risks associated with nanomaterials

There is a disparity between the density of controversies and debates on the health and environmental risks of nanomaterials, and the body of knowledge generated by the human and social sciences (HSS). There are academic publications on "nanos" relating to the ethics or social representations and, to a lesser extent, the legal aspects and the new questions posed to science. However, these publications do not necessarily take into account the risks to health and the environment.

There are hardly any publications on the industrial and economic issues, on risk management practices in industrial or manufacturing units, or on national or European public policy, for example. Several factors may explain this limitation of HSS research:

- the complexity of the problem:
  - nanomaterials are a very broad and potentially intimidating notion from a conceptual perspective for researchers in the human and social sciences;
  - nanomaterials are either heavily industrialised, and their risks assessed with inappropriate conventional methods, or at the pre-industrialisation stage, which limits the fields of study;
- the lack of cooperation between the HSS and other sciences, in addressing the complexity mentioned and the structuring of academic research. Academic structure is currently organised according to discipline and does not promote interdisciplinarity, whether in science, or in the HSS;
- the difficulty some stakeholders have in putting themselves under the spotlight of research in a context where decisional outcomes remain uncertain;
- the lack of funding for research in the HSS.

To overcome this deficiency, funding should continue being developed for HSS research applied to the fields of nanoscience and nanotechnologies, as is regularly discussed in public debates.

These funding programmes should also be designed to enable work in the human and social sciences to produce robust scientific knowledge and a critical examination of their subject, in the epistemological sense, either completely independently or as part of interdisciplinary research. Indeed, it appears that in a number of cases, especially with regard to funding dedicated to science and technology where the HSS have only been included as an aside, the expectations attached to them are, in the best case purely cosmetic, and in the worst case a form of social engineering devoid of any critical weight, whose aim is to enable the hoped-for applications of technological research to easily win the confidence of their markets. This instrumentalisation of HSS research is difficult to measure, indeed there are very few scientific publications on this subject.

In fact, alongside the funding of HSS studies, it is important to promote in the research community recognition of results that cannot rely entirely on a monolithic approach, but must often call on interdisciplinary resources from different HSS or even combining expertise from nanosciences and technologies to ensure relevance. Finally, partnerships between researchers and stakeholders should be considered.

## 7.3 Assessment of exposure and life cycle

Developments in the field of assessment of exposure to nanomaterials are expected, particularly in terms of instruments, measurement criteria and interpretation of results.

To date, it is still difficult to identify and assess the quantity of manufactured nanomaterials found in air or in matrices such as plastic packaging, food, tyres, shoes, rackets, automotive plastics, etc.

Research efforts should therefore be continued because this information is essential to the construction of exposure scenarios.

Similarly, characterising emissions and potential exposure in the workplace during operations using nanomaterials is a difficult task. However, many research projects have been initiated and future results should improve knowledge in terms of metrology, occupational exposure, strategy etc. Moreover, some of this work is also being conducted with the aim of obtaining standardised, harmonised methods (Mandate 461 of the CEN) that can be used both for scientific research and future regulations. For example, in the framework of the European NANoREG<sup>34</sup> project, work is beginning on the generation and characterisation of aerosols for inhalation toxicology studies. This work is being conducted by teams with mixed expertise in the fields of aerosols and toxicology.

Annex 9 presents current knowledge on the indicators to consider, the measurement strategy and the measurement methods and instruments used.

### 7.3.1 Exposure assessment by biometrology

The use of biomarkers of exposure or of early effects could, in the future, complement the measurement of aerosols in workplace atmospheres. This approach would have the advantage of integrating all routes of exposure, taking into account the effectiveness of preventive measures implemented, and overcoming the methodological difficulties of environmental measurements. However, it requires a good knowledge of the toxicokinetic parameters of nanoparticles and the development of sufficiently sensitive and specific assaying techniques.

### 7.3.2 Life cycle

Nanomaterials evolve during their life cycle. It is therefore imperative to take into account how they change in their environment, particularly their dissolution by oxidation (e.g. Ag<sup>0</sup>) or by reduction (e.g. Ce<sup>IV</sup>, Fe<sup>III</sup>). These changes may in fact significantly modify the effects on living organisms or their transfer. Another fundamental aspect is aggregation, which depends on interactions with compounds in their environment (proteins and salts in biological media, self-aggregation *versus* heteroaggregation by organic and/or mineral colloids in aquatic environments, and changes in reactivity, as has been measured and modelled with a n-C60 fullerene) (Hotze, Bottero *et al.* 2010); (Auffan, Pedeutour *et al.* 2010; Botta, Labille *et al.* 2011; Labille, Feng *et al.* 2010).

Finally, the study of the life cycle, from production to disposal, is a complex but essential approach that, especially in the case of an eco-design, also takes into account recycling at the end of life. The study of the life cycle of complex consumer products such as coatings, plastics, cosmetics etc. should include standardised tests that take into account the conditions of normal use and are able to model ageing, as has been done for glass and cement used as matrices for coating metal waste. The release of nanoparticles in their original state, i.e., not surface-coated with the materials in which they were incorporated, is unlikely, as has been demonstrated for sunscreen (Auffan *et al.*, 2010; Labille *et al.*, 2010; Botta *et al.* 2011). It is also important to understand the physico-chemical changes in the surface formulation in order to predict the physical chemistry of the nanomaterials depending on the environment in which they are found. Finally, given the economic and political stakes surrounding recycling, this issue must be considered for products containing nanomaterials.

As an example of one area of work, when the European NANoREG research project was being set up, a consortium of French public researchers designed and proposed an innovative method for assessing the risks associated with nanomaterials, based on an analysis of the life cycle (see Annex 11). Under this approach, there are decision trees corresponding to each stage of the nanomaterial's evolution that are used for conducting toxicology and ecotoxicology tests and

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<sup>34</sup> <http://www.nanoreg.eu/>

exposure measurements (to determine the risk). Some decision trees used for the risk assessment may be common to several stages in the life cycle. Thus, with a minimum number of tests relevant to each stage in the cycle life, it is possible to assess the risks more easily over the entire life cycle.

The "safer by design" approach, which aims to minimise exposure to or (eco)toxicity of nanomaterials from the design stage of an industrial product, implies a good knowledge of not only the characteristics that determine nanomaterial toxicity, but also the parameters that influence their biodistribution. For example, according to this approach, priority is given to the design of nanomaterials with characteristics that promote their excretion rather than their retention. Similarly, inclusion of the nanomaterials in sealed matrices, whenever possible, is another example of implementation of this approach. Understanding the mechanisms that govern the behaviour of nanomaterials in living organisms seems an indispensable step, mainly through knowledge of "bionano" interactions.

- **Definition of the "safer by design" approach:** This aims to control the potential risk of a nanomaterial *a priori* rather than committing to study *a posteriori* the potential health effects and the degree of exposure during the life cycle. It seems more rational than the approaches adopted so far by most players in nanomaterials development. It is commonly implemented today by manufacturers in the pharmaceutical sector. It does not in any way avoid the need for case-by-case studies, but their number is greatly reduced because of the choices made by the operator. The inclusion of nanomaterials in sealed matrices, whenever possible, is an example of implementation of this approach.
- **Implementation:** So far, the "safer by design/by process" approach has relied on voluntary implementation by certain industrial companies manufacturing or using nanomaterials in their processes. The recent regulatory changes, especially the adoption of the REACH Regulation incorporating the principle "no data, no market", applicable to all substances, including those that have been on the markets for a long time, appear to be an explanatory factor for this change in the behaviour of these players. It therefore seems likely, and in any case desirable, that other regulatory changes (introduction of procedures for registration or even marketing authorisation specific to nanomaterials, obligations for public communication regarding assessments of the risks associated with marketed products, etc.) will, in the same movement, broaden its scope.
- **Limitations:** Taking the life cycle of nanomaterials into account in risk assessment, associated with eco-design, is a route that could allow product formulations to be changed in order to limit the risks. The current state of knowledge on changes in manufactured nanomaterials throughout their life cycle does not, however, suggest that such an approach can provide an absolute guarantee against the potential risks associated with nanomaterials. The introduction of due diligence after products designed in this way have been placed on the market could usefully supplement these systems by analysing the evolution of nanomaterials throughout their life cycle.

In order to progress in the area of **assessment of exposure to nanomaterials and characterisation of their life cycle**, the Working Group proposes the following approaches for consideration.

**Regarding exposure:**

- develop measurement protocols and the instruments necessary to identify and quantify the nanomaterials found in any matrix (plastic, air, water, etc.):
  - chemical composition;
  - morphology of the nanomaterials, their aggregates and agglomerates;
  - concentration of the nanomaterials;
- develop aerosolisation methods applicable to any nanomaterials;
- implement harmonised measurement protocols to move towards the establishment of

exposure limits;

- continue developing measurement strategies, either by the successive phase approach (emission, transfer, receipt) or more generally (organisation of a measurement campaign).

#### Regarding the life cycle:

- give priority to studies of waste treatment: destruction or recycling;
- characterise the possible changes in the physico-chemical properties of nanomaterials throughout their life cycle;
- develop harmonised tests that have obtained the consensus of the scientific community for taking into account the life cycle and normal use of consumer products containing nanomaterials.

## 7.4 Hazard identification

### 7.4.1 Characterisation of nanomaterials

Only a combination of several measurement methods can lead to a complete physico-chemical characterisation. It is interesting to note that most recent toxicological studies have appreciated the importance of combining several methods to characterise the physico-chemical properties of nano-objects. However, the lack of standardised measurement procedures, especially in complex biological media, means that it is not yet possible to assess the relevance of the results obtained during toxicology testing. In addition, the lack of use in toxicology studies of reference nanomaterials, whether certified (NRC) or not (NR) (available only recently and still too few or too poorly understood, and often prohibitively expensive), means that metrological traceability<sup>35</sup> is not yet possible, and therefore prevents studies being compared with each other in terms of metrology of the studied physico-chemical parameters. Indeed, metrological traceability and measurement uncertainty are fundamental to obtaining reliable measurements. This point is discussed in the ISO TR 13014: 2012 Standard that deals with the physico-chemical parameters to be provided as part of an assessment of the risks associated with nanomaterials. However, measurement uncertainties (statistical and systematic) are still too rarely documented in characterisation studies and even less so in toxicology studies. Indeed, the variability of measurements from one batch to another or the repeatability between tests may be sources of potential error for the assessment of nanomaterials and are too rarely reported. However, European research projects (Nanogenotox, NANoREG) aim to address this lack of procedures, by developing standard operating procedures (SOPs) with a process of metrological traceability and assessment of measurement uncertainty.

One current scientific and technical challenge in characterising nanomaterials on work sites is the development of innovative concepts and relevant and appropriate methods (portable and easy to use). The European project Nanodevice partly responds to this challenge. Nevertheless, it seems important to emphasise that the technological developments proposed or those to be proposed in the future should take two fundamental aspects into account: metrological traceability and relevance of the physico-chemical characterisations provided.

Sampling and collection of nanomaterials in actual situations are two important parameters for obtaining high-quality characterisations, i.e., representative of actual exposure to nanomaterials during testing. To date, there are no standard protocols that achieve consensus on these two points. Nevertheless, academic, industrial and pre-normative research is underway to try to address these two issues. Examples include the following:

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<sup>35</sup> Property of a measurement result whereby it can be linked to a reference through an unbroken and documented chain of calibrations, each contributing to the measurement uncertainty (source: ISO/IEC GUIDE 99:2007 - International Vocabulary of Metrology - Basic and general concepts and associated terms (VIM)).

- the pre-normative work under Project 3 of VAMAS<sup>36</sup>: "*Techniques for characterizing size distribution of airborne nanoparticles*" conducted in the framework of "*Technical Working Area 34 - Properties of Nanoparticle Populations*". The general objective is to propose methods for characterising the size of nanoparticles in the air, including the entire measurement chain (sampling, analysis, data processing, etc.), that are traceable, easy to implement and whose measurement uncertainties have been estimated;
- TEM work as part of Nanogenotox (see Section 4.2 and Annex 15);
- INRS work using a rotating drum in the framework of Nanogenotox.

Another major difficulty involves the ability to take measurements in matrices similar to biological media or the organs tested in toxicology or ecotoxicology. This is one of the issues to be addressed by several nanomaterial analytical platforms currently being developed (see list in Annex 12).

Apart from the characterisation of nanomaterials prior to their introduction into the exposure environment, characterisation is really most relevant in the exposure environment because it represents what the test system is actually exposed to. However, the usual characterisation methods described above cannot normally be used in complex environments such as biological or environmental matrices representative of real environments. As already mentioned in Section 4.2.2, most of the physico-chemical characteristics are indeed likely to evolve in the exposure environment. Examples include speciation (the different forms in which a chemical element can be present) and the aggregation or agglomeration state etc.. Apart from a few characterisation techniques associated with large instruments, to which access is often limited and which can only be applied to reconstituted environments (i.e. simplified mimicking of real environments), it is generally not possible to determine the state in which nanomaterials are found during exposure, nor how this state changes over time. Implementation of these techniques remains complex (synchrotrons, for example). Nevertheless, efforts have been made within laboratories to develop more usable techniques such as 2D or 3D electron microscopy, or laboratory X-ray nanotomography (microscopy integrated on the above-mentioned platforms).

The results published are unable to confirm whether it will be possible to define a standard protocol adapted to the characterisation of all nanomaterials. Indeed, differences in behaviour from one nanomaterial to another (hydrophilic or hydrophobic nature of the surface, morphology, etc.) in a given solvent indicate that what works for one nanomaterial cannot necessarily be transposed to another. Nevertheless, some studies propose "standard" preparation protocols (for the sample to be characterised) that can be applied to several nanomaterials, but they also show that they are not necessarily suitable for all nanomaterials (Nanoreg, Nanogenotox, etc.).

#### 7.4.2 Toxicology

An increasing abundance of scientific literature is reporting scientific and technological advances in nanotoxicology, especially regarding knowledge of the biological and physico-chemical properties of nanomaterials.

Biokinetic studies should be conducted before toxicology tests. This is because determining the target organ(s) and the level of internal dose would help justify the further use of cells that are specific to or representative of a target organ, as well as the highest concentrations tested in *in vitro* and *in vivo* systems when investigating effects or studying toxic mechanisms of action.

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<sup>36</sup> VAMAS (Versailles Project on Advanced Materials and Standards) is an international network of collaborations on pre-normative research dedicated to advanced materials.

The shape or size in which the nanomaterial is found in the target organ may be associated with certain toxic effects, but these parameters can be difficult to determine. Quantitative detection methods that can be used for nanomaterials associated with biological material should be available soon. Indeed, studies on the internalisation of nanomaterials have been facilitated by the use of new technologies (TOF-SIMS, confocal Raman microscopy, etc.) (Drescher, Giesen *et al.* 2012; Freese, Uboldi *et al.* 2012; Ingle, Dervishi *et al.* 2013; Malfatti, Palko *et al.* 2012; Sun, Chen *et al.* 2013).

Regarding conventional toxicology models, many questions still remain about their relevance to the study of nanomaterials. In general, compared with a standard chemical substance, it appears that the toxicological assessment of nanomaterials will require adaptations both in terms of overall strategy (Which tests should be used?) and experimental procedures, such as for instance those described in the OECD guidelines (How should these tests be conducted?). These guidelines will probably need to change as consolidated toxicological data on different nanomaterials is generated, and according to advances in overall understanding of the mechanisms of action and interferences, as well as development of more suitable models, etc.

Regardless of which test system is implemented, it should be designed so as to reflect realistic human exposure as closely as possible, in terms of both exposure route and level (dose and duration). At the same time, interactions of the nanomaterials with the test system, such as affinity for macromolecules (proteins, lipids, etc.), certain nutrients or growth factors, should be taken into account. Overall, the study of the behaviour of nanomaterials or the properties they acquire depending on the environment in which they are found is a major issue in the use of the experimental data generated.

The absence of non-specific effects or of interference with the test system must be guaranteed to ensure the relevance of the results and the possibility of being able to extrapolate them to humans.

In addition, studies seeking to assess the crossing of biological barriers and elucidate the mechanisms underlying transport of nanomaterials should be implemented more widely. In the event that such crossing of barriers is proven, specific tests should be carried out *in vivo*. For example, many studies show that transcutaneous penetration of nanomaterials is negligible. However, as explained in Annex 6, the relevance of the vast majority of models used may be called into question (*ex vivo* models, or application on healthy skin only). There is a need for the development of appropriate and validated models.

The harmonisation of methods and models is clearly a key issue. Initiatives have been undertaken in this regard in Europe (Nanogenotox, for *in vitro* and *in vivo* genotoxicity) and the United States (Bonner, Silva *et al.* 2013; Xia, Hamilton *et al.* 2013).

Specific guidelines for assessing the genotoxicity of nanomaterials should be proposed. To enable the implemented strategy to be validated, an investigation of reference control nanoparticles (non-genotoxic and genotoxic) should be initiated.

There are still too few available *in vivo* studies on toxicity to the nervous system, toxicity for reproduction or carcinogenesis. This should be addressed by specific research.

Appropriate guidelines on the assessment of immune modulation related to exposure (especially pulmonary exposure) to nanomaterials should be proposed. Mechanistic research to determine the capacity of nanomaterials to interact with the immune system should be undertaken.

Although presently limited, primarily by changes in legislation, experiments *in vivo* are inevitable. Research to develop and validate models of alternatives to animal experiments should therefore continue, given the vast number of toxicity studies to be performed on nanomaterials. In this regard, the development of certain high-throughput approaches seems relevant.

In addition to the harmonisation of the metrics used for exposure, harmonisation when assessing the "biological dose"<sup>37</sup> is also a major challenge, which will allow a comparison of studies conducted by different teams, both *in vivo* and *in vitro* (Donaldson, Schinwald *et al.* 2013; Teeguarden, Hinderliter *et al.* 2007). In addition, suitable dosimetry would facilitate the interpretation of dose-response effects.

### 7.4.3 Ecotoxicology

Ecotoxicity tests do not always reflect realistic exposure scenarios from an environmental point of view. Most work has been conducted with nanomaterials synthesised in the laboratory, which therefore differ from those incorporated in the products available on the market, and are even less like the residues of nanomaterials that may be released into the environment throughout the life cycle of these products. It is therefore necessary to be able to conduct experiments simulating the entire life cycle of nanomaterials. The doses used are often much higher than those likely to be encountered in the environment. This can partly be explained by the fact that there is currently no database on concentrations of nanomaterials found in the environment, due to the technical difficulties of quantifying nanomaterials in complex environmental matrices such as water, soil and sediment. Mathematical models for predicting environmental concentrations of nanomaterials are an alternative to this lack of measured data. In addition, expressing the dose in terms of specific mass is not appropriate and should be supplemented by taking into account the physico-chemical parameters and the aggregation or agglomeration state of the nanomaterials in the exposure environment. There is also a critical lack of studies taking into account the fate, behaviour and toxicity of "mixtures" of nanomaterials, or the toxicity associated with the presence of other substances conveyed to their surface, such as polycyclic aromatic hydrocarbons or polychlorinated biphenyls - PAHs and PCBs (the Trojan horse effect (Auffan, Rose *et al.* 2012).

Furthermore, most ecotoxicity studies have been conducted with a model species (bacteria, algae, invertebrates, vertebrates) exposed to a nanomaterial in a single environment (water, soil or sediment) for periods rarely exceeding three weeks. This work can therefore assess toxicity (lethal and sub-lethal toxicity tests) and the toxic mechanisms of the observed effects of the studied nanomaterials with regard to a species, but are unable to assess the impact of nanomaterials alone or in combination, or that of their residues, on the ecosystem as a whole. To do this, conducting experiments in mesocosms<sup>38</sup>, in the laboratory or in the natural environment, is a credible alternative on which there seems to be consensus at the national, European and US levels (see (MESONNET 2010-2014), NANoREG and iCEINT). Indeed, this type of experimental tool furthers understanding of the fate of nanomaterials (transfer in the water column via processes of aggregation, sedimentation, transformation) and their residues in different environmental compartments (water column, sediment), and helps to study not only their toxicity to organisms of different species (e.g. algae, bacteria, molluscs, fish) but also their trophic transfer<sup>39</sup>, for exposure durations of up to several months. The choice of ecologically relevant species, i.e., those playing an important role in the structure and functioning of ecosystems, is absolutely crucial. Regarding the mechanisms of biokinetics, bioaccumulation and excretion, these are far from being elucidated. It is also essential to be able to locate nanomaterials in organisms and tissues. This is possible in

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<sup>37</sup> The biological dose is the dose that causes a health effect. It is generally calculated for an organ or tissue. For nanomaterials, the issue raised is twofold: to express the biological dose in the most appropriate manner and to encourage harmonisation of the unit in which this dose is expressed.

<sup>38</sup> A mesocosm refers to an experimental system that simulates real-life conditions as closely as possible while controlling a number of environmental factors (FAO (2009) Biosafety of Genetically Modified Organisms: Basic concepts, methods and issues. Food and Agriculture Organization of the United Nations, Rome.

<sup>39</sup> A food chain consists of a succession of living organisms interconnected by their nutritional needs. Trophic transfer refers to the potential transfer of nanomaterials between each constituent living organism in a food chain.

model environments reconstituted using large instruments or through isotopic labelling (whether or not radioactive). It is therefore difficult to consider using this type of analysis for routine or systematic studies. Finally, mesocosms enable work to be conducted on deteriorated products (e.g. cement or exterior paints), and also on sludge from wastewater treatment plants (STEPs) containing nanomaterials that require serious study, given that the sludge produced is either incinerated or applied to agricultural soils.

Among the toxicity mechanisms, while oxidative stress has been observed in most studies, immunotoxicity and genotoxicity have also been reported. However, no ecotoxicity mechanism "specific" to nanomaterials compared with conventional contaminants has so far been elucidated.

The antibacterial properties of many nanomaterials, such as those containing silver, could also lead to changes in bacterial communities (Colman, Arnaout *et al.* 2013) or to the development of bacterial resistance. With this in mind, effluent or sludge from urban wastewater treatment plants (STEPs), for example, are important sources of silver in forms that differ from the original source ( $\text{Ag}^0$ ), but that may change in environments such as soil and surface water into the original reduced form, thereby restarting a "cycle" of oxidation-reduction.

In order to progress in the area of **characterisation of the hazard associated with nanomaterials**, the Working Group proposes the following approaches for consideration.

#### Regarding characterisation of nanomaterials:

- to enhance understanding of the behaviour and effects of nanomaterials, it is necessary to document:
  - the concentration;
  - physico-chemical parameters such as shape, size of a primary particle, size distribution, structure, composition, specific surface area, surface properties, electrical charge, agglomeration state;
  - the dissolution properties due to oxidation or reduction (e.g.  $\text{Ag}^0$  dissolved by oxidation and precipitated in the presence of  $\text{Cl}^-$  or  $\text{HS}^-$  ions);
- develop analytical techniques for characterising nanomaterials, including the corona of proteins or lipids surrounding these nanomaterials, in realistic exposure conditions (concentrations, duration of exposure) and in complex matrices (biological fluids, water, soil/sediment);
- develop analytical techniques for locating nanomaterials in organisms and cells;
- develop techniques for separating the original manufactured nanomaterials from those naturally present in the environment (e.g. use of stable isotopes);
- promote the development of standardised, harmonised protocols and sample preparation methods;
- use reference nanomaterials to calibrate measuring devices.

#### Regarding toxicological and ecotoxicological assessment:

- be able to estimate the quantities of nanomaterials released into the environment, according to the quantities of nanomaterials produced by industry and placed on the market. Mathematical models are alternative tools for achieving this;
- determine dose-response relationships;
- understand the mechanisms of biokinetics, bioaccumulation, distribution and excretion of nanomaterials in living organisms;
- extrapolate *in vitro* results to *in vivo*, and conversely use *in vivo* data to develop relevant *in vitro* models;
- study the fate and transformation of nanomaterials in living organisms in order to better determine their toxicity mechanisms and their cell targets;

- study the fate, behaviour and toxicity of "mixtures" of nanomaterials, or in the presence of other contaminants such as PAHs and PCBs;
- promote the development of tools for early hazard characterisation (QNAR/QSAR analyses, high-throughput platforms, "omics" techniques, etc.);
- consider the advantages and disadvantages of different assessment approaches (case-by-case, categorisation, "safer by design");
- conduct experiments simulating the entire life cycle of nanomaterials. The study of their fate and long-term behaviour, alone or in combination, their interaction with the various health and environmental compartments or with living organisms, their bioavailability, bioaccumulation, biodegradation and their modification of physico-chemical properties is all very important;
- assess the fate, behaviour and impact of nanomaterials and their residues in an ecosystem as a whole. To do this, experiments in aquatic and terrestrial mesocosms are an essential approach, as has been shown for example with metals and pesticides. Study the trophic transfer of nanomaterials in food chains;
- use species representative of different environmental compartments (water, soil, sediment) that play a key role in the structure and functioning of ecosystems;
- identify sub-lethal biomarkers at different levels of biological organisation (sub-individual, individual, population, community) specific to the toxicity of nanomaterials.

## 8 Conclusions of the Working Group and research outlook

### 8.1 The conclusions of the Working Group

#### **Manufactured nanomaterials: substances found on the market whose risks must be assessed**

In the last few decades, manufactured nanomaterials have no longer been confined to the laboratory, but have gradually been integrated into many industrial processes. They are now found in a wide range of everyday products (sunscreen, textiles, food, paint, etc.) and concern a large variety of industrial sectors such as construction, automotive industry, packaging, chemicals, environment, agri-food, energy, cosmetics and health products. This availability on the market and the accompanying controversies have led to questions being asked about the state of available knowledge on assessment of the risks associated with these substances, in particular concerning exposure of the general and working populations and the hazards to health and the environment.

#### **The contribution of previous studies on natural substances or substances unintentionally produced at the nanoscale**

Manufactured nanomaterials are, by definition, distinct from all nanoscale substances present in the natural environment or produced unintentionally through various industrial and domestic processes. But this does not mean that the knowledge produced in the field of unintentional nanomaterials should be ignored. As mentioned in the body of this expert report, the question arises of the use of results from studies conducted on natural or unintentional nanoscale substances (for example, on the ultrafine particles from air pollution or forest fires). These particles often exhibit characteristics that are very different from those of manufactured nanomaterials (complex chemical composition, variable and changeable, presence of chemical entities that themselves have recognised toxicological properties, such as PAHs, nitro-PAHs, VOCs, etc.). However, it might actually prove very useful to draw inspiration from the experimental methodologies developed for them (characterisation, experimental models, realistic dose levels, etc.) and to take advantage of the numerous studies (epidemiological and experimental) conducted on these particles, which have, in some respects, similar behaviour to manufactured nanomaterials.

#### **Difficulties encountered in assessing the risks specifically associated with manufactured nanomaterials**

Concerning nanomaterials, it has proved very difficult to summarise knowledge of their toxicology and ecotoxicology, for the following reasons:

- The research conducted generally highlights the fact that each case is unique, i.e. the toxic and ecotoxic behaviour varies, not only according to the types of nanomaterials, but also within the same family. For example, nanomaterials can differ depending on their manufacturing conditions but also within a single synthesis process (reproducibility). The change in these materials throughout their life cycle (change in the degree of oxidation, whether or not associated with dissolution and precipitation in a mineral form different from the original one, homo- and hetero-aggregation, adsorption, etc.) is an additional source of complexity that should not be neglected;
- The scientific literature to be taken into account when dealing rigorously and thoroughly with the toxic or ecotoxic aspects of nanomaterials is vast. Assessing the risks associated

with nanomaterials requires a multidisciplinary approach, which is essential to achieving a deeper understanding of their risks;

- Finally, there is no evidence that the publications on nanomaterials have adopted a single definition of what constitutes a nanomaterial, providing an additional element of uncertainty. Indeed, although there is now an institutional definition of nanomaterials, recommended by the European Commission<sup>40</sup>, the scientific nature of its content is still being debated. The Working Group found that the institutional definition of manufactured nanomaterials does not take into account many physico-chemical parameters that can define their specific properties. These properties result from their complex chemical composition and size, as well as other parameters that are more complex to measure (see the eight parameters described by ISO TC 229) than those highlighted by the proposed definition.

### **Progress has nevertheless been observed**

The work of the expert group identified significant progress in the knowledge produced in recent years, in the following two areas:

#### **1) Risk assessment:**

- more comprehensive physico-chemical characterisations of the nanomaterials tested, including in nanoproducts, and in complex biological and environmental media, comprising the stability and evolution of physico-chemical parameters in these matrices:
  - more physico-chemical parameters being measured;
  - more frequent combination of measurement methods for a single parameter;
- development or adaptation of toxicological and ecotoxicological tests using more realistic exposures (in addition to acute exposure tests, exploration of chronic exposure tests, adaptation of the concentrations tested, development of studies in terrestrial and aquatic mesocosms);
- attempts at harmonisation and standardisation in physico-chemical characterisation and toxicological and ecotoxicological tests;
- more numerous published studies on the environmental impact;
- better documentation of exposure conditions in scientific articles (e.g. draft standard being prepared on measurement of exposure).

#### **2) Risk management and control:**

- establishment in 2013 of a reporting requirement in France for substances with nanoparticle status, following the work by AFSSET (Afsset 2008; Afsset 2010). Other countries such as Belgium, Italy and Denmark have followed suit (each in their own particular way) and mandatory reporting is now being considered by other countries such as Germany and the United Kingdom;
- guides written on best practice at work and on the related means of prevention (the first of which were published in 2008);
- development of tools for risk assessment based on control banding of risks..

The holding of several public debates, including the national public debate (2009-2010), should also be mentioned as evidence of progress in organising discussion and managing the potential risks associated with manufactured nanomaterials.

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<sup>40</sup> Communication from the Commission to the European Parliament, the Council and the European Economic and Social Committee. Second regulatory review on nanomaterials, 3.10.2012

## Reiterating the observation about the lack of knowledge of the risks associated with nanomaterials

Despite the advances mentioned above, the fact remains that knowledge of toxicity, ecotoxicity and exposure remains fragmented and it is still very difficult to rule on the health risk associated with the use of a given nanomaterial in a particular everyday product. In any event, the risk cannot be excluded.

Uncertainty remains as to:

- the properties (physico-chemical and (eco)toxicological) of the nanomaterials studied and how they may change depending on the environment;
- the methods and techniques available - or that need to be deployed - to characterise these properties (in physico-chemical and (eco)toxicological terms); there are still no suitable simple reference protocols that can be used universally, despite attempts by several national and international research programmes;
- knowledge about the exposure of populations and their environment to nanomaterials.

The establishment in 2013 of an inventory in France, through a mandatory reporting scheme, has the stated aim of learning more about the nanomaterials marketed in France, along with their volumes and uses, and establishing some traceability in the sectors in which they are used. The information reported to define the identity of nanomaterials (physico-chemical characterisation) will most certainly evolve, given predictable changes in characterisation methods. However, the implementation of the reporting scheme will initially help gain insight into the production and importation of nanomaterials in France, with the aim of improving understanding of exposure of populations and the environment to these substances.

Based on the current thinking of the expert group, it seems that two conclusions can be drawn, which can be seen as partially conflicting:

1. a case-by-case analysis of each nanomaterial seems difficult to apply, considering their large number. Such a systematic analysis is not feasible for managing the current situation in the short to medium term, given the time needed and the extensive use of laboratory animals it would imply;
2. while it is possible, and probably even desirable, to establish categories of nanomaterials, their relevance with regard to the data currently available for risk assessment is still being debated.

We therefore consider the following proposals for action to be open to too much criticism to be admissible in the current state of knowledge:

- requests put forward by some stakeholders for a partial or total moratorium, in the absence of an adequate risk assessment. For example, requests for implementation of a partial moratorium on certain products in contact with the human body (cosmetics, food, etc.) or a full moratorium on all nanotechnology products;
- approaches to risk management based solely on a graduated assessment of the risks, mentioned in the report;
- categorisations by effects or according to the physico-chemical properties, which are presently under consideration but not sufficiently advanced.

Given these conclusions, there is a need to propose possible solutions that can be implemented rapidly, while not relying exclusively on the approaches mentioned above. Although the Working Group agrees on the current issues, uncertainties remain, which are a source of debate about both the proposed solutions and how their implementation should be prioritised.

The formation of groups of nanomaterials within assumed risk categories, or the "safer by design/by process" approach seem to be interesting alternatives that should be assessed with a view to demonstrating their effectiveness.

Consequently, alongside the development and validation of the categorisation and "safer by design/by process" approaches, the Working Group recommends continuing risk assessment on a case-by-case basis, which is necessary for the development and assessment of new solutions.

All these approaches should also be systematically accompanied by an assessment of exposure throughout the life cycle of nanomaterials.

## 8.2 The Working Group's recommendations

Based on these findings, the Working Group offers recommendations in terms of public dialogue, regulations, targeting research and assessment of the risks associated with nanomaterials. Given the complexity of the "nano" field and the current uncertainty, these recommendations will need to be reassessed at regular intervals. They are the product of collective discussions at a given point in time based on an analysis of the literature produced up until September 2013.

### Regarding public dialogue

- In the risk governance process for nanomaterials, there should be work towards transparency and greater participation by the groups concerned (citizens' associations, social partners, health professionals, etc.), especially regarding the suggestions made above.

### Regarding regulations

- The text of the Ministerial Order of 6 August 2012 relating to the content and submission conditions of annual declarations of substances with nanoparticle status pursuant to Articles R. 523-12 and R. 523-13 of the French Environmental Code repeatedly stipulates in its annexes that respondents must explain the methods they have used to determine particle size, distribution, shape, etc. The magnitude of the uncertainties persisting here might however justify reinforcing this requirement and insisting that respondents systematically use several characterisation methods and explain in detail all the methods used and their respective results;
- As it stands, REACH is only very partially applicable to nanomaterials, mainly because of the high thresholds in the amount produced, as stipulated by the procedure. It is suggested that these thresholds be lowered to reflect the nature of production of the targeted substances by companies, which is still mainly on a small scale. This does not preclude other forms of regulation being implemented in the future to take account of the specific characteristics of nanomaterials. Similarly, it would seem necessary to develop safety data sheets (SDS) specifically adapted to nanomaterials, that would accompany the substances involved throughout the life cycle of the products;
- So far, the "safer by design/by process" approach has relied on voluntary implementation by certain industrial companies manufacturing or using nanomaterials in their processes. The recent regulatory changes, especially the adoption of the REACH Regulation incorporating the principle "no data, no market", applicable to all substances, appear to be an explanatory factor for this change in the behaviour of these players. It therefore seems likely, and in any case desirable, that other regulatory changes (introduction of procedures for registration or even marketing authorisation specific to nanomaterials, obligations for public communication regarding assessments of the risks associated with marketed products, etc.) will also have the result of broadening its scope. The introduction of due diligence after products designed in this way have been

placed on the market could usefully supplement these systems by analysing the evolution of nanomaterials throughout their life cycle.

### Regarding research

- It is important that public scientific organisations and manufacturers continue their efforts in this field to develop innovative concepts and effective methods (easy to use for sampling, collection and characterisation) for assessing the risks associated with manufactured nanomaterials. High-quality characterisation is needed, i.e. representative of real exposure of workers and consumers to nanomaterials, as well as exposure of the public in general and the environment;
- A major harmonisation effort is still required in analytical procedures, primarily to systematise the detailed characterisation of nanomaterials whose effects are to be studied (physico-chemical parameters, surface state, agglomeration state, etc.) in order to be able to compare the (eco)toxicological studies on these nanomaterials with each other. It therefore seems essential in the future for scientific organisations such as national metrology institutes and standardisation committees to focus their efforts in order to improve the metrological traceability of physico-chemical characterisations of nanomaterials. This can be achieved in particular by developing new nanoscale reference materials, whether or not these have been certified, and by establishing standardised and validated procedures based on consensus to ensure reliable estimation of any measurement uncertainties;
- Similarly, in toxicology, it is necessary to continue efforts to adapt existing models and develop and validate new models, tests or methods for assessing nanomaterial toxicity (cell models that are more representative of the target organs, development of new toxicity tests, new methods of mimicking exposure, high-throughput systems for increasing the speed of investigation, etc.), while working with realistic controlled doses in an attempt to define a comprehensive strategy for assessing nanomaterial toxicity. The availability of reference nanomaterials (i.e. that can be used as positive and negative controls in tests) is necessary to enable these models, tests or methods to be validated;
- It also seems necessary to consolidate knowledge from specific studies on the affinity of nanomaterials for proteins (the *corona*) in order to determine a "signature";
- Concerning the impact on the environment, given all the different media (soil, air, water) and species, the published studies are still a long way from covering the full diversity of situations. Efforts must therefore be continued to learn more about each step of the life cycle, especially with the development of work in mesocosms.

### Regarding risk assessment methods

- Grouping nanomaterials together in categories according to their effects, mainly with the aim of reducing the large number of configurations to be considered, is a goal that seems difficult to achieve in the immediate future. However, it remains a desirable objective, and efforts beyond the industrial sector that initiated this approach should be continued to this end, especially by public research stakeholders;
- To overcome the limitations imposed by the "case-by-case" approach and reduce the number of case studies, the Working Group considers it appropriate to develop and assess the relevance of new alternative approaches to risk assessment;
- Taking into account all the stages, from synthesis to the end of life of the nanomaterial (the "life cycle" approach), would enable the development of strategic frameworks considering the overall problem of assessing the risks associated with nanomaterials (physico-chemical and (eco)toxicological characterisation and exposure).

The collective expert appraisal was validated by the Expert Committee on "Assessment of the risks related to physical agents, new technologies and development areas on 17 December 2013.

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# ANNEXES

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## Annex 1: Internal request letter

Maisons-Alfort, 11 January 2012

### INTERNAL REQUEST

#### regarding the missions of the Working Group on "Nanomaterials and health - food, environment, work"

ANSES may decide independently to investigate any issues within its area of expertise (Article R. 1336-16 of the French Public Health Code).

This document describes the questions asked and the main features associated with an internal request.

#### Themes and objectives:

The proposed internal request aims to respond to the challenges in terms of knowledge and especially monitoring of the hazards, exposure and potential health risks associated with manufactured nanomaterials found in the environment or food-related practices, for the general population and in the workplace.

#### Background to the internal request:

The potential health issues associated with the development, use and environmental dispersion of manufactured nanomaterials are recognised as an important emerging risk. Since 2006, the Agency has published several expert appraisal reports on the health risks related to exposure via food, the environment and the workplace, that highlight the needs in terms of knowledge, monitoring and research on the hazards, exposures and potential risks to human health.

Alongside these expertise activities, the Agency has made a major contribution to the development of new risk assessment methodologies, especially aimed at professionals, and the definition of health and environmental safety tests, at both national and international levels (AFNOR, ISO, OECD, European Commission).

ANSES's expertise work on manufactured nanomaterials includes scientific monitoring, health and environmental risk assessment, methodological developments, studies investigating exposure of specific populations to nanomaterials, and the dissemination of information to different audiences (general and professional populations). The call for research projects in environmental and occupational health also serves to support research projects in the Agency's spheres of competence.

Development of scientific monitoring of manufactured nanomaterials and their potential risks to health and the environment is essential to ensure consistency between the different expertise activities coordinated by the Agency. Accordingly, to meet this need, the Agency proposes establishing a permanent Working Group (WG) on "Nanomaterials and health - food, environment, work" under the auspices of its Expert Committee (CES) on "Assessment of the risks related to

physical agents, new technologies and development areas", whose purpose will primarily be to produce, every year, a review of knowledge on the hazards, exposures and health and environmental risks associated with nanomaterials, for all their uses. The group's work will be carried out in close coordination with the CESs with competence in regulated products at European (REACH, etc.) and national levels, especially with regard to the physico-chemical characteristics of nanomaterials, and with the CESs with competence in risks related to food, air, and water.

## Issues to be addressed by the expert appraisal work:

- **produce an annual review of knowledge on the potential health and environmental risks associated with manufactured nanomaterials for all their uses.**

Such a publication would involve considering all of the literature published on the subject during the previous year and a thorough analysis of the main articles identified by the members of the Working Group (WG). The Agency, supported by this Working Group, intends broadening this work to include a legal and media watch, and the use of analyses and expert reports by international organisations and counterpart health agencies in other countries;

- **identify emerging signs of the hazards and risks associated with manufactured nanomaterials, for all their uses.**

Significant new bibliographic data could be analysed quickly by the WG set up by the Agency, with a view to assessing its importance in terms of contribution to risk assessment. With the help of the monitoring unit, the Agency will ensure that all publications of interest are considered, including "lay" publications;

- **help respond to requests for expert appraisal made to the Agency.**

Some questions put to the Agency on the risks associated with the uses of nanomaterials, particularly in the areas of food, environment or work, can be appraised by the Working Group.

The WG will then focus its activities, insofar as possible, on:

- issues related to the health consequences of uses of nanomaterials for the general population or the environment (food, packaging, industrial products, consumer products, etc.);
- requests for expert appraisal on occupational exposure to nanomaterials;
- requests for support from ANSES's supervisory ministries in drafting a French response during public consultations on international documents (reports, definitions, guides, etc.);

- **propose annual recommendations for targeting research, in particular as input for the Agency's call for research projects in environmental and occupational health;**

- **support the Agency in dialogue with society in the field of risks associated with manufactured nanomaterials.**

The Agency will set up a dialogue committee with stakeholders, coordinated by the "risks and society" unit, and will facilitate the discussions of this dialogue committee with the above-mentioned WG.

The dialogue committee will aim to discuss the state of knowledge, methodologies for

health risk assessment, and scientific debates underway on the hazards and major health issues related to exposure to nanomaterials, in conjunction with the Agency's activities.

The work programme and the work produced by the WG will be presented to the dialogue committee, whose members will include the Chair and Vice-Chair of the Working Group.

This body will also be consulted on the definition of research directions in the context of the call for projects issued by the Agency. The scientific questions raised by the dialogue committee will be relayed to the WG, which may include them in its work, to the extent that this is possible.

### **Planned duration of the expert appraisal:**

The missions are assigned to the Working Group for the duration of its existence. This is a "permanent" group, which is planned to be renewed at least every three years.

**The Director General**

Marc Mortureux

## Annex 2: Review of existing definitions for nanomaterials

This review of standards-based and regulatory definitions relating to the issue of nanomaterials is essentially based on the European report of the Joint Research Centre (JRC)<sup>41</sup>, supplemented by contributions from the Working Group's discussions.

### ► Standards-based definitions

Technical Committee 229 (TC 229) of the International Organization for Standardization (ISO) is the main technical committee responsible for standardisation work related to nanotechnologies. In addition to a number of specific working groups, ISO/TC 229 has established a coordination group to further harmonise work on this issue by the relevant ISO technical committees as well as other organisations, and to identify gaps and cross-cutting opportunities. Within the European Committee for Standardization (CEN), Technical Committee 352 deals with nanotechnologies.

Several of these definitions have already been published in the form of technical specifications (TS).<sup>42</sup>

According to ISO TS 80004-1, the term nanomaterial is defined as follows:

<b>Nanomaterial:</b>	Material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale.
<b>Note:</b>	This generic term is inclusive of nano-object and nanostructured material.

The definitions of the terms nano-object and nanoscale are given below. Thus, nanomaterial is here defined as the sum of two subcategories: nano-objects and nanostructured materials. Note that the two categories are partly overlapping: nano-objects can be nanostructured.

The following core terms related to the definition of nanomaterial were released in August 2008 by ISO/TC 229 and by the CEN through CEN ISO/TS 27687:2008:

<b>Nanoscale:</b>	Size range from approximately 1 nm to 100 nm.
<b>Note 1:</b>	Properties that are not extrapolations from a larger size will typically, but not exclusively, be exhibited in this size range. For such properties the size limits are considered approximate.
<b>Note 2:</b>	The lower limit in this definition (approximately 1 nm) is introduced to avoid single and small groups of atoms from being designated as nano-objects or elements of nanostructures

<b>Nano-object:</b>	Material with one, two or three external dimensions in the nanoscale.
<b>Note:</b>	Generic term for all discrete nanoscale objects.

The CEN ISO/TS 27687:2008 also quotes an existing general definition for particles from the ISO (ISO 14644-6:2007), which it specifically applies to nano-objects:

<sup>41</sup> JRC Reference Reports. "Considerations on a Definition of Nanomaterial for Regulatory Purposes" (2010) available via the following link:

[http://ec.europa.eu/dgs/jrc/downloads/jrc\\_reference\\_report\\_201007\\_nanomaterials.pdf](http://ec.europa.eu/dgs/jrc/downloads/jrc_reference_report_201007_nanomaterials.pdf)

<sup>42</sup> [http://www.iso.org/iso/fr/iso\\_technical\\_committee?commid=381983](http://www.iso.org/iso/fr/iso_technical_committee?commid=381983)

<b>Particle:</b>	Minute piece of matter with defined physical boundaries.
<b>Note 1:</b>	A physical boundary can also be described as an interface.
<b>Note 2:</b>	A particle can move as a unit.
<b>Note 3:</b>	This general particle definition applies to nano-objects.

It is interesting to note that this definition of a particle also includes liquids, e.g. droplets or micelles in emulsions.

The CEN ISO/TS 27687:2008 also gives definitions for particles clustered in agglomerates and aggregates. These definitions were prepared in collaboration with ISO/TC 24/SC 4 (Particle characterisation).

<b>Agglomerates</b>	Collection of weakly bound particles or aggregates or mixtures of the two where the resulting external surface area is similar to the sum of the surface areas of the individual components.
<b>Note 1:</b>	Agglomerates are weakly bonded, for example by van der Waals forces or simple entanglement forces.
<b>Note 2:</b>	Agglomerates are also named 'secondary' particles to distinguish them from the original individual particles named 'primary' particles.

<b>Aggregates</b>	Collection of particles comprising strongly bonded or fused particles where the resulting external surface area may be significantly smaller than the sum of calculated surface areas of the individual components.
<b>Note 1:</b>	Aggregates are held together by strong forces, for example covalent bonds, or those resulting from sintering or complex entanglement.
<b>Note 2:</b>	Aggregates are also named 'secondary' particles to distinguish them from the original individual particles named 'primary' particles.

The ISO/TC 27687:2008 Standard is currently being revised by TC 2296.

► **OECD definitions**

In 2006, the Organisation for Economic Cooperation and Development (OECD) established the Working Party on Manufactured Nanomaterials (WPMN) under the OECD Joint Chemicals Programme. The WPMN definitions, agreed upon in 2007, are as follows:

<b>Nanoscale</b>	Size range typically between 1 nm and 100 nm.
<b>Nanomaterial</b>	Material which is either a nano-object or is nanostructured.
<b>Nano-object</b>	Material confined in one, two, or three dimensions at the nanoscale.
<b>Nanostructured</b>	Having an internal or surface structure at the nanoscale.
<b>Manufactured nanomaterials</b>	Nanomaterials intentionally produced [for commercial purposes] to have specific properties or specific composition.
<b>Note 1:</b>	The WPMN considers that fullerene molecules are included within the scope of manufactured nanomaterials.
<b>Note 2:</b>	The WPMN considers that aggregates and agglomerates are nanostructured materials along the lines of ISO.
<b>Note 3:</b>	Those end-products containing nanomaterials (e.g. tyres, electronic equipment) are not themselves nanomaterials.

► **Definitions of the EU Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR)**

The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) is an independent scientific committee set up by the European Commission (EC). It provides scientific advice to the EC on issues related to consumer safety, public health and the environment. In a 2007 Opinion document, SCENIHR provided several suggestions for definitions:

<b>Nanoscale</b>	A feature characterised by dimensions of the order of 100 nm or less.
<b>Nanostructure</b>	Any structure that is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less.
<b>Nanomaterial</b>	Any form of a material that is composed of discrete functional parts, many of which have one or more dimensions of the order of 100 nm or less.
<b>Nanoparticle</b>	A discrete entity which has three dimensions of the order of 100 nm or less.
<b>Nanosheet</b>	A discrete entity which has one dimension of the order of 100 nm or less, and two long dimensions.
<b>Nanorod</b>	A discrete entity which has two dimensions that are of the order of 100 nm or less, and one long dimension.
<b>Nanotube</b>	A discrete hollow entity which has two dimensions of the order of 100 nm or less, and one long dimension.
<b>Nanoparticulate matter</b>	A substance comprising of particles, the substantial majority of which have three dimensions of the order of 100 nm or less.

The definition of the term nanomaterial was the subject of a new Opinion<sup>43</sup> in 2010 that describes the complexity of determining size thresholds (upper and lower limits) common to all nanomaterials. According to the experts, the previously defined thresholds were not based on purely scientific criteria. A lower limit of 1 nm is suggested. The SCENIHR proposes a differentiated approach considering several thresholds for the upper size limit.

An example is given with two upper thresholds, a high one at 500 nm and a low one (critical threshold).

- **Category 1:** median size of particles greater than 500 nm for materials for which further information is missing

If the median size of the material is above 500 nm it is assumed that the size distribution at the lower end will always be above the designated lower threshold of 100 nm. Thus, no further information regarding possible nanospecific properties may be needed and classical risk assessment can be performed taking into consideration the particulate nature of the material.

- **Category 2:** median size of particles between 100 and 500 nm

When the median size is below 500 nm, a material is considered to be a nanomaterial and a more detailed nanospecific risk assessment is necessary taking into consideration possible nanospecific characteristics of the material.

When this size is between 100 nm and 500 nm, the nanospecific risk assessment may be waived when additional information is provided that the number size distribution demonstrates that the material has less than 0.15% (or any specified percentage) of the number size distribution below the 100 nm threshold. For dry materials, the volume specific surface area ( $< 60 \text{ m}^2/\text{cm}^3$ ) may be used as an additional qualifier. In these cases a classical risk assessment can be performed taking into consideration the particulate nature of the material.

- **Category 3:** median size of particles between 1 and 100 nm

The material is considered to be a nanomaterial and nanospecific risk assessment has to be performed when more than 0.15% (or any specified percentage) of the number size distribution is below 100 nm. For dry materials, the volume specific surface area may be used as an additional qualifier.

#### ► Definitions of the EU Scientific Committee on Consumer Products (SCCP)

In 2007, the EU Scientific Committee on Consumer Products issued an opinion on the safety of nanomaterials in cosmetic products<sup>44</sup>. This European body proposed a glossary of terms "in the absence of internationally agreed definitions," derived from an earlier report of the British Standards Institution (BSI) from 2005.

<b>Nanoscale</b>	Having one or more dimensions of the order of 100 nm or less.
<b>Nanoparticle</b>	Particle with one or more dimensions at the nanoscale.

<sup>43</sup> SCENIHR Opinion: "Scientific basis for the definition of the term "nanomaterial" (2010) available via the following link [http://ec.europa.eu/dgs/jrc/downloads/jrc\\_reference\\_report\\_201007\\_nanomaterials.pdf](http://ec.europa.eu/dgs/jrc/downloads/jrc_reference_report_201007_nanomaterials.pdf).

<sup>44</sup> [http://ec.europa.eu/health/ph\\_risk/committees/04\\_sccp/docs/sccp\\_o\\_123.pdf](http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_123.pdf).

<b>Nanomaterial</b>	Material with one or more external dimensions, or an internal structure, on the nanoscale, which could exhibit novel characteristics compared to the same material without nanoscale features.
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► **European definition for the Cosmetic Products Regulation**

The amendment to the European Regulation on cosmetic products in 2009<sup>45</sup> specifically introduced the obligation to label nanomaterials in the list of ingredients of these products, from 1 January 2013.

The following definition is used:

<b>Nanomaterial</b>	An insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm
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It is also mentioned that this definition shall be adapted to *“technical and scientific progress and to definitions subsequently agreed at international level”*.

► **European definition for the Regulation concerning novel foods and novel food ingredients**

A revision of the “*Novel food*”. Regulation of the European Parliament and of the Council on novel foods and novel food ingredients was proposed and discussed in 2011. This revision proposed considering that food containing or consisting of nanomaterials be regarded as novel, and therefore subject to assessment and authorisation.

<b>Engineered nanomaterial</b>	Any intentionally produced material that has one or more dimensions of the order of 100 nm or less, or is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic to the nanoscale.
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► **European definition relating to the provision of food information to consumers**

Article 18 of Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 establishes an obligation for specific labelling for nanomaterials, identical to that established by the Cosmetic Products Regulation. In this context, it applies to ingredients present in the form of engineered nanomaterials, which are defined in its Article 1.

<b>Engineered nanomaterial</b>	Any intentionally produced material that has one or more dimensions of the order of 100 nm or less or that is composed of distinct functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic of the nanoscale. Properties that are characteristic of the nanoscale include: i. those related to the large specific surface area of the materials considered;
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<sup>45</sup> Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products:

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF>

	and/or ii. specific physico-chemical properties that are different from those of the non-nanoform of the same material.
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### ► Definition of the American Chemistry Council (ACC)

The American Chemistry Council (ACC), representing leading companies in the chemicals sector, gives the following definition in the area of nanotechnologies:

<b>Engineered nanomaterial</b>	Any intentionally produced material that has a size in 1, 2 or 3 dimensions of typically between 1-100 nanometres. It is noted that neither 1 nm nor 100 nm is a ‘bright line’ and data available for materials outside of this range may be valuable. Buckyballs are also included even though they have a size < 1 nm.
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Aggregates and agglomerates with size greater than 100 nm are included in this definition if breakdown may occur creating particles in the 1-100 nm range during the life cycle.

However, the following are specifically excluded from this definition of “engineered nanomaterials”:

- Materials that do not have properties that are novel/unique/new compared to the non-nanoscale form of a material of the same composition;
- Materials that are soluble in water or in biologically relevant solvents. Solubility occurs when the material is surrounded by solvent at the molecular level. The rate of dissolution is sufficiently fast that size is not a factor in determining a toxicological endpoint;
- Particles for which the size distribution is such that particles with dimensions between 1 and 100 nm represent less than 10% of this distribution. This 10% threshold may be established according to a distribution by mass or surface area, whichever is more inclusive;
- Micelles and single polymer molecules.

### ► National definitions of nanomaterials

#### ❖ Australia

The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) concludes that there is no agreed national or international definition of nanomaterials. In 2009 it proposed the following working definition:

*“... industrial nanomaterials are those industrial materials intentionally produced, manufactured or engineered to have specific properties or specific composition, and one or more dimensions typically between 1 nm and 100 nm. This size range refers to individual particle size, and does not take into account agglomeration of particles.”*

#### ❖ Canada

In an interim policy statement, Health Canada provides the following *ad-hoc* definition of the term “nanomaterials”<sup>46</sup>:

<sup>46</sup> <http://www.hc-sc.gc.ca/sr-sr/pubs/nano/pol-eng.php>

"Health Canada considers any manufactured substance or product and any component material, ingredient, device, or structure to be nanomaterial if:

- a. It is at or within the nanoscale in at least one external dimension, or has internal or surface structure at the nanoscale, or,
- b. It is smaller or larger than the nanoscale in all dimensions and exhibits one or more nanoscale properties/phenomena.

For the purposes of this definition:

- i. The term "nanoscale" means 1 to 100 nanometres (nm), inclusive;
- ii. The term "nanoscale properties/phenomena" means properties which are attributable to size and their effects; these properties are distinguishable from the chemical or physical properties of individual atoms, individual molecules and bulk material; and,
- iii. The term "manufactured" includes engineering processes and the control of matter".

#### ❖ Denmark

The Danish Ministry of the Environment defines "nanomaterials" in the following way:

*"Nanomaterials can be defined as materials which are less than 100 nanometres in length along the shortest side or have structures which have such small dimensions but are built into larger materials (i.e. nanostructured surfaces). A nanometre is a millionth of a millimetre. Nanomaterials can be produced from existing chemical substances or completely new chemical compounds, and can be made from one or more substances. The small size of the materials is [the] reason for their special characteristics."*

#### ❖ United Kingdom

In 2004, the Royal Society & the Royal Academy of Engineering published a report in which the following definition for nanomaterials was given:

*"Although a broad definition, we categorise nanomaterials as those which have structured components with at least one dimension less than 100 nm. Materials that have one dimension in the nanoscale (and are extended in the other two dimensions) are layers, such as thin films or surface coatings. Some of the features on computer chips come in this category. Materials that are nanoscale in two dimensions (and extended in one dimension) include nanowires and nanotubes. Materials that are nanoscale in three dimensions are particles, for example precipitates, colloids and quantum dots (tiny particles of semiconductor materials). Nanocrystalline materials, made up of nanometre-sized grains, also fall into this category."*

The report was followed up by an action in the UK, from 2006 to 2008, to establish a voluntary reporting scheme for engineered nanoscale materials, which was organised by the Department for Environment, Food and Rural Affairs (DEFRA). In the guidelines on this scheme<sup>47</sup>, DEFRA gives the following definition:

*"Nanoscale materials are defined as having two or more dimensions up to 200 nm."*

It is stated that the definition will be reviewed according to the ongoing work of the BSI, CEN and ISO. The guidelines go on to specify that the focus of this scheme is materials that:

- are deliberately engineered (i.e. not natural or unintentional by-products of other processes);
- have two or more dimensions broadly in the nanoscale; and
- are "free" within any environmental media at any stage in a product's life cycle.

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<sup>47</sup> <http://archive.defra.gov.uk/environment/quality/nanotech/documents/vrs-nanoscale.pdf>

### ❖ United States

In the USA there is no official definition for "nanomaterial". In a concept paper on application of the Toxic Substances Control Act (TSCA), the US Environment Protection Agency (US-EPA) defines "engineered nanoscale materials" as follows<sup>48</sup>:

*"Engineered nanoscale material' is any particle, substance or material that has been engineered to have one or more dimensions in the nanoscale."*

*"The term 'engineered' is intended to mean that the material is 1) purposefully produced and 2) purposefully designed to be a nanoscale material..."*

*"The term 'nanoscale' is generally used to refer to the scale measured in nanometres ( $1.10^{-9}$  m). For the purposes of the Program, nanoscale is the size range between the atomic/molecular state and the bulk/macro state. This is generally, but not exclusively, below 100 nm and above 1 nm..."*

However, the paper also states that:

*"The description given herein should not be considered to be definitive for any purpose other than for EPA's Nanoscale Materials Stewardship Program; this definition is only applicable within the context of the Program as a guideline for determining if a material is appropriate for inclusion in the Program."*

More recently, the National Nanotechnology Initiative (NNI) formulated the following general definition:

*"Nanomaterial is a term that includes all nanosized materials, including engineered nanoparticles, incidental nanoparticles and other nano-objects, like those that exist in nature.*

*When particles are purposefully manufactured with nano-scale dimensions, they are called engineered nanoparticles. There are two other ways nanoparticles are formed. Nanoparticles can occur as a by-product of combustion, industrial manufacturing, and other human activities; these are known as incidental nanoparticles. Natural processes, such as sea spray and erosion, can also create nanoparticles."*

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<sup>48</sup> <http://www.epa.gov/oppt/nano/nmsp-conceptpaper.pdf>

## Annex 3: DGCIS Survey

The main markets concerned by nanomaterials are transport, construction, healthcare, agro-industry, luxury goods and defence. As stressed in earlier surveys, SMEs are strongly represented, and make up a large part (62%) of this industrial sector in France, especially in the nanomaterial "production" and "processing/integration" parts of the chain. The Île-de-France and Rhône-Alpes regions together host 55% of companies. Of particular interest is the recent creation of a website<sup>49</sup> that focuses on documenting "nano" activities, launched on the initiative of *NanoThinking*, a strategic consulting firm specialising in innovation in nanotechnologies. This site adds to the historical site created by the DGCIS<sup>50</sup> entitled "Database of nanomaterials players in France".

### French producers of nanomaterials

Industrial production of nanomaterials in France lacks significant structure: a large number of start-ups, with fairly low profiles, make up the population of companies producing nanomaterials.

Thus, according to the survey by the DGCIS, of the 40 to 50 producers identified:

- 80% are SMEs;
- 50% of these producer SMEs were only founded in the last 5 years;
- 75% have R&D and industrial activity, the others solely perform R&D.

Regarding the industrial sectors of origin, the producers mainly come from the chemicals, healthcare and microelectronics sectors.

It was found that 90% of nanomaterials produced in France are nanoparticles (mainly titanium dioxide, silica and cerium dioxide), totalling 135,000 tonnes annually. Nanofibres and nanotubes each account for several dozen tonnes produced per year. Finally, nanolayers and surface nanostructured materials come third in quantity produced, although actual amounts produced are low.

### Integrators/processors of nanomaterials

In the second link of the chain, between 30 and 40 companies were identified, and reported the processing and integration of nanomaterials.

Approximately 100 tonnes of nanomaterials are purchased per year, of which 95% are nanoparticles processed and integrated into semi-finished products (mainly nanoboehmites and ceramic oxides such as titanium dioxide and silica). Nanofibres and nanotubes on the one hand, and nanofilms, nanolayers and nanocoatings on the other, each represent a volume of one tonne per year purchased.

In terms of workforce, about 1500 people work on the processing and integration of nanomaterials into semi-finished products in all these companies. The breakdown by type of structure is more balanced than in the "production" link since 50% are SMEs. The most heavily represented sectors of origin are construction, chemicals, microelectronics, plastics and healthcare.

The main characteristic is the leadership of a player from the French construction industry.

### Users of nanomaterials

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<sup>49</sup> <http://www.nanothinking.com/nanotechmap#>

<sup>50</sup> <http://www.nanomateriaux.org>

Finally, for the last link in the chain, represented by 60 to 90 users of nanomaterials, the survey estimated that 720 tonnes of nanomaterials were used in products in France, and that 500 people were dedicated to this activity in the companies surveyed. Nearly half of users are SMEs.

Nanomaterials used in the marketed products are mainly nanoparticles and aggregates used historically (carbon black, nano silica, titanium dioxide, etc.). Nanotubes and nanofibres (such as carbon nanotubes) are currently only used at a pre-industrialisation stage.

## Annex 4: Ethical issues relating to nanotechnologies and nanomaterials

It now seems widely accepted that "nanotechnologies pose ethical problems". This assertion is most often taken to mean that the development of nanotechnologies is liable to manifest or convey a threat to different values or principles considered important to the life of humans or to their existence in society, such as those concerning respect for autonomy, justice, freedom, dignity, right to privacy, etc.

Since the early 2000s, a wealth of literature has been devoted to all these questions, emanating from various stakeholders (researchers, associations, official bodies, "the general public", etc.). From the point of view of academics and "ethicists", significant work on nanotechnologies has taken place at international level. Many books have been published and a specialist journal is devoted to this topic (*NanoEthics*, published by Springer). Below we present a number of issues addressed repeatedly in all of these publications, which for convenience will be referred to generically as "ethics of nanotechnologies".

### A. Recurring problems discussed by the "ethics of nanotechnologies"

The issues most discussed in the academic literature can be summarised in the form of different problems.

#### - A problem of definition

Does the development of nanotechnologies really pose entirely new ethical issues (which would justify the emergence of a new discipline), or is it just based on other types of classical problems already encountered with other (if not all) types of technology? Either position has its defenders, who can be distinguished mainly by a) the means of identifying ethical problems and issues, and b) the means to be used to resolve them. For some "conservatives", the issues are not new, and the situations created by the development of nanotechnologies can be addressed by applying sets of principles and standards that have already been well defined, such as those already in force in the field of bioethics. For others, nanotechnologies create new problems, but the traditional principles still apply ("*The problems are new, not the principles*"). And for others, nanotechnologies cause radically new problems, justifying the development of new methods to deal with them (situations related to the convergence of disciplines are often mentioned, such as the development of nanorobots introduced into the human body and allowed to make autonomous decisions). Finally, other authors, noting similarities between the problems posed by different fields of innovation, prefer to speak of the ethics of "*New and Emerging Science and Technology (NEST)*"<sup>51</sup>.

#### - A problem of status

Who should be responsible for identifying and resolving ethical issues related to nanotechnologies? Again, opinions diverge between the temptation to resort to convention and entrust this task to ethicists (more or less associated with the technological developments, such as in the case of projects supported by the European Commission), to the general public, by organising arenas for dialogue and public debate, or to the scientists themselves, for example through the promotion of codes of conduct<sup>52</sup> (this latter option sometimes comes up against the

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<sup>51</sup> On all these issues see for example F. Doridot, "The Different Models of Ethical Governance for Nanotechnology", in *Ethical Governance of Emerging Technologies Development*, F. Doridot & Alii (eds.), IGI Global, Hershey, USA, 2013.

<sup>52</sup> Such as what was done at European level with the "NanoCode" project (see <http://www.nanocode.eu/>).

scientist's classic posture, which involves shifting responsibility for making good use of his discoveries and inventions onto society<sup>53</sup>).

- **A problem of purpose**

The issue of nanotechnologies revives the old problem of the moral neutrality of technology. Are nanotechnologies a neutral instrument that can be used for good or bad, or do they have an intrinsic morality (which is then often considered bad)? The first position frequently gives rise to sectoral ethics, which seek to examine, often on a case-by-case or scope-by-scope basis, the problems posed by the development or use of nanotechnologies and the ways of ensuring their satisfactory resolution. The second position, which can also be described as "substantial", leads to an analysis of the "true nature" of nanotechnologies (even though, in the eyes of many players, this "umbrella" term would actually cover very different realities and issues). For example, this second position is expressed in the analyses of Jean-Pierre Dupuy on the "metaphysical research programme" supposed to characterise nanotechnologies<sup>54</sup>. Some radical opponents of nanotechnologies also subscribe to this idea, denouncing the advent of an inherently alienating and anti-humanist "Nanoworld"<sup>55</sup>.

- **A problem of method**

Should the ethics of nanotechnologies consist of an "ethics of the future" and rely on forecast scenarios to detect any emerging issues well in advance, in order to counteract them? Or should it accompany nanotechnology development step by step, and conduct for instance a "continuous standards-based assessment"? Although the first position has been heavily criticised, the scenarios method still attracts a broad following, especially in the Netherlands<sup>56</sup>.

- **A problem of scope**

The ethics of nanotechnologies looks at various subjects from different perspectives. Once confined to issues of moral responsibility attached to the dissemination of products with uncertain hazards (whether for operators, consumers or the environment), it gradually began addressing more economic and political issues (admissibility of promises used to justify nanotechnology development, adverse effects of nanotechnology development on the socio-economic balance, increase in the rift between rich and poor countries, etc.), before turning to the question of the profound transformations in humans, society and nature induced by the advent of nanotechnologies. Jean-Pierre Dupuy therefore proposed to assign to it, as a very broad scope, the study of the *effects* of the development of nanotechnologies, where he distinguishes between: effects on relationships of dominance (or power effects), effects on the relationship to nature (or ontological effects), effects on the relationship to knowledge (or epistemic effects), effects on the very possibility of ethics (or ethical effects) and effects on categories (or metaphysical effects)<sup>57</sup>.

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<sup>53</sup> Often mentioned as the origin of this posture is the nascent scientific community's search for independence in the 17<sup>th</sup> century, when confronted by religious and political power. A form of expression is found later in Max Weber (1919).

<sup>54</sup> See for example Jean-Pierre Dupuy, *Nanotechnologies*, in Canto-Sperber, M. (dir.), *Dictionnaire d'éthique et de philosophie morale*, Presses Universitaires de France, Paris, 2004, pp. 1319-1322.

<sup>55</sup> See for example Pièces et Main d'œuvre (PMO), *Aujourd'hui le Nanomonde*, Editions l'Echappée, 2008.

<sup>56</sup> See for example: Rip, A. and H. Te Kulve (2008), *Constructive Technology Assessment and Socio-Technical Scenarios. The Yearbook of Nanotechnology in Society*, Volume 1: Presenting Futures. E. Fisher, C. Selin and J. M. Wetmore, Springer: 49-70.

<sup>57</sup> See for example Dupuy (2004) (cited above).

## B. Main issues addressed by the ethics of nanotechnologies

The literature on the ethics of nanotechnologies addresses various recurring issues, which we can try and present briefly as follows. We have chosen not to consider issues specific to nanotechnology applications in the field of medicine, which are explicitly outside the scope of ANSES's work in the field of nanomaterials, but which nevertheless are among the issues most often discussed by the ethics of nanotechnologies.

### - The link to the transhumanist project

The development of nanotechnologies is often criticised for its link to the transhumanist project. Transhumanism is a movement originating from the United States that advocates the use of science and technology to improve the physical and mental characteristics of human beings. Nanotechnologies have been associated with it, especially in the United States, where some of their supporters are known transhumanists. Transhumanist views are expressed in the landmark American report by the National Nanotechnologies Initiative (NNI) in 2002, which encourages Nano-Bio-Info-Cogno (NBIC) convergence with the aim of improving "human performance", and benefiting society through interactions between human beings and intelligent machines. There is now a transhumanist association in France, which is calling for more freedom in the use of new technologies for free self-transformation.

The matter of "human enhancement" is loaded with ethical issues. In particular, it raises questions about freedom and autonomy of the subject, health and safety, justice and equity (including the risk of a growing social disadvantage for people with no access to enhancement techniques), as well as issues relating to the concept of human dignity, about which major changes in meaning are to be feared. The ethical debate surrounding human enhancement in particular demonstrates the conflict between the value of freedom (promoted by supporters of the right to enhancement) and respect for human dignity, conceived by some in a very conservative way.

The question is more generally asked in literature on the "type of society" targeted by the development of nanotechnologies. Some thinkers (such as Jean-Pierre Dupuy in France) have also wanted to see in nanotechnology research the expression of an essentially reductionist "metaphysical programme", showing the dangerous ambition of secreting uncertainty and loss of control within a living nature completely rebuilt from a materialist point of view.

#### Further reading:

- Article by Jean-Pierre Dupuy, "Quand les technologies convergeront", [www.resogm.org/IMG/pdf/RDM\\_023\\_0408.pdf](http://www.resogm.org/IMG/pdf/RDM_023_0408.pdf)
- Report by the NNI, "Converging Technologies for Improving Human Performance", [www.wtec.org/ConvergingTechnologies/Report/NBIC\\_report.pdf](http://www.wtec.org/ConvergingTechnologies/Report/NBIC_report.pdf)
- Website of the French Transhumanist Association: <http://transhumanistes.com/>
- Article from the *Nanoethics* journal on the issue of human enhancement: Patrick Lin & Fritz Allhoff, "Untangling the Debate: The Ethics of Human Enhancement", *Nanoethics*, Springer, 2008. (easily available on the Web).

### - Doubts about the promises in terms of energy and environment

Nanotechnologies promise society energy savings from the implementation of applications such as fuel cells, more efficient batteries, high-efficiency solar panels, reinforced insulation, etc. They also pledge to help reduce the environmental impact of human activities, whether through the action of nanoparticles for treatment and remediation (water, soil, etc.) or the promotion of "bottom-up" construction approaches rather than impact reduction. But these benefits have yet to demonstrate their ability to be assessed in terms of the harmful effect of nanoparticles, or of taking fully into account the life cycle of nanoproducts. Several studies have emphasised the importance of the environmental footprint of nanoproducts compared to conventional products, and the persistence at the nanoscale of highly energy-intensive production processes (this is the case for instance with

carbon nanotubes). Evaluating the actual environmental relevance of nanotechnologies can only be done by reviewing rigorous assessments with the inclusion of external factors, and to date the results for nanotechnologies remain uncertain, and even unfavourable for some. (External environmental factors refer to all a production process's impacts on the environment, and the indirect costs that will be incurred to deal with them.) These issues are supplemented by political and economic difficulties, such as that of market domination by Western patents, compromising poor countries' access to nanotechnology solutions, particularly in the field of water decontamination.

Further reading:

- Special edition of the *Journal of Industrial Ecology*, Volume 12, Issue 3, Special Issue on Nanotechnology, <http://onlinelibrary.wiley.com/doi/10.1111/jiec.2008.12.issue-3/issuetoc>

- Article by Donald C. Maclurcan, *Nanotechnology and Developing Countries – Part 2: What Realities?*, available from:

<http://www.azonano.com/article.aspx?ArticleID=1429>

- **Issues about surveillance and respect for privacy**

Nanotechnologies raise concerns in this area, mainly via their applications in the field of IT and telecommunications, which include the development of smaller and smaller RFID chips. The proliferation of data collection of all kinds made possible by these chips raises fears in some quarters about jeopardising personal privacy, heralding a surveillance society where any right to anonymity or oblivion is prohibited. Although it is now technologically possible to deactivate the identification processes associated with these chips, the extra cost then gives rise to a trade-off between economic development and ethical respect, which many observers agree is unhealthy. The development of implants of all kinds is also often incriminated, especially insofar as it gives rise to *de facto* discrimination (between "implanted" and "non-implanted" individuals) that then promotes the generalised implementation of implants. Although similar risks already exist for macro-scale technologies, some consider that the scale of invisibility achieved with nanoscale devices brings with it new risks of irreversible control phenomena – this was particularly highlighted in France by the French Data Protection Authority (CNIL).

Further reading:

- *Sous l'œil des puces: La RFID et la démocratie*, Michel Alberganti, Actes Sud, 2007.

- ObservatoryNano project report, *Ethical and Societal Aspects of Nanotechnology Enabled ICT and Security Technologies*, April 2011,

<http://www.observatorynano.eu/project/document/3525/>

- **The issue of military applications**

The military field is very active in nanotechnologies. Many states invest massive sums, and the field finances and leads a number of innovations. Some observers predict the imminent arrival of nanotechnology weapons that will relegate nuclear weapons to ancient history. And some recent conflicts seem to have already demonstrated the use of bombs made more lethal by the use of nanotechnology processes.

Many aspects of military nanotechnology research raise ethical concerns. For instance, the "future soldier" programme, as defined in particular by the United States, includes the use of different implants and sensors to improve combatants' mental and physical performance, thereby coming back to issues such as respect for human dignity and human enhancement. Research is underway to hybridise living organisms and electronic systems in the form of controllable insects and nano-drones, touching on ethical issues relating to respect for living things. The development of highly sophisticated, virtually undetectable surveillance systems (cybersurveillance, biomonitoring, etc.), while it may be strategic for the military, also leads to fears about abuses, such as concealed use

in surveillance of civilian populations. The growing autonomy of robots and military artefacts made possible by nanotechnologies forcefully rekindles all the fears about loss of control associated with "nano" objects. More generally, many specialists also worry about the risks of a breakdown in the current balance of global deterrence, in the event that new weapons with overwhelming superiority are invented (especially nano-biological weapons). Lastly, many civil society stakeholders and international organisations repeatedly point to the imbalance between investments made globally in new weapons, and those devoted to supporting peace efforts.

Further reading:

- See "Military, Arms Control, and Security Aspects of Nanotechnology", Jürgen Altmann and Mark Avrum Gubrud, in D. Baird, A. Nordmann and J. Schummer (dir.), *Discovering the Nanoscale*, Amsterdam, IOS Press, 2004. Available online from:

<http://books.google.fr/books?hl=fr&lr=&id=O-LM6eWUbmIC&oi=fnd&pg=PA269&dq=Military,+Arms+Control,+and+Security+Aspects+of+Nanotechnology&ots=mceL2U1KtA&sig=63V-IV6aoKAomWX3mlwQdVMD53Q#v=onepage&q=Military%20Arms%20Control%20and%20Security%20Aspects%20of%20Nanotechnology&f=false>

In December 2013, in France,, the Ministry of Economy and Finance, the Ministry of Productive Recovery, the Ministry of Defence, the Ministry of Agriculture, Food and Forestry, the Ministry of Ecology, Sustainable Development and Energy, the Ministry of Higher Education and Research, the General Council for the Environment and Sustainable Development, and the General Inspectorate for Administrative Affairs jointly published a report entitled "*The industrial deployment of nanotechnologies and synthetic biology in the territories, a precursor of future manufacturing*".

(<http://www.ladocumentationfrancaise.fr/var/storage/rapports-publics/144000176/0000.pdf>).

### C. Outline of the recommendations from official bodies

#### In France:

##### i) **The General Mining Council (CGM) and the General Information Technology Council (CGTI)**

In 2004, a report<sup>58</sup> was published by the General Mining Council and the General Information Technology Council, on the topic of nanotechnologies. This science of the infinitely small covers multiple areas of application: information technology (increased storage possibilities of nanomaterials), medical technologies (bioactive implants, help with tests through the use of DNA microarrays, targeted treatment of diseased cells, etc.), ecotechnologies (detection and neutralisation of micro-organisms and pesticides, for example), energy technologies (improved energy savings in transport) and so on. In addition to the industrial challenges posed by nanotechnologies, the report presents the types of risks associated with this new technique, particularly the environmental ones. It also highlights the ethical issues raised by the transformation of living things: "The natural non-living, the living and the artefact are in the process of merging".

The report concludes with 13 recommendations, of which the most important in the short term, from the point of view of public action in France, are the creation of interministerial coordination in

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<sup>58</sup> "*Les Nanotechnologies: Ethique et prospective industrielle*" – J.-P. Dupuy, F.Roure, La Documentation Française, Nov. 2004.

<http://www.ladocumentationfrancaise.fr/var/storage/rapports-publics/054000313/0000.pdf>

synergy with all stakeholders, and, echoing this, the establishment of a review body that can oversee the effective implementation of this public policy:

- 1. Draw up and implement in the very short term a continuous interministerial coordination function capable of promoting the development of convergence in technologies with high transformational capacity, starting with nanotechnologies.
- 2. Support AFNOR's standardisation effort as part of WG 166 of the European Committee for Standardization, including the requirement for a joint EU/US secretariat like the ISO by encouraging industry participation.
- 3. Create a technological network for nano-bio-info-cogno meta-convergence, in addition to the RMNT, RNTL, RNRT, RIAM and RNTS networks, likely in particular to encourage French proposals for FP7 calls for tender.
- 4. Support the recommendation of the European Commission's high-level expert group on "Foresighting the New Technology Wave" (NTW), which is seeking to create a European societal observatory on converging technologies.
- 5. Promote the observation of nanotechnologies in France in all its scientific, technological and social dimensions, by providing public support for the adaptation and institutionalisation of the OMNT's role, and cultivate a constructive dialogue on its results between all the stakeholders.
- 6. Mobilise the human and financial resources needed to develop criteria for ongoing normative assessment for the national and international community, as well as their presentation and implementation of the corresponding methodology (*relevant criteria for peer review*).
- 7. Launch a research programme on the new regulations and subsidiarity in convergent technologies, including those for international trade and customs issues.
- 8. Educate young people about the value of multidisciplinary training, and improve the image of nanotechnologies and emerging technologies in general secondary school curricula by rapidly updating training and educational materials for technology teachers in secondary education.
- 9. Mobilise the INRS, Ineris, the INVS, and players in healthcare and consumer protection in support of prevention of physical and societal risks, starting with product classifications and databases.
- 10. Include in the missions of the DRIREs territorial monitoring of facilities of any kind dealing with nanoparticles and nanomaterials, and ensure liaison with the national and European regulatory authorities.
- 11. Ensure that the National Consultative Ethics Committee (CCNE) for the Life Sciences has the necessary means to fulfil its mission with regard to nanotechnologies and meta-convergence, and to participate in international discussions on these crucial topics, in conjunction with the CNRS national ethics council, in particular.
- 12. Encourage major research organisations, starting with CNRS and INSERM, to build platforms for research on the ethical and social implications of nanotechnologies, viewed from the perspective of meta-convergence.
- 13. Establish an operational review body to take responsibility for the implementation of public policy in nanotechnologies, as a continuation of the proposal outlined in the report of the Academy of Sciences and the Academy of Technologies.

## ii) COMETS

In 2006, the COMETS<sup>59</sup> issued an opinion on the ethical issues of nanoscience and nanotechnologies: The eight recommendations issued by this Ethics Committee at the time are listed below:

- 1. With a view to holding a consultation, help rally the parties interested in developing a research programme: industrial companies, consumer groups, patient organisations, non-governmental organisations, etc. These players' opinions are essential for enlightening decision-makers about society's expectations. CNRS, because it covers all the fundamental disciplines and is concerned by the applications, must play a leading role in this consultation.
- 2. Integrate concerns about the ethics of research at several levels in the career of researchers - initial training, assessment, formulation of research projects.
- 3. Produce short guides on ethics for researchers, or dossiers in an accessible language summarising the results of the many existing studies.
- 4. Open ethical spaces in research centres as places for debate, where scientists, engineers and technicians can express their opinions and hold discussions, with the participation of researchers in the human and social sciences.
- 5. Stimulate the interest of researchers in the human and social sciences in the field of nanoscience and nanotechnologies.
- 6a. Establish procedures for identifying and arbitrating conflicts of interest in relationships with industry;
- 6b. Ensure the transparency of funding sources and, if possible, of results from projects conducted jointly by CNRS and industry.
- 7. Concerning relations with the public:
  - Present the expected benefits of nanoscience and nanotechnologies without downplaying the possible harmful aspects;
  - Place greater emphasis on the implications of this research for humans, on the issues related to the choice of nanoscience as scientific priorities; and, if possible, avoid focusing solely on the economic and industrial issues;
  - Dare to consider the very long term issues, by helping to identify the fantasies they can inspire.
- 8. Establish forums for dialogue and/or participate in public debates organised at local, national, European and international level.

## iii) CCNE

In February 2007, the National Consultative Ethics Committee (CCNE) for Life Sciences and Health issued an opinion (No. 96)<sup>60</sup> on the *"Ethical issues raised by nanosciences, nanotechnologies and health"*.

Its recommendations were as follows:

- 1. Ensure the availability of sufficient information on the alarming and ambivalent capacity of molecular manmade nanosystems to pass through biological barriers, in particular between blood and brain. Similarly, information must be forthcoming on the low or non-existent

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<sup>59</sup> COMETS is a consultative body of 12 members, researchers or engineers in a broad variety of disciplines, reporting to the CNRS board. Established in 1994, it develops thinking on the ethical aspects raised by the practice of research, makes recommendations and raises staff awareness. Opinion on the ethical issues of nanoscience and nanotechnologies.

<sup>60</sup> <http://www.ccne-ethique.fr/sites/default/files/publications/avis096en.pdf>

biodegradability which could have major consequences on health except for some specific therapeutic indications.

- 2. As a matter of urgency, intensify research and development on nanometrology to design more instruments for the detection and identification of nanoparticles, in particular those created specifically for the formation of nano-objects and nanostructures.
- 3. Underline the disparity between too little development (or publication) of fundamental research and the accelerated production of commercial technological applications. As a result, some essential decisions and choices may be bypassed. More support is urgently required for the development of fundamental research on nanosciences, without prejudice to the freedom of research. Ethical aspects must be evaluated in projects to be financed by national and European organisations and private foundations. Scientists now being trained, in particular future PhDs in nanosciences and nanotechnologies, should be required to include in their doctoral theses a summary of ethical issues relating to their research. In the European research arena and on the global scene, states must implement strategies which include such ethical reflection in the "knowledge triangle": research, education and transfer.
- 4. Encourage integrated multidisciplinary research to ensure that the design of new nanomaterials and nanosystems is combined with a study of their primary effects on the environment, on health and their positive and negative biological implications. The separation of these approaches into calls for different projects (ANR and FP7) does not guarantee that sufficient research is carried out on risk assessment before such innovations emerge from the confines of research laboratories and go into industrial production. Risk evaluation must include the complete life cycle of nanoproducts. This requires an upgrading of industrial toxicology using human and technical resources on a par with standard procedures in the field of innovative technology. Industrial financing of research on risks is an ethical priority, even though it may and should be complemented by more extensive investment in public and fundamental research.
- 5. Give priority to the array of protective measures required for workers in contact with nanomaterials and to the confinement of premises used for their study and production. Give priority to research on adverse effects with particular attention to low-dose toxicity for highly vulnerable populations, in particular workers in contact with nanomaterials who could be exposed despite protective measures. For precautionary reasons, pregnant women should be excluded from such employment. Monitoring of fetuses and newborns should be prescribed by regulation in the event of professional or accidental exposure. Animal research on the effects of nanoparticles should be greatly intensified, even for nanomaterials devoid of any purely medical application (nanocosmetics). As regards occupational medicine and the work of site security and hygiene committees, laboratories, research teams and production sites must be required to draw up a code of good practices and to implement special procedures for monitoring the protection and supervision of research and industry personnel engaged in the manufacture of nanometric products.
- 6. Ensure a climate of trust by reporting regularly and clearly on scientific progress to the research community, both public and private, supported by European regulations for the mandatory registration of all new nanostructures together with their possible consequences on biological reactivity. A European law similar to REACH must be enabled for nanoproducts. European reflection on standards for the protection of intellectual property rights and models for licensing agreements more appropriate to nanotechnologies should also include new knowledge-sharing and research product-sharing procedures designed to increase the attention given to ethical considerations.
- 7. Encourage networking and information-pooling among the various agencies: Biomedicine, AFSSAPS, AFSSA and the French Institute for Public Health Surveillance (InVS). The greatest attention must be given to the respect of relevant principles, such as privacy, informed consent before exposure to these innovations and the protection of personal safety. Industrialists must be required to provide information and clear specific labelling of products containing manufactured nanoparticles so that consumers can refuse to use them if they so wish. The collection and transparency of information on the pharmacovigilance of nanomedical products

will be achieved by an extension of the scope of competence of existing agencies involved in the supervision of medicines and implanted devices.

- 8. Develop the dissemination of scientific, technological and industrial cultural material in the field of nanosciences and nanotechnologies. Set up an effective information system for the public and society through the organisation of public contradictory debates. These would be decentralised to regions and be the subject of public reports including the responses given by researchers and industrialists to questions, expectations and fears expressed during the debates. Making publicly available a maximum amount of trustworthy information and not hiding behind the pretext of industrial confidentiality to abstain from doing so, should become a practical obligation.
- 9. Finally, determined vigilance must be exercised regarding the serious consequences for individual liberties and for respect for human dignity if identification and interconnection capacities were developed without the knowledge of those concerned. Any possibility of military applications being adapted for civilian purposes should be fully and publicly debated with due regard for individual rights before any transfer takes place.

In conclusion, the ethical dimension of the use of nanomaterials can be studied under two headings. On the one hand, the philosophical man-machine problem raised by nanosystems, which remains a threat to the respect for human beings. This important intellectual subject must not however be allowed to overshadow a second and much more urgent question which is the covert intrusion of nanoparticles with more regard for technological performance and commercial profitability than for the perception of potential risks. This second question, more than the first, makes it very necessary to raise awareness so as to avoid outright rejection by society of new techniques more concerned at this point with competing in the race for innovation than with respect for the physical and mental integrity of individuals.

Controlling the consequences of scientific and technological progress is the responsibility of society as a whole; it cannot be the sole concern of economic players or associations. We must not allow nanotechnology to supersede nanoscience.

### International:

In 2006, in Quebec, the Commission on the Ethics of Science and Technology (CEST1) published a position statement entitled "*Ethics and Nanotechnology: a basis for action*". This paper provided a portrait of nanoscience and nanotechnologies in order to identify the ethical issues accompanying their appearance. At the end of its statement, the Commission recognised that it was far from having exhausted the subject of nanotechnologies. In fact, many questions remained unresolved, suggesting that the issues discussed would soon be multiplied by the increasing number of discoveries and applications. The Commission also concluded its deliberations with this observation: Serious reflection on the ethical and social issues raised by technology is only beginning and it is important to continue thinking, discussing, expressing views on nanotechnologies and the best way to ensure their harmonious development. That is why the Commission sees the need to continue addressing more focused questions about the responsible management of nanotechnologies that the State could raise, as it will have to make decisions regarding this field in the future (CEST, 2006, p. xi).

In the 2011 supplement to the position statement "*Ethics and Nanotechnology: a basis for action*" entitled "*Ethical Issues of Nanotechnologies in the agri-food sector*", the CEST (Quebec) issued a new opinion that was adopted at the 51<sup>st</sup> session of the CEST on 25 and 26 August 2011.

Several recommendations, a unified vision. The Commission understands that the recommendations made in this document are a whole and should be read as such. These recommendations are minimum measures which the government should implement to assume its responsibilities regarding the protection of the environment and public health.

- 1 The Commission recommends that the Québec Research Fund – Nature and technologies (FRQ–NT) and the Québec Research Fund – Health (FRQS), develop a research financing

strategy so that issues regarding the risk inherent in nanotech applications in the agri-food sector be properly studied.

The Commission recommends that the Québec Research Fund – Society and culture (FRQ–SC) ensure that the NE3LS network promotes research on the cultural and social stakes regarding the development of nanotechnologies in the agri-food sector.

The Commission recommends that these organisations conduct a continuous assessment of financing to ensure that the objectives are properly attained.

- 2. The Commission recommends that the Minister of Agriculture, Fisheries and Food:
  - Implement a scientific and technological watch network for the development of nanotech applications in the agri-food sector to ensure the development and maintenance of expertise within the public service, especially of the professionals working for the MAPAQ;
  - Commit the funds required to ensure a scientific and technological watch and to ensure that this expertise is available within the Ministry on the one hand, and on the other hand, to ensure participation in activities (conferences and seminars) to maintain and develop this expertise;
  - Create links for cooperation and work in relationship with the federal organisations concerned.
- 3. The Commission recommends that the government of Quebec implement an interdepartmental mechanism which includes the Ministry of Agriculture, Fisheries and Food, the Ministry of Health and Social Services, and the Ministry of Sustainable Development, Environment and Parks, in order to facilitate the exchange of information regarding the state of scientific knowledge about the risks inherent in nanotech applications in the agri-food sector.
- 4. The Commission recommends that the Minister of Agriculture, Fisheries and Food of Quebec develop an Internet portal for nanotechnologies specifically concerning the agri-food sector using the GMO portal as a model. This portal could make available to Quebec’s population a public forum featuring information that is factual, independent and easily accessible to the various clienteles concerned. It should have postings made by a watch network set up beforehand (recommendation 2) and be connected to social networks to ensure that dissemination of information would be as extensive as possible.
- 5. The Commission recommends that the government of Quebec use the procedure of the Bureau for Public Audience on the Environment (BAPE) to establish a permanent consultation mechanism with Quebec’s population, which would allow considering the perceptions of risk regarding food safety and the overall perception of risk, as much as specific projects which may challenge food safety.
- 6. The Commission recommends that the government of Quebec encourage the competent Canadian regulatory authorities, especially the Health Products and Food Branch at Health Canada, to continue their work regarding the assessment of the safety of products containing synthesised nanoparticles or which are derived from nanotechnologies.

The Commission recommends that the government of Quebec require from the federal government that any food which is manufactured or prepared using nanotechnologies or which contains synthesised nanoparticles be automatically considered as a “novel food” and be systematically subject to the regulations which apply to “novel foods”.
- 7. The Commission recommends that the Minister of Agriculture, Fisheries and Food of Quebec pay special attention to the inspection of any food which was manipulated or came in contact with materials derived from nanotechnologies or which contains synthesised nanoparticles.
- 8. The Commission recommends that the Minister of Agriculture, Fisheries and Food of Quebec take the measures required to amend the regulations so that all test data required under regulations for certification purposes be rendered public on a compulsory basis, including the results of previous tests submitted for certification purposes and which were insufficient to obtain this certification.
- 9. The Commission recommends that the government of Quebec take the measures required to ensure that the actors in the agri-food industry who knowingly use nanotechnologies properly

inform consumers. On this point, labelling is in the Commission's opinion, still a choice option to properly respect the right to information. The Commission believes however on the one hand that the extent to which cultural and social stakes may or may not contribute to warrant the labelling of these products must be assessed, and on the other hand, it must give broader consideration to the ethical criteria which should apply to this practice.

In 2008, the COMEST (UNESCO) issued a report<sup>61</sup> entitled "*Nanotechnologies and ethics. Policies and actions*". It contained a number of findings and recommendations, of which we have selected only a few elements for inclusion in this summary.

Given that nanotechnologies are developing very quickly, UNESCO should establish an international commission for nanotechnologies and ethics, responsible for monitoring the development of ethical issues and the emergence of new problems in this field, and providing timely responses.

**Ethical research and ethics in connection with legal issues:** Research in ethics needs to be developed in association with nanotechnologies. Ethical considerations are still insufficient within the vast financial effort devoted to nanotechnologies. Ethicists should be encouraged to address nanotechnologies, and teams in scientific research on nanotechnologies should endeavour to be in close interaction with ethicists and philosophers. The ethics programme of UNESCO can play a role here in providing an international platform for the ethics of nanotechnologies, in acting as a clearing house for information regarding ethical issues, and in establishing a database of relevant information concerning ethics and policies (as part of the Global Ethics Observatory). It is also important to address the legal context (e.g. consumer legislation, occupational health legislation, criminal negligence claims against corporations, laws regarding technology development, production and dissemination). This research would need to be interdisciplinary.

**Promotion of ethical, legal and social issues (ELSI) research:** UNESCO should promote ELSI research as an important tool within the national technology research frameworks of countries, recommending that a certain percentage of the nanotechnologies research budget be allocated for ELSI research, as is currently the case for human genome research.

**Nanotechnologies and development:** Even when interdisciplinary, scientific research alone cannot solve value problems regarding nanotechnologies. Social science research, more debate and awareness, as well as explicit examination and articulation of the ethical principles involved are needed. Therefore UNESCO could assist countries in identifying technologies that are most appropriate and relevant for development. It is necessary to distinguish the actions to be undertaken at an international level and issues that need to be addressed from a national or local perspective. For example countries whose national resources may be replaced by nano-engineered materials, for example, should rather look for the best use of their resources and for specific nanotechnologies research.

In this debate the following topics should be considered:

- the utility of particular nanotechnologies for development;
- the comparative advantages and disadvantages of a given nanotechnology for a given country;
- the ability to turn research effort into applications that are useful for development;

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<sup>61</sup> "*Nanotechnologies and ethics. Policies and actions*", Report of the World Commission on the Ethics of Scientific Knowledge and Technology (COMEST), UNESCO Publications, 2008.

<http://unesdoc.unesco.org/images/0015/001521/152146e.pdf>

- the possible environmental risk;
- risk assessment and management;
- the impact of the intellectual property regime in terms of risk-benefit assessment;
- sharing of benefits (similar to the provisions of the *Universal Declaration on Bioethics and Human Rights*);
- international cooperation between developing and developed countries (similar to the provisions of the *Universal Declaration on Bioethics and Human Rights*);
- cost-benefit analysis of alternative technologies and actions versus those offered by nanotechnologies;
- management of social transformations resulting from structural changes in the global economic system due to nanotechnologies.

## Annex 5: Regulations

*NB: this review focuses on regulations applicable in France and within the European Union, even though other countries have also adopted more or less restrictive rules regarding nanomaterials. This choice was dictated by the need for the summary to provide information that was relevant for ANSES's supervisory Ministries and the French public. Only standards legally applicable in France have therefore been briefly described.*

The degree to which nanomaterials are taken into account in the standards system has greatly evolved over recent years. The standards adopted have gradually been tightened, from non-binding standards, communications and then recommendations in 2004, to the adoption of legal texts more or less specifically devoted to the issue of nanomaterials since 2009. Two main themes can be identified from this normative evolution. On the one hand, there seems no reason to challenge the predetermined legal classifications into which nanomaterials are gradually being integrated, which can give the impression of a fragmented development of standards (I). However, on the other hand, a trend is now becoming clearer, through the search for a more general working definition to understand these objects: that of a more comprehensive documenting of their production and import into the countries concerned (II).

### 1. A fragmented development of standards

#### The approach chosen by the European Commission: adapt without causing an upset

In its 2004 Communication "Towards a European strategy for nanotechnology"<sup>62</sup> the European Commission very clearly stated the need to submit the existing European regulations to review and to potential amendments based on a proactive approach to risk.

In 2005, however, in the Communication that describes this strategy<sup>63</sup>, the message seems to have been downplayed in favour of a less abrupt and more sector-specific approach. Indeed, the Commission confirms this, in Section 6 devoted to public health, safety, environmental and consumer protection, stating that it intends to implement several types of measures to ensure a high level of protection of human health, consumers, workers and the environment. Among these measures, the adoption of possible regulatory measures only comes in fourth place (point 6.1,d) after "Identify and address safety concerns associated with applications and use of N&N" (point 6.1,a), "Promote safe and cost-effective measures [understood to mean technical measures] to minimise exposure of workers, consumers and the environment to manufactured nanoscale entities" (point 6.1,b), and "Develop with Member States, international organisations, European agencies, industry and other stakeholders, terminology, guidelines, models and standards for risk assessment throughout the whole life cycle of N&N products" (point 6.1,c).

This strategy, tending to a minimum adaptation of the sector-specific regulations without seeking a more specific approach for all the issues associated with nanomaterials, is confirmed by the two recommendations published by the Commission in 2008<sup>64</sup> and 2012<sup>65</sup> on the regulatory aspects of

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<sup>62</sup> Communication from the European Commission COM(2004) 338 final - Brussels, 12.5.2004 Towards a European strategy for nanotechnology.

<sup>63</sup> Communication from the Commission to the Council, the European Parliament and the Economic and Social Committee COM(2005) 243 final - Brussels, 07.6.2005 Nanosciences and nanotechnologies: An action plan for Europe 2005-2009

<sup>64</sup> Communication from the Commission to the European Parliament, the Council and the European Economic and Social Committee COM(2008) 366 final - Brussels, 17.6.2008 Regulatory aspects of nanomaterials.

nanomaterials. The Commission concludes that in principle the existing regulations cover the potential risks associated with nanomaterials, which are "*similar to normal chemicals/substances in that some may be toxic and some may not*". According to the Commission, "*Important challenges relate primarily to establishing validated methods and instrumentation for detection, characterization, and analysis, completing information on hazards of nanomaterials and developing methods to assess exposure to nanomaterials*" and that "*Overall the Commission remains convinced that REACH sets the best possible framework for the risk management of nanomaterials, [...] but more specific requirements for nanomaterials within the framework have proven necessary [...]*".

This approach is the subject of strong criticism, including from the European Parliament, which accuses the Commission of failing to engage in the construction of a clear regulatory framework specific to nanomaterials<sup>66</sup>. We will see that the recommendation adopted in 2011 concerning the definition of nanomaterials is a partial response to this desire. In the hypothetical expectation of a change in strategy, however, the regulatory activity affecting five specific sectors should be highlighted.

### Sectoral adaptations

Provisions specifically dedicated to nanomaterials have, since 2009, been incorporated in several pieces of sector-specific legislation of the European Union.

#### **a) Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives**

Article 12 of the Regulation stipulates that "*When a food additive is already included in a Community list and there is a significant change in its production methods or in the starting materials used, or there is a change in particle size, for example through nanotechnology, the food additive prepared by those new methods or materials shall be considered as a different additive and a new entry in the Community lists or a change in the specifications shall be required before it can be placed on the market.*"

#### **b) Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products**

Article 2 of the Regulation defines the notion of nanomaterial as "*an insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm*". The Regulation also stipulates in its Articles 13 and 16, a specific notification procedure<sup>67</sup> for cosmetic products containing nanomaterials, and for the following information to be made available by 11 January 2014 at the latest: a "*catalogue of all nanomaterials used in cosmetic products placed on the market, including those used as colorants, UV-filters and preservatives in a separate section, indicating the categories of cosmetic products and the reasonably foreseeable exposure conditions*". These provisions are accompanied by a new requirement for labelling of cosmetics containing nanomaterials, since Article 19 of the

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<sup>65</sup> Communication from the Commission to the European Parliament, the Council and the European Economic and Social Committee, COM (2012) 572, Brussels, 02.10.2012, Second regulatory review on nanomaterials.

<sup>66</sup> European Parliament Resolution of 24 April 2009 on regulatory aspects of nanomaterials (2008/2208(INI)).

<sup>67</sup> Including information on the toxicology of the nanomaterials used, foreseeable exposure conditions and safety data.

Regulation stipulates that "*All ingredients present in the form of nanomaterials shall be clearly indicated in the list of ingredients. The names of such ingredients shall be followed by the word 'nano' in brackets*".

**c) Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food**

The Regulation establishes specific requirements applicable to the manufacture and marketing of plastic materials and articles:

- "intended to come into contact with food; or
- already in contact with food; or
- which can reasonably be expected to come into contact with food".

The authorisations issued to substances (listed in Annex I) "*based on the risk assessment of the conventional particle size of a substance do not cover engineered nanoparticles*" (recital 23). This discrimination between nanoparticulate and non-nanoparticulate substances is specified in Article 9 of the Regulation, where it is stated that "*Substances in nanoform shall only be used if explicitly authorised and mentioned in the specifications in Annex I*". Furthermore, the derogations concerning compliance with the restrictions and specifications stipulated in the Regulation or with the obligation on manufacturing with substances in the Union list, relative to substances contained in the layers of certain plastic materials and articles, where such substances are contained in a plastic layer that is not in direct contact with food and is separated from the food by a layer acting as a functional barrier, are not applicable to substances in a nanoscale form (Articles 13 and 14 of the Regulation).

**d) Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers**

The Regulation (Article 18) provides for a specific labelling requirement for nanomaterials identical to that implemented by the Cosmetic Products Regulation. Here, it applies to ingredients present in the form of manufactured nanomaterials, which are defined in its Article 2, as: "*any intentionally produced material that has one or more dimensions of the order of 100 nm or less or that is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic of the nanoscale*".

*Properties that are characteristic of the nanoscale include:*

- those related to the large specific surface area of the materials considered; and/or*
- specific physico-chemical properties that are different from those of the non-nanoform of the same material*".

**e) Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products**

Regulation (EU) No 528/2012 contains several innovations including the creation of a Union list of approved active substances. It specifies in its Article 4 paragraph 4 that "*The approval of an active substance shall not cover nanomaterials except where explicitly mentioned*". The notion of "*nanomaterial*" is defined in a manner consistent with Recommendation 2011/696/EU on the definition of nanomaterials.

A biocidal product may only be placed on the market or used if an authorisation has been granted for the biocidal product by the competent authority. The authorisation may only be granted in the event of a positive assessment of the risks presented by the biocidal product and if the active substances used in the biocidal product appear on the Union list of approved active substances. If

nanomaterials are present in the biocidal product, Article 19 of the Regulation states that the risk to human health, animal health and the environment must be examined separately. The simplified authorisation procedure for biocidal products described in Articles 25 *et seq* of the 2012 Regulation is not applicable to biocidal products containing nanomaterials (Article 25 c).

Authorised biocidal products shall indicate on a label "*the nanomaterials contained in the product, if any, and any specific related risks*" (Article 69 paragraph 2 of the Regulation) [and following each reference to nanomaterials, the word "nano" in brackets]. This obligation to provide information about the presence of nanomaterials extends to "*treated articles*" as defined in Article 3 paragraph 1 l) of the Regulation. Finally, as part of their duty of surveillance, Member States shall submit to the European Commission a report including in particular "*information on the use of nanomaterials in biocidal products and the potential risks thereof*" (Article 65 paragraph 3 d).

Finally, it should be noted that other European regulations deal with nanomaterials in their recitals but without devoting any special provisions to them.

**f) Commission Regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food**

According to recital 14 of the Regulation: "*Intelligent packaging systems provide the user with information on the conditions of the food and should not release their constituents into the food. Intelligent systems may be positioned on the outer surface of the package and may be separated from the food by a functional barrier, which is a barrier within food contact materials or articles preventing the migration of substances from behind that barrier into the food. Behind a functional barrier, non-authorised substances may be used, provided they fulfil certain criteria and their migration remains below a given detection limit. Taking into account foods for infants and other particularly susceptible persons, as well as the difficulties of this type of analysis affected by a large analytical tolerance, a maximum level of 0.01 mg/kg in food should be established for the migration of a non-authorised substance through a functional barrier. New technologies that engineer substances in particle size that exhibit chemical and physical properties that significantly differ from those at a larger scale, for example, nanoparticles, should be assessed on a case-by-case basis as regards their risk until more information is known about such new technology. Therefore, they should not be covered by the functional barrier concept*".

**g) Directive 2011/65/EU of the European Parliament and of the Council of 8 June 2011 on the restriction of the use of certain hazardous substances in electrical and electronic equipment**

According to Recital 14 of the Directive, "*As soon as scientific evidence is available, and taking into account the precautionary principle, the restriction of other hazardous substances, including any substances of very small size or with a very small internal or surface structure (nanomaterials) which may be hazardous due to properties relating to their size or structure, and their substitution by more environmentally friendly alternatives which ensure at least the same level of protection of consumers should be examined. To this end, the review and amendment of the list of restricted substances in Annex II should be coherent, maximise synergies with, and reflect the complementary nature of the work carried out under other Union legislation, and in particular under Regulation (EC) No 1907/2006, while ensuring the mutually independent operation of this Directive and that Regulation. Consultation with the relevant stakeholders should be carried out and specific account should be taken of the potential impact on SMEs*".

## 2. A dedicated construction at the embryonic stage: define in order to identify?

The years 2009 and 2010<sup>68</sup> can be seen as very important with regard to the definition of the field of nanomaterials and/or nanoparticles. This was then echoed by various national, European or international bodies<sup>69</sup>.

Despite the fragmentary efforts undertaken, as we have seen, to adapt the existing regulatory frameworks to nanomaterials, the lack of concrete social and economic assessment of their deployment continues to be felt. To identify the nanomaterials that are already present (and are therefore potential sources of exposure) in Europe, the Commission chose to respond positively to the call by the Parliament and the European Council, and on 18 October 2011, adopted a definition of nanomaterials. The French government also seems to be following this trend, having set up, with the *Grenelle* Acts and their implementing decrees, the mandatory reporting of substances with nanoparticle status.

### A- The European definition of nanomaterials

Striving for a balance between the results of the work by the SCENIHR and those of the JRC, with a desire to propose a definition that is both broad (including aggregates and agglomerates) but nevertheless excludes nanostructured materials, while avoiding terms considered too vague such as "typically but not exclusively" that had been approved by the ISO, and taking into account the comments it had received during the public consultation launched in December 2010, the European Commission produced a definition of nanomaterials in October 2011<sup>70</sup>. The text finally adopted bears the imprint of all these constraints (for example, regarding the number size threshold for the presence of the targeted nanoparticles, agglomerates or aggregates, which is set at 50%, subject to exceptions).

The definition, intended to serve as a basis for any revision of sectoral legislation that should be specified in relation to nanomaterials, is as follows: "*Nanomaterial* means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm.

*In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50% may be replaced by a threshold between 1 and 50%.*"

The recommendation states, however, that "*By derogation from point 2, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials*" and gives the following definitions, intended to supplement the first:

"For the purposes of point 2, "particle", "agglomerate" and "aggregate" are defined as follows:

- a) "particle" means a minute piece of matter with defined physical boundaries;
- b) "agglomerate" means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components;
- c) "aggregate" means a particle comprising of strongly bound or fused particles".

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<sup>68</sup> Nanoscience and nanotechnologies: hopes and concerns, Louis Laurent; Nanomaterials: A review of the definitions, applications, health effects. How to implement secure development Eric Gaffet, Comptes Rendus de l'Académie des Sciences, Physique.

<sup>69</sup> Canada: see [http://www.hc-sc.gc.ca/sr-sr/consult/\\_2010/nanomater/draft-ebauche-eng.php](http://www.hc-sc.gc.ca/sr-sr/consult/_2010/nanomater/draft-ebauche-eng.php)

Australia: Adjustments to NICNAS new chemicals processes for industrial nanomaterials, [http://www.nicnas.gov.au/Publications/Chemical\\_Gazette/pdf/2010oct\\_whole.pdf#page=14](http://www.nicnas.gov.au/Publications/Chemical_Gazette/pdf/2010oct_whole.pdf#page=14)

<sup>70</sup> Commission Recommendation of 18 October 2011 on the definition of nanomaterial (2011/696/EU)

It then states that "*Where technically feasible and requested in specific legislation, compliance with the definition in point 2 may be determined on the basis of the specific surface area by volume. A material should be considered as falling under the definition in point 2 where the specific surface area by volume of the material is greater than 60 m<sup>2</sup>/cm<sup>3</sup>. However, a material which, based on its number size distribution, is a nanomaterial should be considered as complying with the definition in point 2 even if the material has a specific surface area lower than 60 m<sup>2</sup>/cm<sup>3</sup>.*"

Finally, the Recommendation stipulates that "*The definition set out in points 1 to 5 will be reviewed in the light of experience and of scientific and technological developments. The review should particularly focus on whether the number size distribution threshold of 50% should be increased or decreased.*"

The memo published by the Commission at the same time as its recommendation warns, however, that this definition, which is particularly broad, is not intended to be adopted as such in all sectoral legislation potentially affected by nanomaterials, which should instead adapt the definition to their particular issues. The Commission reaffirms its position on the regulation strategy that it has been implementing since 2004 and explains the choice of the legal instrument of the Recommendation as allowing Member States to apply this definition with greater flexibility in their own systems.

## **B- The French reporting requirement for substances with nanoparticle status**

Providing for the organisation of a national public debate, as well as the establishment of a reporting requirement for substances with nanoparticle status, Article 42 of the Act of 3 August 2009<sup>71</sup> and Article 185 of the Act of 12 July 2010<sup>72</sup> are now about to be implemented. Since 1 January 2013, in fact, as a result of the information contained in the implementing decrees adopted last February<sup>73</sup> and the Order<sup>74</sup> detailing its contents, a report must be submitted by all "*persons who manufacture, import or distribute substances with nanoparticle status, in a pure state or contained in mixtures, without being bound, or in materials intended to release such substances in normal or reasonably foreseeable conditions of use*".

Subject to the reporting requirement are "*substances with nanoparticle status, in a pure state or contained in mixtures, without being bound*" and "*materials intended to release such substances in normal or reasonably foreseeable conditions of use*" that are produced, imported or distributed in amounts of at least 100 grams per year.

The notions of substances with nanoparticle status and substances contained in mixtures without being bound are defined in the implementing decree. According to this, in fact, "*substance with nanoparticle status*' means a substance as defined in Article 3 of Regulation (EC) No 1907/2006, manufactured intentionally at the nanoscale, containing particles, unbound or in aggregate form or in agglomerate form, of which a minimum proportion of the particles, in a number size distribution, have one or more external dimensions between 1 nm and 100 nm." The decree specifies, however, that "*this minimum proportion may be reduced in specific cases when justified for reasons of environmental protection, public health, safety or competitiveness. This is specified by a joint Order of the ministers responsible for the environment, agriculture, health, labour and industry*" and, as also specified by the Commission in 2011, "*By derogation from this definition, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external*

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<sup>71</sup> Planning Act No 2009-967 of 3 August 2009 on the implementation of the environmental *Grenelle*.

<sup>72</sup> Act No 2010-788 of 12 July 2010 on a national commitment for the environment.

<sup>73</sup> Decree No. 2012-232 of 17 February 2012 on the annual reporting of substances with nanoparticle status pursuant to Article L.523-4 of the French Environmental Code and Decree No. 2012-233 of 17 February 2012 on designation of the bodies referred to in Article L. 523-3 of the French Environmental Code.

<sup>74</sup> Order of 6 August 2012 on the content and submission conditions of annual reporting of substances with nanoparticle status, pursuant to Articles R. 523-12 and R. 523-13 of the French Environmental Code.

*dimensions below 1 nm should be considered as nanomaterials*" before adding, also on the same model, the terms of particle, agglomerate and aggregate.

Regarding a substance with nanoparticle status contained in a mixture without being bound, it is defined by the decree as a *"substance with nanoparticle status intentionally incorporated in a mixture from which it is liable to be extracted or released under normal or reasonably foreseeable conditions of use"*.

As for materials intended to reject such substances, they are not covered by any specific definition but must, according to the REACH (Registration, Evaluation and Authorisation of Chemicals) Regulation, cited by the French texts, be interpreted restrictively.

Reports are submitted electronically until 1 May of each year, to the French Agency for Food, Environmental and Occupational Health & Safety (ANSES), which administers the scheme on behalf of the Ministry of Ecology, Sustainable Development and Energy. The reports relate to the previous calendar year and specify the identity of the respondent, the identity of the nanoparticle substance, the quantity produced, imported or distributed in year N-1, its uses, and the identity of any professional users to whom it was sold. The precise content of the report is detailed in the Order adopted on 6 August 2012. The legal regime governing the reporting requirement, in turn, stipulates that if a manufacturer, importer or distributor has not submitted their report before 1 May of the past year, or within two months of a reminder sent by ANSES if the original report was incomplete, the Minister for the Environment may order payment of a fine of not exceeding 3000 euros and a daily penalty of 300 euros with effect from the day of the corresponding decision and until the obligation has been met. Finally, pursuant to Article L. 523-2 of the French Environmental Code, the administrative authority may require the respondent to disclose *"all available information on the hazards of these substances and the exposure they are likely to lead to, or useful in the assessment of risks to health and the environment"*, subject to the same penalties as the conventional report in the event of failure to respond within two months.

Many uncertainties remain since this reporting requirement was adopted. Because it does not concern retailers who sell finished products to the general public, in particular, it is difficult to see it as a means of achieving the objectives set by the legislature: traceability of nanoparticle substances will only, in fact, be partial and consumer information will take no account of the products actually in consumers' hands. It therefore seems likely that the real objective pursued by the legislature was rather to allow the documenting of nanomaterials present in France, an objective also apparently sought by the European Commission even if it has not, until now, actually set up any general requirement to implement it. The next few months should give us more information on the benefits and practical significance of this new scheme, which, it should be stressed, has nevertheless already begun to inspire our European neighbours<sup>75</sup>.

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<sup>75</sup> V. Pineros Garcet, J (2012) *Nanomaterials registries: EU Member States Initiatives. Federal Public Service for Health, Food Chain Safety and Environment, on behalf of the Member States nano-databases harmonization group. Presentation at CEFIC, 02.03.2012, Brussels.*

## Annex 6: Toxicity

### 1. Percutaneous penetration

Regarding the critical analysis of the literature on percutaneous penetration of nanoparticles, we drew on a report<sup>76</sup> produced by AFSSAPS, and adopted by this agency's Cosmetology Commission on 15 March 2011.

#### TiO<sub>2</sub> nanoparticles

The results from numerous reported *in vitro* and *ex vivo* skin penetration studies on human and animal skin indicate that the presence of TiO<sub>2</sub> nanoparticles is limited to the upper layers of the skin (*stratum corneum* and pilosebaceous *infundibulum*).

However, these studies were conducted over short periods (maximum 72 hours) with particles that were not characterised according to current knowledge in terms of size, crystalline form, coating, etc. In addition, some studies did not use standardised and validated protocols according to the recommendations of Europe's Scientific Committee on Consumer Safety (SCCS) or the Organisation for Economic Cooperation and Development (OECD).

A study by the FDA (US Food and Drug Administration; Sadrieh *et al*, 2010) remains to this day the relevant study chosen by AFSSAPS for analysing skin penetration of TiO<sub>2</sub> nanoparticles, since it:

- i) was conducted *in vivo*;
- ii) involved the assessment of dermal penetration of TiO<sub>2</sub> nanoparticles representative of those marketed for cosmetic products;
- iii) was conducted on the miniature pig, a species that is an appropriate model for the extrapolation of results to humans, because of the strong similarities in the skin of these two species, in terms of permeability and structure;
- iii) was carried out over a relatively long time with repeated applications (application of products 4 times a day, 5 days a week for 22 days), compared to other studies from the scientific literature that took place over short periods (up to 72 hours).

Thus, Sadrieh *et al.* (2010)<sup>77</sup> concluded as to the presence of large quantities of TiO<sub>2</sub> nanoparticles (coated and uncoated) and submicron TiO<sub>2</sub> particles (300-500 nm) in the *stratum corneum*, as well as the presence of a few isolated particles of TiO<sub>2</sub> in the dermis for animals treated with these three types of particles. Furthermore, this study revealed statistically significant quantities of TiO<sub>2</sub> in the left inguinal lymph node in the group treated with uncoated TiO<sub>2</sub> nanoparticles and in the right inguinal lymph node in the group treated with submicron TiO<sub>2</sub> particles (300-500 nm).

Because of the presence of uncoated TiO<sub>2</sub> nanoparticles and submicron TiO<sub>2</sub> particles (300-500 nm) in the inguinal lymph nodes, these results were unable to definitively confirm that systemic absorption in the miniature pig did not occur.

It would therefore be necessary to quantify the amount available in the inguinal lymph nodes and to elucidate the mechanisms of penetration. It is nevertheless important to remember that the TiO<sub>2</sub> nanoparticles used in cosmetics are generally coated.

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<sup>76</sup> "Etat des connaissances relatif aux nanoparticules de dioxyde de titane et d'oxyde de zinc dans les produits cosmétiques en termes de pénétration cutanée, de génotoxicité et de cancérogenèse" [Review of knowledge on titanium dioxide and zinc oxide nanoparticles in cosmetic products in terms of dermal penetration, genotoxicity and carcinogenicity] Report adopted by the Cosmetology Commission on 15 March 2011, Request No 2008BCT0001, AFSSAPS – 14/06/2011

<sup>77</sup> "Lack of significant dermal penetration of titanium dioxide from sunscreen formulations containing nano- and submicron-size TiO<sub>2</sub> particles", Sadrieh N., Wokovich A.M., Gopee N.V., Zheng J., Haines D., Parmitter D. *et al.* (2010). *Toxicological Sciences* 115: 156-66.

## ZnO nanoparticles

Concerning ZnO nanoparticles, few studies are available compared to those available for TiO<sub>2</sub> nanoparticles. *In vitro* dermal absorption studies (animal and human skin models) and studies in volunteers have been conducted showing that the presence of ZnO nanoparticles is limited to the upper layers of the skin (*stratum corneum* or *stratum granulosum*).

A recent study (Gulson *et al.*, 2010<sup>78</sup>) showed a statistically significant increase in levels of radiolabelled zinc (<sup>68</sup>Zn) measured in the blood and urine of human volunteers treated with ZnO nanoparticles. However, according to the authors this increase is still small compared to the levels of endogenous zinc in humans. They also point out that it is not possible to determine whether the <sup>68</sup>Zn was absorbed in the form of ZnO particles or as soluble Zn<sup>2+</sup> ions, or both.

## Influence of the quality of the skin and tests

The study findings are valid for healthy, undamaged skin. The results reported in the literature concerning damaged skin seem contradictory and it is likely that any skin lesion of pathological or exogenous origin may promote the absorption of nanoparticles.

In addition, it has been observed in some studies involving nanoparticles other than TiO<sub>2</sub> and ZnO (for example quantum dots and fullerenes) that there may be an impact on dermal penetration from mechanical effects (e.g. flexion of the skin), which would lead to an increase in dermal penetration, resulting in the presence of particles in the deeper layers of the epidermis and in the dermis.

## Assessment:

In conclusion, based on the available data, it is not possible to rule out systemic penetration of nanomaterials after dermal application.

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<sup>78</sup>"Small amounts of zinc from zinc oxide particles in sunscreens applied outdoors are absorbed through human skin", Gulson B., McCall M., Korsch M., Gomez L., Casey P., Yalchin O. *et al.* (2010). - Toxicological Sciences 118: 140-149.

## Annex 7: Safety by design/by process

It should be emphasised that the "toxicity and ecotoxicity" fields are at the intersection between two approaches based on very different principles:

- i) current principle: studies are conducted on a case by case basis and take place *a posteriori*;
- ii) revised principle: studies focus on "safer by design/by process"<sup>79</sup>

The toxic effects specifically induced by the nanoscale dimension of nanoparticles have now been addressed by numerous studies<sup>80</sup>. However it should be noted that these results are still highly fragmentary. They do not concern the products really likely to lead to consumer exposure<sup>81</sup> nor do they examine all the potential routes of exposure - ingestion for example is hardly considered. Finally, it should be noted that nearly 80% of the publications dating from before 2007<sup>82</sup> (Hansen *et al.*) only very partially describe the nanoparticles studied; such uncertainties mean that it is

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<sup>79</sup> *Maximizing safe design of engineered nanomaterials: the NIH and NIEHS research perspective* Sally S. Tinkle 2009 John Wiley & Sons, Inc. Volume 2, January/February 2010

*Hazard Reduction in Nanotechnology* Lucas Reijnders Journal of Industrial Ecology Volume 12, Number 3297 (2008)

*Are classical process safety concepts relevant to nanotechnology applications?* Paul R Amyotte Proc. Nanosafe 2010: International Conference on Safe Production and Use of Nanomaterials - Journal of Physics: Conference Series 304 (2011) 012071 IOP Publishing

*The 5 principles of "Design for Safer Nanotechnology"* Gregory Morose Journal of Cleaner Production 18 (2010) 285–289

Preface to "Développons les Nanomatériaux ? E. Gaffet "Développons les Nanomatériaux ? Sophie Carenco - ISBN-978-2-7288-0474-0, Collection "Sciences Durables" - Editions Rue d'Ulm (2012), Presse de l'Ecole Normale Supérieure

*Examining the Holy Grail of Nanotechnology: Safe By Design* Sally S. Tinkle <http://www.azonano.com/article.aspx?ArticleID=2508>

<sup>80</sup> Auffan, M., Achouak W., Rose J., Roncato M. A., Chaneac C., Waite D. T., Masion A., Woicik J. C., Wiesner. M. R., Bottero J.Y., (2008), "Relation between the redox state of iron-based nanoparticles and their cytotoxicity toward *Escherichia coli*". Environmental Science & Technology 42(17):6730-6735.

Donaldson, K., Murphy F. A., Duffin R., Poland C. A., (2010), "Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma". Particle and Fibre Toxicology 7.

Liu, A. H., K. N. Sun, J. F. Yang, D. M. Zhao, (2008a), "Toxicological effects of multi-wall carbon nanotubes in rats", Journal of Nanoparticle Research 10(8):1303-1307.

Muller, J., I. Decordier, P. H. Hoet, N. Lombaert, L. Thomassen, F. Huaux, D. Lison, M. Kirsch-Volders, (2008), "Clastogenic and aneugenic effects of multi-wall carbon nanotubes in epithelial cells". - Carcinogenesis 29(2):427-433.

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

Takagi, A., A. Hirose, T. Nishimura, N. Fukumori, A. Ogata, N. Ohashi, S. Kitajima, J. Kanno, (2008), "Induction of mesothelioma in p53+/- mouse by intraperitoneal application of multi-wall carbon nanotube". Journal of Toxicological Sciences 33(1):105 - 116.

Warheit, DB, Laurence BR., Reed KL, DH Roach DH., Reynolds GAM, Webb TR, (2004), "Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats", Toxicological Sciences 77(1):117-125.

<sup>81</sup> Ostrowski A.D., Martin T., Conti J., Hurt I., Herr Harthorn B. (2009) - J Nanopart Res 11:251–257),

<sup>82</sup> S. F. Hansen, B. H. Larsen, S.I. Olsen, A. Baun, "Categorization framework to aid hazard identification of nanomaterials", Nanotoxicology 2007, 18

impossible to establish any correlation between the physico-chemical parameters and the toxicity and ecotoxicity results.

In addition, as stressed in the first estimate of the cost and duration of the work required to conduct nanotoxicology studies on existing nanomaterials<sup>83</sup> (Choi *et al.* 2009), such an approach would cost US industry between 249 million and 1.18 billion dollars and would take over 50 years.

This has led to a radical call to consider the need for responsible and sustainable industrial development, also known as "safer by design" or "safer by process". Using as a starting point the specific properties of nanomaterials and how they evolve throughout their life cycle, Morose *et al.* (Morose *et al.* 2010) developed a mnemonic device to help remember what is meant by this new design of nanomaterials, namely SAFER: S for size, surface and structure<sup>84</sup>, A for Alternative materials<sup>85</sup>, F for Functionalization, E for Encapsulation<sup>86</sup> and R for Reduce the quantity.

This is a real paradigm shift, proposing a move away from the current approach that verifies the safety of nanoparticles and/or nanoproducts *a posteriori*, by designing nanoparticles and/or nanoproducts, from the very earliest stages of development and implementation, with the fewest possible risks to human health and the environment. This therefore involves integrating nanomaterial risk management as an element in the same way as other parameters in fields such as development, implementation, or design.

To implement such a change<sup>87</sup>, the proposal is to move from a short-term phase involving data collection, implementation of good practices in industrial health and safety, publication of good practice guides and assessment of toxicity, to a longer-term phase based on evidence of toxicity, reduction of toxicity, effective atmospheric control measures, a continuous improvement in health and safety best practices, and limits on the use of certain nanoparticles if the risk is too high.

Such an approach has already been suggested and was taken on board by the European Commission in October 2011<sup>88</sup>. It proposes for example, when assessing the safety of nanomaterials in cosmetics, exploring the possibility of developing assessment criteria that use several approaches, including a category-based approach, rather than on a case-by-case basis, whereas others that are more conventional are closer to the case-by-case approach.

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<sup>83</sup> Choi J.Y., Ramachandran G., Kandlika M. (2009) – *The impact of toxicity testing costs on nanomaterial regulation* - Environ. Sci. Technol., 43 (9), pp 3030–3034

<sup>84</sup> *Size-Controlled Dissolution of Organic-Coated Silver Nanoparticles* Rui Ma, Clément Levard, Stella M. Marinakos, Yingwen Cheng, Jie Liu, F. Marc Michel, Gordon E. Brown, Jr., Gregory V. Lowry Environ. Sci. Technol. 2012, 46, 752–759

<sup>85</sup> 'Safe-by-design' nanoparticles show reduced toxicity Cordelia Sealy NanoToday, Sept 2011, pp 113 <http://journals.elsevier.com/17480132/nano-today/1/36-news/162-safe-by-design-nanoparticles-show-reduced-toxicity>

*Decreased Dissolution of ZnO by Iron Doping Yields Nanoparticles with Reduced Toxicity in the Rodent Lung and Zebrafish Embryos* Tian Xia, Yan Zhao, Tina Sager, Saji George, Suman Pokhrel, Ning Li, David Schoenfeld, Huan Meng, Sijie Lin, Xiang Wang, Meiyang Wang, Zhaoxia Ji, Jeffrey I. Zink, Lutz Mädler, Vincent Castranova, Shuo Lin, Andre E. Nel [www.acsnano.org](http://www.acsnano.org), in press

*Role of Fe Doping in Tuning the Band Gap of TiO<sub>2</sub> for the Photo-Oxidation-Induced Cytotoxicity Paradigm* Saji George et al. J. Am. Chem. Soc., 2011, 133 (29), pp 11270–11278

<sup>86</sup> *Perspectives on Supplying Attenuation Grades of Titanium Dioxide and Zinc Oxide for Sunscreen Applications* David Schlossman, Yun Shao, Pascal Delrieu, Kobo Products Inc.

*Rate constant of the first order reaction of oxydation of acetalydehyde* M. Kobayashi and al., *Cosm & Toil.*, Vol. 112, No. 6, (1997) p83

<sup>87</sup> WTEC (2010), "Nanotechnology long-term impacts and research Directions 2000 – 2020" - [http://www.wtec.org/nano2/docs/ChaptersPdf/Ch04\\_NanoEHS\\_draft\\_2010-0929.pdf](http://www.wtec.org/nano2/docs/ChaptersPdf/Ch04_NanoEHS_draft_2010-0929.pdf)

<sup>88</sup> European Commission (2011), *EC Requests Guidance on Safety Assessment of Nanomaterials in Cosmetics*.



## Annex 8: Comparative review of existing methods of risk assessment adapted to nanomaterials or nanoproducts

This annex has been written based on the work of another ANSES Working Group tasked with establishing a risk assessment method adapted to the issues of consumer products containing manufactured nanomaterials. This expert appraisal work was still ongoing at the time of writing this report, and has been provided for the purposes of the annual review.

In response to the challenges posed by nanomaterials in terms of health risk assessment, several alternative risk assessment approaches and tools for guiding action (risk management) in such a context of uncertainty are currently available.

Individually designed to meet different purposes (e.g. help in the prevention of occupational risks, prioritisation of risks for nanoproducts, etc.), applied to specific objects (e.g. nanomaterials, nanoproducts, nanoparticles only, etc.) and according to different targets (e.g. consumers, general population, workers, etc.), the operating principles and rationales applied for each of these products are just as diverse.

The Working Group first compiled a list of these various tools and methodological approaches, before subsequently analysing them.

Identifying the existing methods mainly entailed drawing on the knowledge of each of the Working Group's experts, supplemented by a literature search. Given the obvious methodological benefits in terms of risk assessment presented by tools designed to help manage occupational risks, these were also considered, despite their having been designed for the work environment, a domain specifically excluded from the scope of the targeted risk assessment method.

A selection of the most relevant alternative risk assessment approaches and risk management tools among all those identified are discussed in this section. Their respective rationales and risk assessment approaches are briefly described here, as well as their purpose and areas of application. Finally, the salient features of each of these tools, and their advantages and disadvantages in relation to the objectives of this expert appraisal work, are outlined in a summary table.

### 1. Precautionary matrix for synthetic nanomaterials (FOPH-FOEN)

#### ► Purpose

This matrix<sup>89</sup> was created by the Swiss federal offices for public health (FOPH) and the environment (FOEN) as part of the Swiss "Synthetic Nanomaterials"<sup>90</sup> action plan dated 9 April 2008. It aims to conduct an initial objective analysis of the potential risks of a manufactured nanomaterial and its applications on the basis of current knowledge, and to determine, at each stage of the life cycle, whether special "nanospecific" measures should be taken to protect workers, consumers and the environment. The purpose of this matrix is also to identify potential sources of risk during the different stages of the life cycle of manufactured nanomaterials (production, use and disposal).

The authors warn, however, that *"this approach should not in any way be considered a risk assessment as such."*

Intended to serve as a working tool for a wide range of players involved in the safety of workers, consumers or the environment who are not necessarily specialists in risk assessment (from industry, trade, commerce, government, insurance, research laboratories, etc.), the precautionary matrix relies on a limited number of assessment parameters. However, the authors point out that

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<sup>89</sup> <http://www.bag.admin.ch/nanotechnologie/12171/12174/index.html?lang=en>

<sup>90</sup> <http://www.bafu.admin.ch/publikationen/publikation/00574/index.html?lang=en>

its use by non-specialists should only be considered under the supervision of specialists because of the knowledge needed to characterise some of these parameters, and also to properly exploit the results.

Finally, the method is not static but designed to evolve based on feedback and new scientific knowledge.

### ► Scope

The objects to which use of this method relate do not exactly correspond to the definition of nanomaterials provided by the ISO. Firstly, these objects are restricted to nanoparticles, nanofibres (nanoscale objects with respectively 2 or 3 dimensions at the nanoscale) and their aggregates grouped under the term "NPR" (for nanoparticles and nanorods). Secondly, the authors recommend considering a field at the nanoscale extending up to 500 nm (for the record, ISO defines the nanoscale as a "size range from approximately 1 nm to 100 nm, typically, but not exclusively"). Materials with a surface or volumetric nanostructure, provided they contain none of the particles defined above, are excluded from this category.

The precautionary matrix is applied to each stage of the life cycle of the nanomaterial or the application considered. The types of risks considered (health risks for workers, health risks for consumers, environmental risks) depend on the stage of the life cycle considered. In detail, the processes considered and the types of risks considered are summarised in Table 7.

**Table 7: "Target" groups to be considered depending on the type of process studied**

Process considered	Target group(s)
Research & development	- Employees - Environment
Production*	- Employees - Environment
Use	- Consumers - Environment
Recycling/disposal	- Employees - Environment

\* Includes the processes of primary production, processing, storage, packaging and transport

### ► Operating principles

The precautionary matrix is part of a procedure ultimately leading to completion of a new matrix for each of the different stages or processes identified. It is also recommended to complete a separate matrix for employees with different activity profiles for the same stage, or for different consumer groups.

The general principle proposed is a semi-quantitative assessment model. It involves, for each completed matrix, calculating a dimensionless score, characterising the needs in terms of precaution by combining the scores (also dimensionless) allocated to each of the criteria considered, using the following formula:

$$V = N \times (W \times E + S)$$

where:

**V:** assessment of needs in terms of precaution

**N:** nanorelevance

**W:** potential effect of NPRs on health and the environment

**E:** potential exposure of workers or consumers, or potential dispersion into the environment

**S:** score conditional on the level of information about the life cycle

These different needs in terms of precaution (employee/worker/environment) are then characterised according to the scores obtained: either the nanospecific risk can be considered low

and the situation does not require additional risk studies, or the score exceeds the threshold of action and further steps must therefore be taken (additional risk studies, risk reduction actions, etc.).

The calculation is centred on the combination of a score for potential effect (W), corresponding to a level of potential hazard, with a score for potential exposure/environmental dispersion (E). This result is complemented by a score (S) representing the level of knowledge about the product's life cycle. The less knowledge there is, the higher the score, tending in this case to trigger additional actions. Lastly, the nanorelevance term (N) only serves to verify that the product studied does indeed correspond to the definition of NPR or NPR aggregate.

The main criteria W and E are based on a limited number of assessment parameters such as:

- reactivity and stability of NPRs;
- their immediate physical environment (air, aerosols, fluids, solid matrices, etc.);
- NPR quantities concerned (by weight);
- frequency of use;
- their number.

Ultimately, the grid resembles a questionnaire with closed responses for each of these parameters, i.e. the response provided is guided by a set of predefined answers, if possible objectified by quantitative thresholds, and a score is allocated to each of these responses. It should be noted that in the event of inability to answer due to insufficient knowledge, the authors recommend considering the highest score.

#### ► Advantages/Disadvantages

One of the unique features of this model is the use of a score relative to the state of knowledge for assessing the level of risk incurred. The authors propose increasing the scores when the requested data are unavailable.

Another interesting feature is the calculation of scores from scenarios considered "normal" as well as from "worst case" scenarios.

This tool has a solid methodological basis, in several respects. Firstly, the matrix resembles a questionnaire with closed responses, i.e. the answer to each assessment criterion is not subjective but is guided by a set of scores dependent on quantitative thresholds referring to recent evidence (published in 2008, revised in 2011). These scores are then integrated into a model whose consistency (weighting of these scores, consistency of units, etc.) has been verified by an external expert statistician. Lastly, the matrix is accompanied by a full explanatory document on its use.

However, limitations to this tool have also been noted. Regarding the assessment of the potential effects of nanomaterials, this potential effect is calculated based on only two criteria:

- level of redox and/or catalytic activity of the nanomaterial;
- stability in relevant media (physiological and environmental conditions for calculating respectively the effects for humans and the environment).

This model is based on a particular toxicological mechanism: the formation of reactive oxygen species (ROS). However, toxicological studies have shown that the effects associated with exposure to nanomaterials do not exclusively concern the formation of ROS. This score, depending solely on physico-chemical parameters combined with toxicokinetic data (mainly on clearance) does not take into account the toxicological and ecotoxicological studies that might be available, which are however highly relevant (information on penetration of the skin barrier for dermal exposure, for instance). Nor can this model be used to distinguish the characteristics associated with the different routes of exposure (respiratory, oral, dermal).

## 2. Graduated risk management (Control Banding) tools

There are several graduated risk management (control banding) tools currently available that can be applied to the case of nanomaterials. The first suggested application for nanomaterials was proposed by Maynard *et al.*<sup>91</sup>. Although they have rather similar aims and general operating principles, these various products differ slightly in their detailed operating principles (types of factors taken into account, probabilistic or deterministic approach to estimate exposure, etc.). This section therefore covers the different tools identified, with a particular focus on the one developed by ANSES.

### ► Purpose

Originally developed for the pharmaceutical industry to ensure the safety of workers involved in processes using products for which little information was available, the graduated risk management method is essentially an alternative proposed with a view to conducting a qualitative risk assessment and implementing protective measures for employees exposed to products on which data are lacking.

Its purpose is to provide a structured analytical approach that overcomes the uncertainties and gaps in knowledge, and enables the most appropriate practical operational protection/control measures to be proposed *a priori*.

For some of these methods, the stated objective is also to provide a tool that is relatively simple to use and interpret, aimed at organisations without the means to call on the expertise of an occupational hygienist.

### ► Scope

The many tools derived from the graduated risk management approach are similar to tools assisting in the prevention of health risks in the workplace. When adapted specifically for research, production or processing of nanomaterials, only nanospecific health risks are concerned.

The definition of nanomaterials used and the type of nanomaterials taken into account differ slightly according to the tool.

### ► Operating principles

Graduated risk management can be likened to a qualitative or semi-quantitative approach to the assessment and management of occupational risk. The general principle involves assigning a "band" to a product depending on the levels of hazard and probability of exposure to this product at the work station. In this process, each of these bands corresponds to a risk management strategy.

This principle is based on the assumption that it is possible to act in a context of uncertainty, because although the toxicological effects of the nanomaterials and/or the actual exposure to these products may be unknown, the range of control strategies to be applied with regard to these risk levels are broadly equivalent to those for chemicals and are therefore known.

### ❖ COSHH essentials<sup>92</sup>

This method, developed by the British agency responsible for occupational health and safety (UK Health and Safety Executive), is an integral part of the toolbox helping small and medium

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<sup>91</sup> Maynard AD (2007) Nanotechnology: The Next Big Thing, or Much Ado about Nothing? *Annals of Occupational Hygiene* 51(1), 1-12.

<sup>92</sup> [www.coshh-essentials.org.uk](http://www.coshh-essentials.org.uk)

companies meet their obligations in terms of occupational risk prevention as defined by COSHH<sup>93</sup> national legislation.

The tool offers five hazard groups. The allocation of one of these groups to a product or a substance is based on labelling data, initially the "R" risk phrases defined by European labelling regulations and, by extension, the risk phrases of the Globally Harmonised System (GHS) of classification and labelling.

Although not specifically designed for nanomaterials, an adapted version of this tool can help refine the allocation of groups depending on physico-chemical characteristics specific to nanomaterials (size, morphology, surface, crystallinity, reactivity, solubility), enabling their hazard level to be reduced or increased.

The exposure potential is characterised according to the emission potential of the type of process considered (dustiness potential, physical state, quantity, nature of the process).

At the end of the analysis, one of four levels of protection is recommended (good industrial hygiene practices, local air extraction, contained process that can be opened, totally contained process).

#### ❖ British Standards Institution

The BSI guide defines four categories of hazard for manufactured nanomaterials (in descending order of hazard level: fibrous, CMAR<sup>94</sup>, insoluble, soluble) and incorporates information about benchmark exposure levels (BELs), which give an indication of the control levels for nanomaterials in these groups and, on the basis of a graduated risk management approach, provide indications of risk control (mainly through control of exposure).

It should be noted that these BELs mainly result from an expert decision-making process and are not determined on the basis of established knowledge.

#### ❖ Stoffenmanager

This method, intended for the assessment and management of risks from dermal and respiratory exposure in small and medium enterprises, has an online application<sup>95</sup>. It combines a framework for assigning hazard bands similar to that of *COSHH Essentials* with a simplified method of assigning an exposure band, is easy to understand and can be used by non-experts. This regularly updated method offers a module specifically developed for nanomaterials (*Stoffenmanager Nano 1.0*) which has not yet been tested as extensively as the original *Stoffenmanager*.

Exposure is calculated by an algorithm of exposure by inhalation based on the source-recipient approach, using various input factors (tasks, existing control measures, general ventilation and product characteristics) graded on a logarithmic scale. Because it uses a dust-diffusion model, the tool is not suitable for fibrous materials.

Based on the results of validation studies, the Working Group concluded that the exposure estimated by *Stoffenmanager* is generally correct and sufficiently conservative, although in some specific cases, results could be improved by adapting the model.

#### ❖ Control Banding NanoTool

This system involves calculating a severity score for the nanomaterial (between 0 and 100) to be combined with a score for probability of exposure (also between 0 and 100), in order to graphically determine the risk level score (see Figure 6).

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<sup>93</sup> COSHH: Control of Substances Hazardous to Health <http://www.hse.gov.uk/coshh/>

<sup>94</sup> Nanomaterials for which the larger particle sizes have already been classified as carcinogenic, mutagenic, asthmogenic or reproductive toxin.

<sup>95</sup> <http://nano.stoffenmanager.nl/>

This model predominantly considers respiratory exposure but also takes into account the dermal route of exposure.

		Probability			
		Extremely Unlikely (0-25)	Less Likely (26-50)	Likely (51-75)	Probable (76-100)
Severity	Very High (76-100)	RL 3	RL 3	RL 4	RL 4
	High (51-75)	RL 2	RL 2	RL 3	RL 4
	Medium (26-50)	RL 1	RL 1	RL 2	RL 3
	Low (0-25)	RL 1	RL 1	RL 1	RL 2

RL 1: General Ventilation  
 RL 2: Fume hoods or local exhaust ventilation  
 RL 3: Containment  
 RL 4: Seek specialist advice

Source: [http://controlbanding.net/uploads/CB\\_Nanotool\\_description\\_Version2\\_3-16-10\\_.pdf](http://controlbanding.net/uploads/CB_Nanotool_description_Version2_3-16-10_.pdf)

**Figure 6: Determination of the risk level (RL) scores based on the severity score (y-axis) and the probability score (x-axis) for NanoTool 2.0.**

The calculation of the severity score includes toxicological data on the "parent" material in addition to the physico-chemical characteristics and toxicological properties of the nanomaterial. The specific data on the nanomaterial and the "parent" material account for respectively 70% and 30% of this severity score.

The number of workers exposed is included as a factor when calculating the score for probability of worker exposure, thus indicating that the type of risk assessed by this model does not correspond exactly to an individual risk (independent of the number of people exposed).

The factors taken into account and the ranges of their associated scores are summarised in Table 8.

One of the most interesting features of this system is that it makes allowance in the calculation for a lack of available data to characterise these factors. Thus, in such a case of insufficient knowledge, the score used is 75% ( $\frac{3}{4}$ ) of the maximum interval for the score associated with this parameter. Therefore, this rule does not quite correspond to a precautionary principle in which the risk would be increased (maximum score) according to the lack of data. The justification for this choice is as follows: a conservative approach would involve treating an unknown risk as equivalent to a high risk, and would not therefore lead to recommendations on technical risk control measures (such as total containment of the process), but would specifically require specialist advice to be sought (RL 4). However, since the authors anticipated that information would be unavailable for many of the factors regarded as important, they felt that this choice would cancel out the benefits of graduated risk management. A safety mechanism was therefore planned for this system: when the user enters "data unavailable" for all the score determinants, the level of control will automatically be "containment" (RL 3).

**Table 8: Factors taken into account for calculating scores of severity and probability of exposure, and "target" ranges to be considered depending on the type of process being studied**

Severity score (0 - 100)			Probability score (0 -100)	
Factor		Score range	Factor	Score range
Specific to the nanomaterial	Surface reactivity	0 - 10	Quantities handled**	6.25 - 25
	Particle morphology	0 - 10	Dustiness	0 - 30
	Particle dimension	0 - 10	Number of employees	0 - 15
	Solubility	0 - 10	Frequency	0 - 15
	Carcinogenicity	0 - 6	Duration	0 - 15
	Toxicity to reproduction	0 - 6		
	Mutagenicity	0 - 6		
	Dermal toxicity	0 - 6		
	Ability to cause asthma	0 - 6		
"Parent" material	Toxicity of "parent" material*	0 - 10		
	Carcinogenicity	0 - 4		
	Toxicity to reproduction	0 - 4		
	Mutagenicity	0 - 4		
	Dermal toxicity	0 - 4		
	Ability to cause asthma	0 - 4		

\*: Existing occupational exposure limits of the "parent" material taken into account

\*\* : In weight for the task concerned

This tool, one of the first to be proposed, has been assessed in a real situation<sup>96</sup>. However, determining scores may require significant expertise.

❖ Control banding tool (ANSES, 2011)<sup>97</sup>

One of this tool's stated aims is to provide a means for graduated management of health risks specifically adapted to nanomaterials, and intended for organisations lacking the resources to conduct the extensive toxicological studies required for a proper risk assessment process. The approach is simple, accessible and highly operational.

<sup>96</sup> Zalk DM, Paik SY, Swuste P (2009) *Evaluating the Control Banding Nanotool: a qualitative risk assessment method for controlling nanoparticle exposures*. *Journal of Nanoparticle Research* **11**(7), 1685-1704.

<sup>97</sup>

<http://www.anses.fr/en/content/tool-control-banding-risks-associated-nanomaterials> ?

One special feature of this approach is that it immediately excludes nanomaterials meeting the definition of biopersistent fibres, assigning the highest hazard band to them.

The hazard band is assigned to other types of nanomaterials according to the toxicological properties of the reference substance (ideally, the "parent" material or an "analogous" material) characterised using the labelling data from the CLP<sup>98</sup> Regulation (see **Erreur ! Source du renvoi introuvable.**) adjusted for the following mitigation/aggravation factors specific to the nanomaterial:

- solubility, to determine its biopersistence or its biokinetic behaviour (ability to cross biological barriers);
- reactivity, to determine the potentially increased capacity of the nanomaterial, compared to the reference substance, to generate reactive species (a mechanism of inflammatory stress).

		Toxicity level labelling				
		HB1	HB2	HB3	HB4	HB5
<b>Classification and labelling</b>		 <b>Warning</b> Eye irrit. 2 Skin irrit. 2 And all H-phrases not otherwise listed	 <b>Warning</b> Acute tox. 4   <b>Warning</b> STOT-SE 2	 <b>Warning</b> Acute tox. 3   <b>Warning</b> STOT-RE 2   Skin Corr. 1 Eye Dam. 1   <b>Warning</b> Skin sens. 1 STOT-SE 3 (resp. irritant)	 <b>Danger</b> Acute tox. 1-2   <b>Danger</b> STOT-SE 1 STOT-RE 1 Repro. Tox 1A - 1B   <b>Warning</b> Carc. 2 Repro. 2	 <b>Danger</b> Resp. sens. 1 Carc. 1A - 1B Muta. 1A - 1B   <b>Warning</b> Muta. 2

Figure 7: Hazard bands considered for the “parent” material based on the allocation of hazard groups in the e-COSHH Essentials tool.

<sup>98</sup> The CLP Regulation refers to Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures.

The logic diagram for allocation of hazard bands is shown in Figure 8.

**Figure 8: Diagram showing how a nanomaterial is allocated to a hazard band in the ANSES control banding tool.**

The exposure potential in this model is similar to an emission potential, and allocation of exposure bands (see **Erreur ! Source du renvoi introuvable.**) takes into account:

- the physical characteristic of the material and its matrix (dustiness potential, aerosol, liquid, solid);
- any more or less dispersive process it undergoes.

Physical form	Solid	Liquid	Powder	Aerosol
Emission Potential	EP1	EP2	EP3	EP4
Specific cases of band modification due to the natural tendency of the material				
	Friable solid <sup>99</sup> (+2 bands)	Highly volatile liquid <sup>100</sup> (+1 band)	Highly or moderately dusty powder <sup>101</sup> (+1 band)	-
Specific cases of band modification due to process operation				
	Dust generated by external forces <sup>102</sup> (+3 bands) Melting (+1 band) Dispersion in liquid (+1 band)	Powder generated by evaporation (+1/+2 band according to dustiness of the powder) Spraying (+2 bands) No generation of aerosol during process: (-1 band)	Spraying (+1 band)	-

Figure 9: Allocation of exposure bands in the ANSES control banding tool

Combining these hazard and exposure bands leads to a control level being determined from among five predefined levels (no specific measure, local ventilation, ventilation with a hood, contained process, and contained process with review by a specialist).

		Emission potential bands			
		EP1	EP2	EP3	EP4
Hazard bands	HB1	CL1	CL 1	CL 2	CL 3
	HB2	CL1	CL 1	CL 2	CL 3
	HB3	CL1	CL 1	CL 3	CL 4
	HB4	CL 2	CL 2	CL 4	CL 5
	HB5	CL 5	CL 5	CL 5	CL 5

Figure 10: Matrix of control levels (CL) to be implemented with regard to the combination of the hazard level and emission potential in the ANSES control banding tool

<sup>99</sup> Material whose matrix is likely to release particles under low stress (Hansen *et al.*, 2007)

<sup>100</sup> INRS ND 2233

<sup>101</sup> Respirable fraction according to EN 15051

<sup>102</sup> External forces such as for instance, mechanical forces, electrical forces, lasers, etc.

This method's weakness lies in the difficulty of defining and characterising a material's solubility and reactivity (increment factors for the hazard band).

#### ► Advantages / disadvantages

The various approaches associated with graduated risk management tend to compensate any knowledge gaps, especially concerning toxicology, by taking into account parameters that are more easily accessible, such as physico-chemical properties and toxicity data available for the nanomaterial in question or materials of a similar nature and physico-chemical form (parent material or chemical analogues).

These tools are attractive because firstly, they have an apparently simple structured approach leading to conventional practical protection/control measures (filters, isolation measures, etc.), and secondly, they have been successfully tested by the pharmaceutical industry on issues relatively similar to those encountered for nanomaterials.

However, several disadvantages should be mentioned. Firstly, despite the apparent simplicity of these methods, the lack of available information frequently means that there is a need for expert judgment.

Regarding their effectiveness, the graduated risk management approach is confronted by currently irreducible unknowns such as the specific toxicity of a given type of nanomaterial (novel health effects, new diseases, etc.). Regardless of the method developed, it is still evident to their respective designers that these tools cannot be used to demonstrate that the risks are being adequately controlled. They must be understood as a strategy to be followed as an interim measure, enabling an initial selection of control measures to be proposed pending further information on exposure, toxicity and risk. There is in fact to date no theoretical or empirical tool able to scientifically estimate the toxicity of a type of nanomaterial based only on physico-chemical data and the toxicological properties of the parent material. Predicting the toxicity of nanomaterials from the consideration of these various factors cannot currently be guaranteed.

Finally, as this graduated risk management approach is an integral part of occupational risk prevention, few efforts are made to distinguish the different routes of exposure. In most cases, only the respiratory route is considered, with the oral route never being taken into account. Although exposure via ingestion proves to be largely irrelevant in such an occupational context, its inclusion is necessary for the assessment method targeted by the work of the Working Group.

### 3. NanoRiskCat

#### ► Purpose

NanoRiskCat (NRC) is a tool that was developed by the Danish Environmental Protection Agency for industrial companies using nanoproducts or integrating manufactured nanomaterials in the products they market, as well as for regulators and the public. The purpose of this methodological approach is to assess, prioritise and inform about the potential exposure and effects of nanoproducts, with the aim of helping these various players take decisions on issues of nanoproduct safety with respect to health and the environment.

The method therefore adopts a simple approach (decision tree) expressing the results in very simple terms (logos associated with colours), to enable it to be used as a communication tool for a non-specialist audience.

Concerning its application, this assessment/communication system is currently being used by the Danish nanoproducts database<sup>103</sup>.

► **Scope**

The objects targeted for assessment by this method are products containing nanomaterials within the meaning of the definition given by the ISO.

For each product, three targets have been selected for exposure arising from its use (professional user, consumer and the environment) and two targets have been selected for its effects (humans and the environment). Products are categorised for each targeted exposure and effect.

Therefore, although exposure of the professional nanoproduct user is taken into account and clearly distinguished from that of the consumer, it should be noted that the risks associated with production of the nanoproduct are not addressed by the assessment. This method is firmly aimed at assessing the risks associated with the use of the product.

► **Operating principles**

Each risk assessment for a product containing nanomaterials is carried out on the basis of five criteria: three relating to the potential for exposure to/dispersion of nanomaterials (for the professional user, the consumer and the environment), and two relating to their health and environmental effects.

For each of these five criteria, the analysis is carried out according to a specific framework and the result is expressed using a coloured indicator according to an intuitive colour code, reflecting the four possible levels:

- Red: strong indication of exposure or effects;
- Yellow: moderate indication of exposure or effects;
- Green: low indication of exposure;
- Grey: Insufficient data available for assessment.

This colour coding is then supplemented by a phrase for indications of effects. The tool proposes two tables containing nineteen standard phrases for "effect on human health" indications, and twelve phrases for "effect on the environment" indications. These phrases explain the choice of colour.

Ultimately, the result is expressed in a detailed format and also in summary form as shown in **Erreur ! Source du renvoi introuvable.**

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<sup>103</sup> The Nanodatabase: <http://nano.taenk.dk/>

Indications regarding exposure/dispersion			Indications regarding effects	
Professional user	Consumer	Environment	Human health	Environment
●	●	●	●	●
			< Explanatory phrase * >	< Explanatory phrase ** >

\*: See the list of predefined phrases in NanoRiskCat that can assist the user in detailing the evidence justifying the estimated level of hazard to human health, represented by the colour indicator.

\*\* : See the list of predefined phrases in NanoRiskCat that can assist the user in detailing the evidence justifying the estimated level of hazard to the environment, represented by the colour indicator.

**Figure 11: Simplified expression of results for NanoRiskCat**

One of the key features of this assessment method is that it expresses the results of the analysis as estimates of exposure and hazard levels for different targets. Their interpretation in terms of risk is left to the reader's discretion. This choice is justified firstly, by the value of this information for issues of risk management and, secondly, because it avoids introducing an element of subjectivity into the analysis. Indeed, systematically identifying risk levels by combining the hazard and exposure/dispersion levels essentially relies on expert choices that are not easy to justify.

To obtain these analysis results, the method is applied in successive stages and combined with a framework for categorising the different items (exposure and effect).

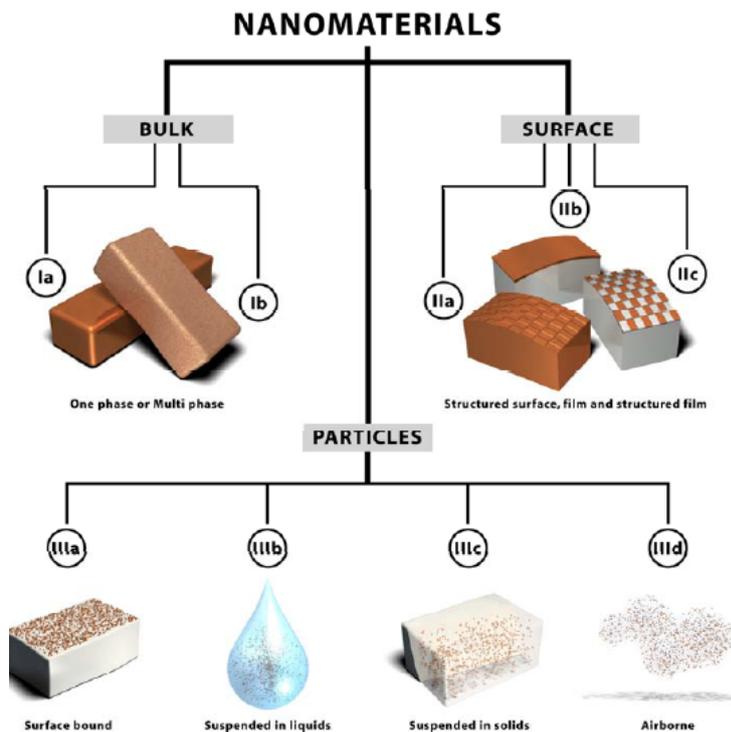
#### 1) Description of the product

The description of the nanomaterial and the product containing it is the first step. Information on the nanomaterial's physico-chemical parameters are collected (source, production, process, appearance, chemical composition, physical form, scale, purity, size of nano-objects, water solubility, agglomeration state, aggregation state, CAS number) and the stages of the material's life cycle are described according to the structure set out by the *Nano Risk Framework*.

#### 2) Assessment of exposure level

When specific exposure data are available, these are used. When this information is not known, the level of exposure is assessed according to the availability of the nanomaterial in the article (see Figure 12) and the REACH category descriptors<sup>104</sup> (PROC: process category, PC: product category, FC: technical functions, AC: article's emission category and ERC: environmental release category).

<sup>104</sup> *Guidance on information requirements and chemical safety assessment, Appendix R12*



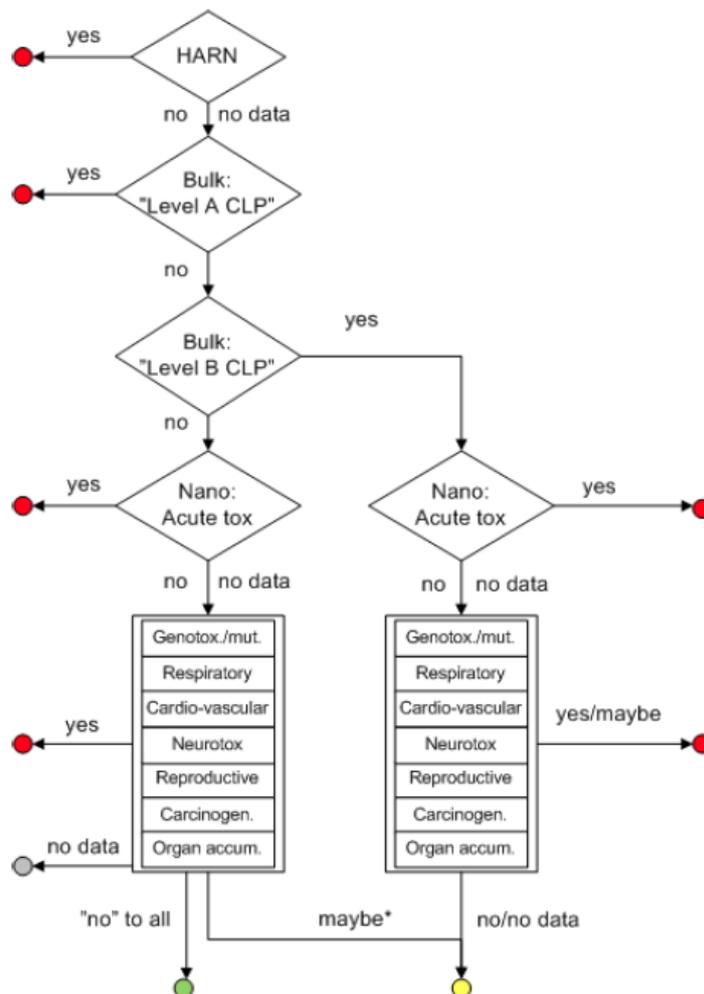
Source: Hansen 2007<sup>105</sup>

Figure 12: Categorisation of the product matrix for NanoRiskCat

<sup>105</sup> Hansen SF, Michelson ES, Kamper A, Borling P, Stuer-Lauridsen F, Baun A (2008) Categorization framework to aid exposure assessment of nanomaterials in consumer products. *Ecotoxicology* 17(5), 438-447

### 3) Assessment of the potential hazard to human health

The potential hazard to humans is assessed with the aid of a relatively simple decision tree (Erreur ! Source du renvoi introuvable.).



Source: Hansen 2011<sup>106</sup>

**Figure 13: NanoRiskCat decision tree for determining the potential hazard for humans**

According to this rationale, a maximum potential hazard (red indicator) is assigned immediately to fibrous type nanomaterials (whose length/diameter ratio is greater than  $10^{107}$ ).

Information about the toxicity of the nanomaterial's "parent" material is then taken into account using labelling data. Based on the realistic assumption that the level of hazard of a nanomaterial is at least as high as that of its "parent" material, the nanomaterial's hazard level is highest when these labelling data correspond to toxicological effects regarded as strong (class A). If the CLP labelling categorisation applied to the non-nanoscale material only indicates effects considered to be moderate (class B), the hazard potential of the nanomaterial is incremented.

The remainder of the assessment is based on toxicological data specific to the nanomaterial for several types of effects (acute toxicity, mutagenicity/genotoxicity, cardiovascular effects,

<sup>106</sup> Hansen SF, Baun A, Alstrup-Jensen K (2011) NanoRiskCat – a conceptual decision support tool for nanomaterials. Danish Ministry of the Environment

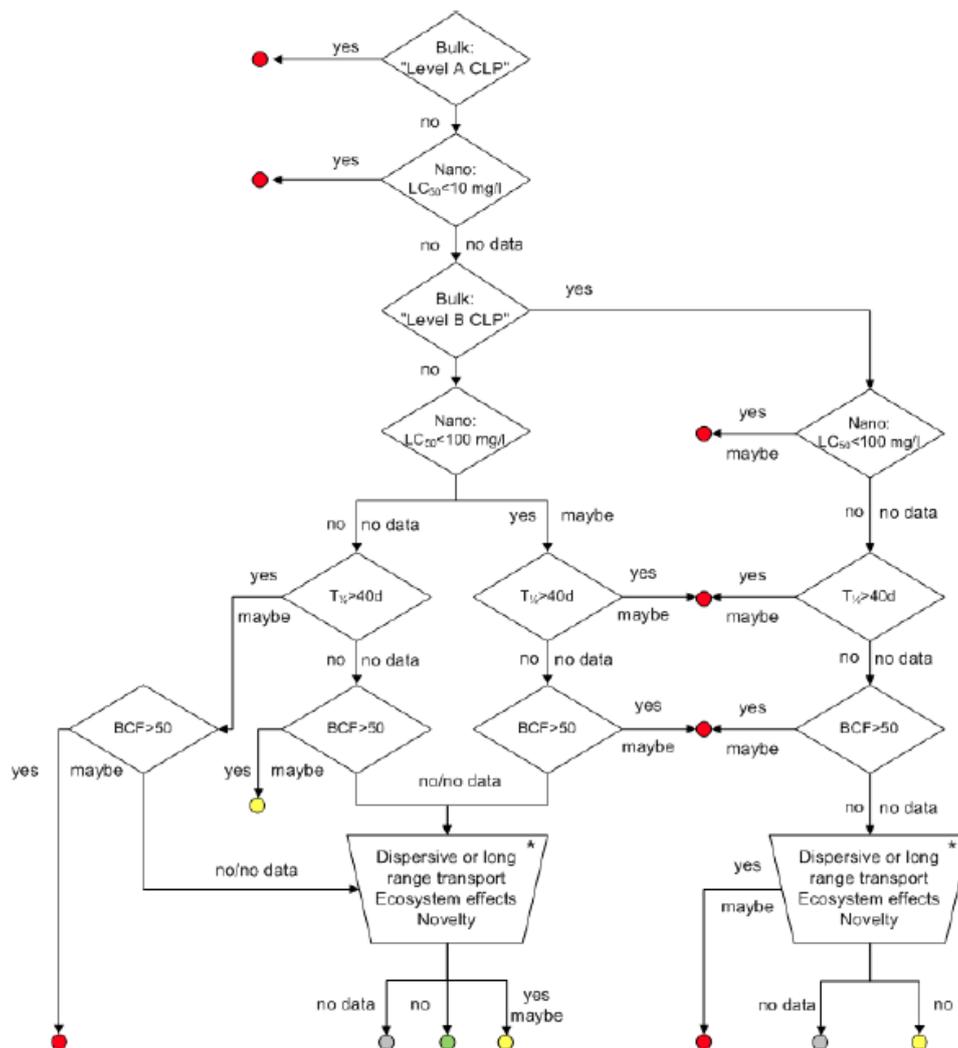
<sup>107</sup> HARN: High Aspect Ratio Nanoparticles

respiratory effects, neurotoxic effects, reproductive effects, carcinogenic effects) and on bioaccumulation. Once a relevant effect is found, this scheme recommends considering the highest hazard level. Allowance is made in the decision tree for the absence or presence of uncertainties relating to these data.

#### 4) Assessment of the potential hazard to the environment

The potential hazard represented by the nanomaterial to the environment is also assessed according to a decision tree (see Figure 14) taking into account the CLP labelling data for the "parent" material, and ecotoxicological data specific to the nanomaterial combined with limit values from REACH.

One of the characteristics of this method is that it incorporates other types of data at the end of the chain. Thus, if the nanomaterial has a high potential for dispersion in environmental compartments or if it is not already present in the environment (new material), this potential hazard is incremented as a precautionary measure.



Source: Hansen 2010<sup>108</sup>

Figure 14: NanoRiskCat decision tree for determining the potential hazard for the environment

► Advantages/disadvantages

This method has several major points of interest. Firstly, its aims resemble those of the expert appraisal work contained in this report, as it applies specifically to finished products containing manufactured nanomaterials. Thus, its application should enable nanoproducs to be compared with each other.

The logic behind its use, based on a highly structured, visual approach, is simple enough to place it within the reach of non-specialists in risk assessment, although in practice, the responses to be given at each of the steps still require, in most cases, the application of significant expert skills.

The results at the end are expressed in a very visual way that makes them easy to understand. Moreover, the logic diagrams offered provide visual explanations tracing the reasoning behind these results.

<sup>108</sup> Hansen SF, Baun A, Alstrup-Jensen K (2011) *NanoRiskCat - A conceptual decision support tool for nanomaterials*. Danish Ministry of the Environment.

Finally, the assessment method has been designed to be used even when data are lacking. Traceability of the results enables areas of uncertainty to be distinguished.

However, when examined in detail, several limitations and disadvantages were also identified. Concerning the methods for determining levels of exposure and environmental dispersion, these consist of simple generic assessments that only include specific features of the product's use to a very limited degree. The use of REACH descriptors is interesting, but also raises the issue of the relevance of these classifications for nanoproducts in the context of nanospecific risks. Another drawback is that the source load (quantity of manufactured nanomaterials in the product) is not included in these assessments despite the relevance of this parameter.

Concerning the method of assessing potential hazards to human health and the environment, no distinction is made between the different routes of exposure and environmental compartments. The proposed assessment processes are mainly based on the most reliable data, whether toxicological or ecotoxicological, but are unable to incorporate other types of information, such as properties that are relevant for hazard assessment (physico-chemical reactivity for example), if the above types of data are unavailable. In addition, many (eco)toxicological thresholds are expressed using conventional units ( $\text{mg.kg}^{-1}$  for example) whose relevance to nanomaterials is still under discussion.

This method ultimately produces data on exposure and hazard levels. These data are obtained before the risk level has been determined and are therefore very useful to risk management. The estimated risk level is also valuable in the context of risk management. However, it is up to the user to interpret the level of risk on this basis.

Finally, the effective operation of this method has not yet been sufficiently validated, and because it is a recent method, no feedback has yet been documented.

#### **4. A prudent approach to nanotech environmental, health, and safety risks (Lux Research, 2005)**

##### **► Purpose**

The consulting firm *Lux Research* has developed a quick and easy-to-use "turnkey" tool for the qualitative assessment of the nanospecific risks of a finished product throughout its life cycle<sup>109</sup>.

##### **► Scope**

This method ultimately aims to determine an estimated level of nanospecific risk to health for the entire life cycle of a product containing manufactured nanoparticles. The results also express the estimated potential intrinsic hazard of manufactured nanoparticles and the estimated potential exposure for three phases of the life cycle (production/use/end of life).

##### **► Operating principles**

Starting from the standard health risk assessment framework, the risk level of a product is defined from the intrinsic hazard level of the nanomaterial and the level of exposure/dispersion during the life cycle of the finished product.

In detail, the hazard level, characterised by a three-point scale (low, moderate and high), is directly related to the type or family of nanomaterial used (fullerenes, titanium dioxide, zinc oxide, etc.). The classification of this level is predetermined according to several criteria stated by the authors.

Similarly, the rating of the exposure level, still based on a scale of three (low, moderate and high) is predefined according to the product category, while distinguishing the stages of the product's life

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<sup>109</sup> LuxResearch (2005) A prudent approach to nanotech environmental, health, and safety risks. New York.

cycle (production/use/end of life). A number of criteria are specified for estimating these exposure levels.

The level of nanospecific risk associated with the product, still defined according to three levels (low, moderate and high), comes from combining the hazard levels and exposure levels estimated in the previous steps. The result, in the form of a table cross-referencing the type of nanomaterial with the type of application and incorporating an intuitive colour code, provides a schematic presentation of the risks.

#### ► Advantages/disadvantages

The synthetic presentation of these results makes it much easier to compare the risks according to the products. However, although relatively simple to use, this assessment model does have several major methodological shortcomings.

To begin with, it is a pity that only one potential hazard is considered for the entire life cycle. Indeed, nanomaterials injected into the matrix of a product may change during the life of this product. The nanomaterials emitted (dispersed in the environment and to which the population is exposed) and thus their toxicity may then differ from that of the initial nanomaterials. Therefore, a toxicity assessment should ideally be performed for each phase of the life cycle. In addition, a nanomaterial's toxicity can depend on many factors that may or may not be specific to the type of nanomaterial. Yet in this model, the potential hazard is set for a group or family of nanomaterials.

Continuing this reasoning, several questions remain about these ratings, which were predefined by the authors. On the one hand, the criteria listed for these assessments are very general and do not seem exhaustive (their relevance and weight were not explained in the document provided). On the other hand, the methods used to obtain these ratings (hazard and exposure) from the criteria mentioned are not explained. Similarly, the overall risk assessment process based on the proposed ratings is not described. These various points make it impossible to update the rating parameters as scientific knowledge advances (this document was published in 2005) or to extend the categories used (types of nanomaterials and applications), in order to adapt the tool as it stands to our current problem.

There are other methodological issues, particularly regarding the exact nature of the type of risk estimated, because the target populations differ according to the phase of the life cycle concerned: the concepts of risks to workers, consumers and the general population are not differentiated.

## 5. Multi-criteria decision analysis (MCDA)

#### ► Purpose

*"Multi-criteria analysis is a technical science devoted to elucidating understanding of a decision-making problem and its resolution. It aims to make explicit a coherent family of criteria allowing conception, justification and transformation of preferences within the decision process."<sup>110</sup>*

Generally speaking, multi-criteria analysis should enable different options to be assessed when no single one is perfect, and should help reconcile different types of information (technical/technological, design, environmental, economic and social data, as well as data on emerging risks for which the uncertainties are high). Multi-criteria analysis combines all these inputs with cost/benefit data. Linkov *et al.* showed that multi-criteria analysis can help manage the

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<sup>110</sup> Lehoux N, Vallée P (2004) 'Analyse multicritères.' In (Ecole Polytechnique de Montréal: Montréal) Available at [http://www.performance-publique.budget.gouv.fr/sites/performance\\_publique/files/files/documents/performance/controle\\_gestion/qualite\\_et\\_CG/Analyse\\_multicriteres/1\\_Multi\\_criteres2004.pdf](http://www.performance-publique.budget.gouv.fr/sites/performance_publique/files/files/documents/performance/controle_gestion/qualite_et_CG/Analyse_multicriteres/1_Multi_criteres2004.pdf) [Verified 2014]

risks associated with nanomaterials by developing and better identifying the materials and methods of production despite the high degree of uncertainty<sup>111</sup>.

► **Scope**

There are many fields of application: choice of a development site, an environmental project, the use of a particular technology. In the case of nanotechnologies, MCDA focuses essentially on the technology or nanomaterials that can be used.

► **Operating principles**

The operating principle was expressed schematically by I. Linkov *et al.*<sup>111</sup> (see **Erreur ! Source du renvoi introuvable.**). The procedure to be followed can be summarised in five steps:

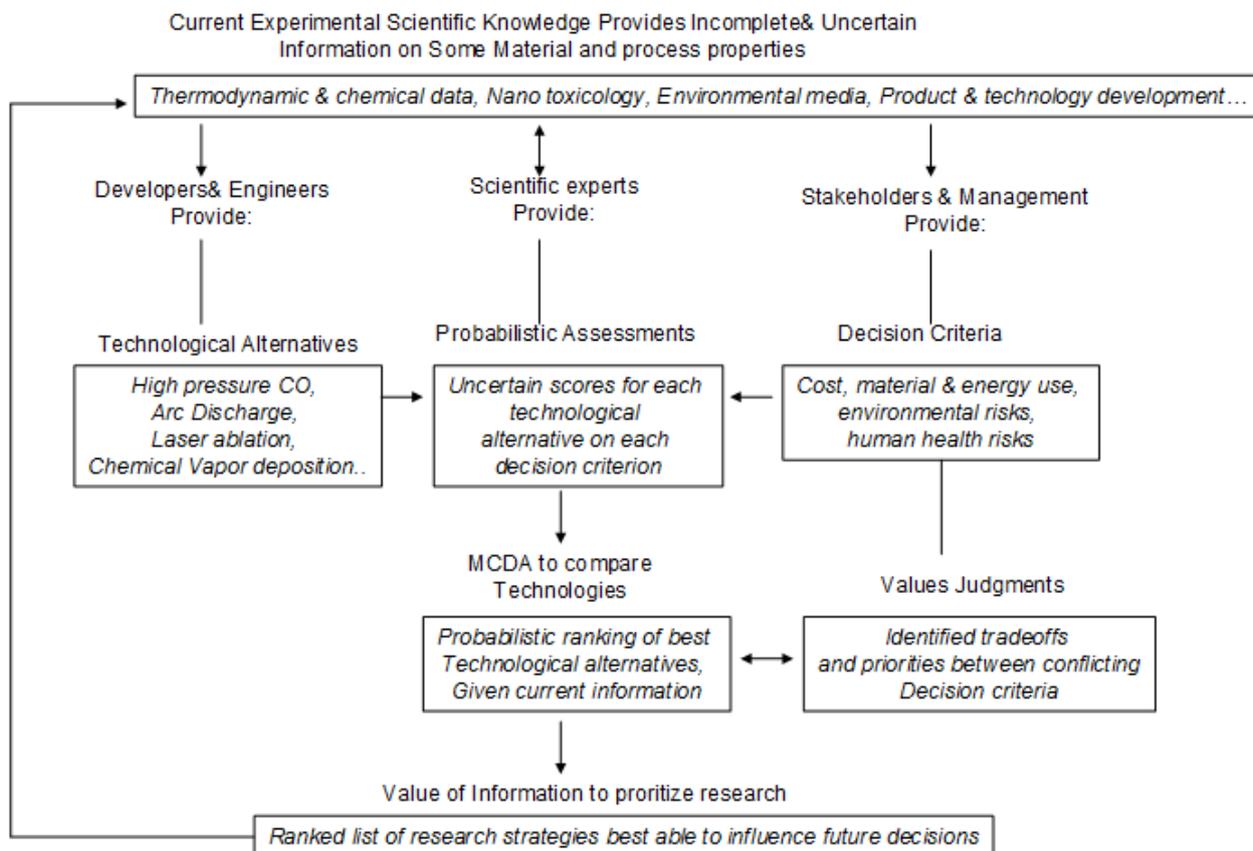
- identify the overall objective of the process and the type of decision
- make a list of possible or conceivable solutions
- make a list of criteria to consider
- assess each solution in view of each of the criteria
- aggregate these assessments in order to designate the solution with the best rating

Several methods can be used to aggregate the assessments or the ratings relative to each criterion:

- 1) Complete aggregation methods (top-down approach) that attempt to aggregate the  $n$  criteria in order to reduce them to a single one. Transitive judgments are assumed, e.g.:  $a > b$ ,  $b > c$  so  $a > c$ . These methods include WSM (*Weight Sum Method*), WPM (*Weight Product Method*), AHP (*Analytical Hierarchy Process*), MAUT (*Multi-Attribute Utility Theory*);
- 2) Partial aggregation methods (bottom-up approach) that attempt to compare potential actions or rankings with each other and to establish outranking relations between these elements. One example is ELECTRE, a family of so-called outranking methods that is based on a comparison of actions;
- 3) Local aggregation methods that seek a starting solution and then go on to perform an iterative search to find a better solution. Examples include the cone of improvement method or GOAL Programming.

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<sup>111</sup> Linkov I, Bates ME, Trump BD, Seager TP, Chappell MA, Keisler JM (2013) For nanotechnology decisions, use decision analysis. *Nano Today* **8**(1), 5-10.



Source: Linkov *et al.* 2013

**Figure 15: Positioning of a multi-criteria analysis approach within a decision making process**

### ► Advantages/disadvantages

Use of this decision support tool offers transparency, replicability and quantitative rigor. Moreover, this method can be used to explore and analyse interactions and interconnections between a vast number of parameters from different sectors.

The disadvantage lies in the fact that it does not take into account possible uncertainties for each parameter. MCDA therefore needs to be accompanied by a Monte-Carlo analysis to assess the distributions of the best degrees of dominance for each alternative.

In summary, MCDA seems to be difficult to implement in an assessment of the health risks associated with nanoproducts.

## 6. Green Screen™ v1.2

This method for comparing the hazards of chemicals was developed by the American NGO Clean Production Action<sup>112</sup> on the scientific basis of the findings of the US EPA's programme "Design for environment"<sup>113</sup> (mainly from the report by the Furniture Flame Retardancy Partnership<sup>114</sup>).

The NGO NRDC (Natural Resources Defense Council) commissioned an independent consulting firm to adapt and apply this method, which is free to use, to compare the analyses of two types of

<sup>112</sup> <http://www.cleanproduction.org/>

<sup>113</sup> <http://www.epa.gov/dfe/inde.g.htm>

<sup>114</sup> <http://www.epa.gov/dfe/pubs/flameret/ffr-alt.htm>

silver nanomaterials (metallic silver and silica-silver composite), where the silver substance was in its ionic form. The results of this study are available on the website of the NRDC<sup>115</sup>.

Although it is not really a risk analysis process specifically applied to nanomaterials, but rather a method for comparing the hazards of conventional chemicals, this work was nevertheless analysed by the Working Group because of, firstly, the debates following its application to nanomaterials<sup>116</sup> and, secondly, the methodological benefits of certain aspects of this work.

#### ► Purpose

This methodological approach proposes a transparent assessment of the intrinsic hazards associated with chemicals for a wide range of effects and then an interpretation of this information that is relevant to industry and risk managers, by classifying these substances according to four hazard classes which are combined with recommendations on uses.

Designed to express the results of the hazard analysis in a very synthetic manner by substance, this method is intended to be used to compare several chemicals for a given use, in order to prioritise the most suitable alternatives on the basis of their hazards (it could be used to search for a substitute substance or to design products according to the "safer by design" approach, for instance).

Based on the principles of simplicity and transparency, this approach aims to be easy to understand and use by the general public. Because it only uses official classification data published by health agencies and/or existing labelling for the substance considered, special expertise skills are unnecessary.

#### ► Scope

This method applies to conventional chemicals and, by extension, to nanomaterials.

The method addresses hazards to humans (health effects and physical hazards) as well as environmental hazards (ecotoxicity and factors relating to the fate of the substance in the environment).

#### ► Operating principles

The method is applied in three successive stages.

##### 1) Hazard assessment and classification

The first step is to establish the hazard levels for each of the eighteen effects to be considered (see Table 9) from the five levels proposed (very strong, strong, moderate, low or unknown). This classification is fully guided by a table listing the information sources to be consulted<sup>117</sup> and determining the hazard classes based on the information collected<sup>118</sup>.

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<sup>115</sup> [http://docs.nrdc.org/health/files/hea\\_13061001b.pdf](http://docs.nrdc.org/health/files/hea_13061001b.pdf)

<sup>116</sup> [http://switchboard.nrdc.org/blogs/jsass/greenscreen\\_hazard\\_assessment.html](http://switchboard.nrdc.org/blogs/jsass/greenscreen_hazard_assessment.html)

<sup>117</sup> [http://www.cleanproduction.org/library/greenScreenv1-2/GreenScreen\\_1-2\\_InfoSources.pdf](http://www.cleanproduction.org/library/greenScreenv1-2/GreenScreen_1-2_InfoSources.pdf)

<sup>118</sup> [http://www.cleanproduction.org/library/GreenScreen\\_v1\\_2-2e\\_CriteriaDetailed\\_2012\\_10\\_10w\\_all\\_Lists\\_vf.pdf](http://www.cleanproduction.org/library/GreenScreen_v1_2-2e_CriteriaDetailed_2012_10_10w_all_Lists_vf.pdf)

**Table 9: Effect categories analysed by chemical within the *GreenScreen™* method.**

Ecotoxicity and fate in the environment	Toxicity to humans (Group I)	Toxicity to humans (Group II)	Physical hazards
<ul style="list-style-type: none"> <li>Acute aquatic ecotoxicity</li> <li>Chronic aquatic ecotoxicity</li> <li>Other ecotoxicity studies (if available)</li> <li>Persistence</li> <li>Bioaccumulation</li> </ul>	<ul style="list-style-type: none"> <li>Carcinogenicity</li> <li>Mutagenicity and genotoxicity</li> <li>Toxicity for reproduction</li> <li>Toxicity for development</li> <li>Endocrine activity</li> </ul>	<ul style="list-style-type: none"> <li>Acute toxicity</li> <li>Systemic toxicity and effects on organs</li> <li>Neurotoxicity</li> <li>Skin sensitisation</li> <li>Respiratory sensitisation</li> <li>Dermal irritation</li> <li>Eye irritation</li> </ul>	<ul style="list-style-type: none"> <li>Reactivity</li> <li>Flammability</li> </ul>

Source: <http://www.cleanproduction.org/Greenscreen.v1-2.php>

This information is then summarised in a single line for each substance as shown in Figure 16:

Chemical Name	Group I Human					Group II Human							Ecotox		Fate		Physical	
	C	M	R	D	E	AT	ST	N	SnS	SnR	IrS	IrE	AA	CA	P	B	Rx	F
Chemical 1	M	L	L	M	M	L	L	M	L	DG	L	H	L	L	vH	M	L	L
Chemical 2	L	L	M	M	H	L	H	M	L	L	M	M	H	H	vH	vH	L	L
Chemical 3	L	L	M	H	DG	L	H	DG	L	DG	L	L	L	DG	M	M	L	M

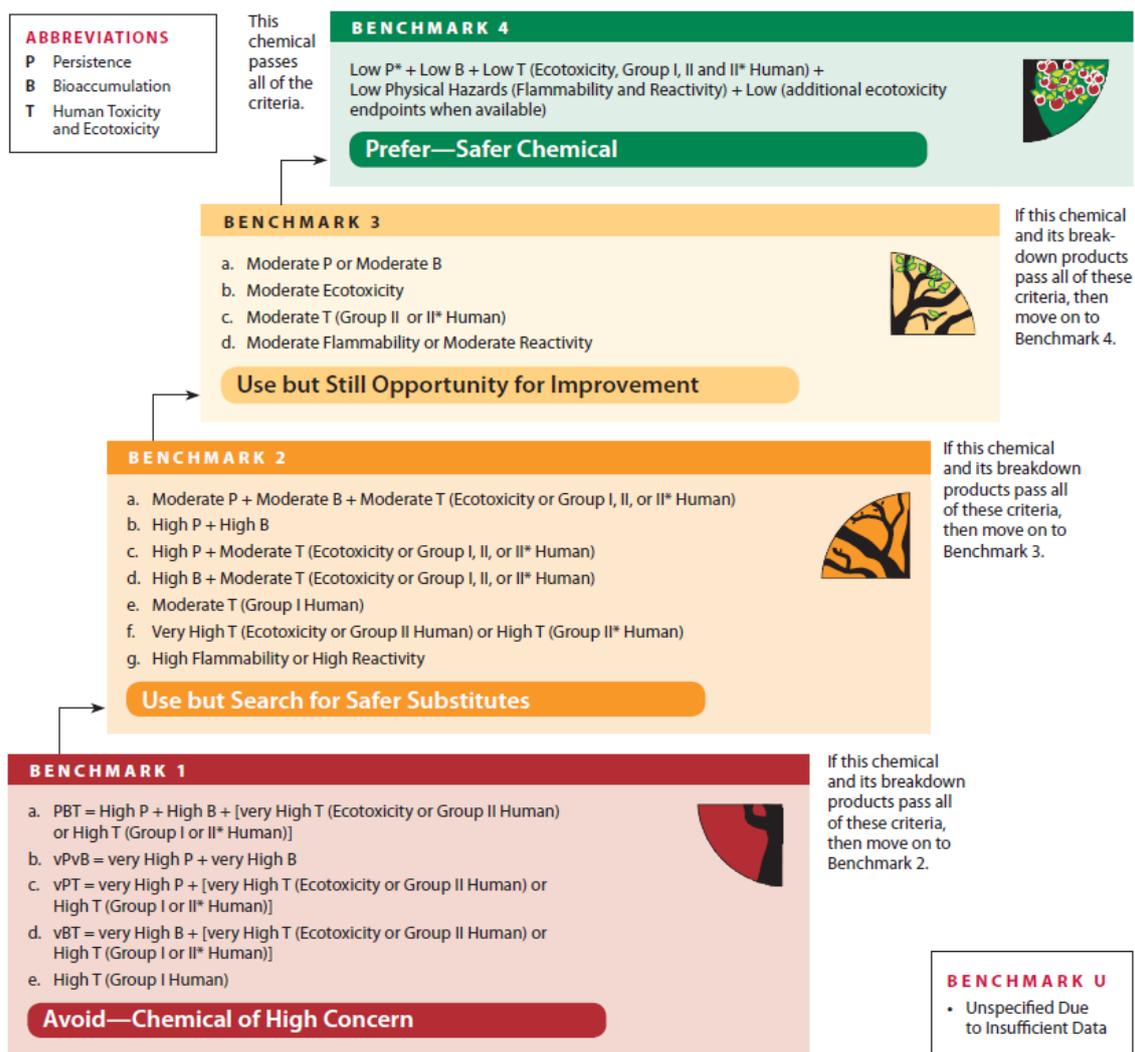
Source: [http://docs.nrdc.org/health/files/hea\\_13061001a.pdf](http://docs.nrdc.org/health/files/hea_13061001a.pdf)

**Figure 16: Example of representation of classification and comparison of chemical hazards in *GreenScreen™***

When applying this method to nanomaterials, adjustments must be made in particular to ensure that the assessment is based on the use of data specific to the nanomaterial in question, taken from reference studies in which the nanomaterial has been sufficiently characterised. On this last point, the characterised physico-chemical parameters mentioned as eligibility criteria in the study (Card and Magnusson, 2010) are similar to those determined by the ISO for risk assessment (ISO, 2008).

2) “Benchmark” scores - assigning a hazard class for the substance

The user is then asked to assign one of four hazard classes to the substance according to the hazard levels obtained for each of the effects considered in the previous step. Each of these classes is associated with a very general management recommendation. The approach also has the option of not assigning a hazard class when too many data are unavailable. The criteria for determining the hazard class are explained in Figure 17.



Source: [http://docs.nrdc.org/health/files/hea\\_13061001a.pdf](http://docs.nrdc.org/health/files/hea_13061001a.pdf)

Figure 17: Management recommendations based on the hazard categories in *GreenScreen™*

### 3) Characterising results and taking decisions

This last step involves processing and analysing the data generated according to the specific goals of the study, in order to assist in management choices. Several options are proposed, including grouping together various substances analysed by hazard classes in an effects analysis table (see Figure 17) or identifying the gaps in existing knowledge.

#### ► **Advantages/disadvantages**

The intended objectives of this method differ substantially from those of the work in this report: for *GreenScreen* the aim is to compare the hazards of substances intended for the same purpose, in order to determine the best possible solution in terms of health, and not to be able to compare the levels of risk associated with the use of possibly different products. The authors justify this focus on the hazard by the following arguments:

- In general, reducing risk via a reduction in the hazard level is a more efficient strategy than one based on reducing exposure;
- For health issues concerning end-users of the materials (consumers, professional users), reducing exposure is not always a possible lever for action in practice;

- For an analysis seeking to compare substances for a given application and the same type of product, it is often found that the exposure potential is equivalent;
- Lastly, addressing health issues from the perspective of comparing hazard levels commits the user to a continuous improvement process (seeking less hazardous substances). In contrast, the same issue seen through the prism of risk assessment could lead the user to seek a more binary solution (acceptable/unacceptable level of risk).

Proposing to determine the effects to consider is highly relevant. However, the type of information sought (data already appraised and published by health agencies) seems insufficient for the issue of nanomaterials. Indeed, an assessment mechanism based on this single level of information does not incorporate indices that are small but that might possibly be found in the literature (*in vitro* studies or a combination of physico-chemical parameters identified as possibly hazardous, for example). It is true that the user will require new skills to process this more detailed information, but it is particularly relevant. Few data meeting the quality criteria ideally required are currently available, from among the large quantity already called for. It seems unlikely that these data can be obtained in the medium term, for each effect and specific to each existing nanomaterial. It seems even less likely that the data required for future nanomaterials can be generated quickly. In such a context in which the use of these materials is growing rapidly, it is important to remain attentive to the health signals, try to interpret them and provide input for management decisions as early as possible, without waiting for higher levels of evidence, with a view to anticipating the possible occurrence of risks.

Lastly, another identified limitation of this method is that it considers a single hazard level, regardless of the possible changes occurring to the nanomaterial during its life cycle.

## 7. Nano Risk Framework (DuPont 2007)

### ► Purpose

Intended for risk managers who wish to make their decisions on the basis of a risk assessment, this document, published by DuPont, does not exactly offer a risk assessment method but consists more broadly of a complete reference framework for nanospecific risk management, including a risk assessment component<sup>119</sup>.

This reference framework, applicable to products containing manufactured nanomaterials, is part of an iterative process of continuous improvement and therefore includes various management support elements (assessment of the effectiveness of risk management, predicted costs and timeframes, methodological framework of actions to be taken based on an analysis of the situation).

### ► Scope

The risk assessment component of this document relates very broadly to the nanospecific risks to humans and the environment from manufactured nanomaterials or products containing them, during their entire life cycle. The risks to human health relate to occupational health risks as well as those for the general population (whether or not they are consumers).

The definition of the term manufactured nanomaterial which the authors used to build this framework resembles very closely the one provided by the ISO. Concerning nanoproducts, the authors state that this tool applies to products containing a substance of which as little as 10% (by mass) can be considered to be at the nanoscale.

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<sup>119</sup> DuPont (2007) Nanorisk Framework

### ► Operating principles

The method is divided into six distinct and successive stages as shown in Figure 18.

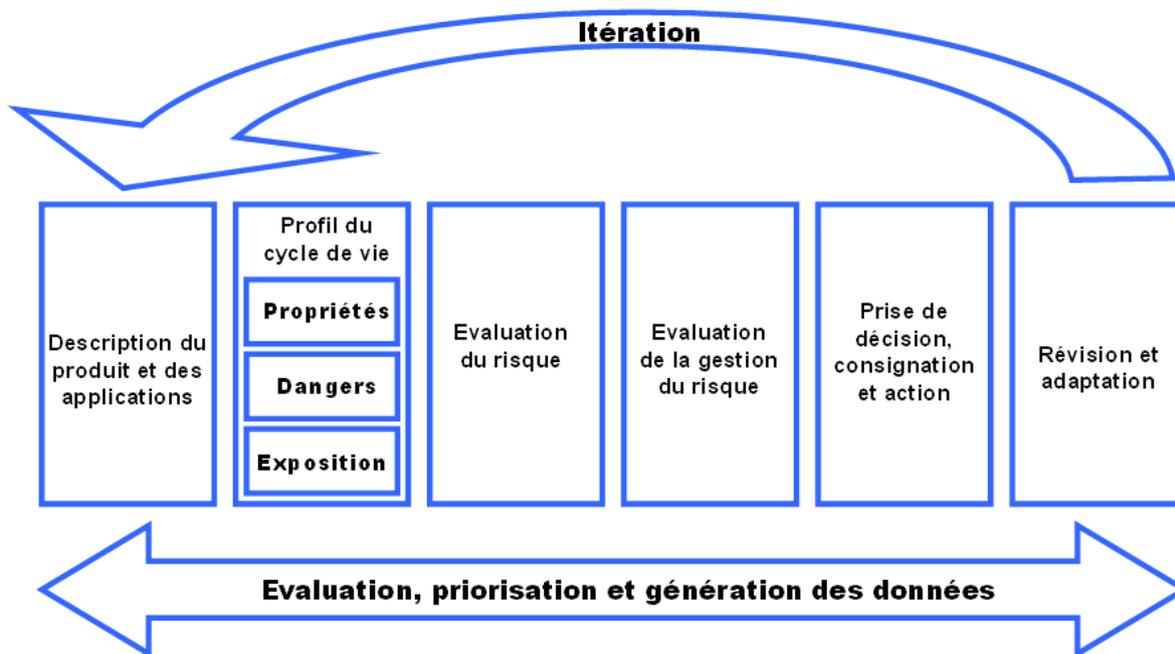


Figure 18: Explanatory diagram of the *Nano Risk Framework* risk management model

#### 1) Describe material and its applications

A descriptive framework is supplied through a list of questions relating to the description of the nanomaterial and its expected uses (see lists below). Various information is collected, including:

- characterisation parameters and physico-chemical properties of the materials considered;
- their origin (identity of suppliers, manufacturing processes, etc.);
- their stage of development (research, production pilot, pre-commercial demonstration or marketing);
- Identification of reference materials, substitute materials and "bulk" materials (of the same chemical composition as the nanomaterial but whose dimensions are larger than those of the nanoscale);
- known and expected uses and applications, the new properties offered by the nanoscale form compared to existing alternatives;

This basic description should cover each expected use of the material – existing or new – including use by the consumer and post-use or end-of-life management (disposal or recycling).

#### 2) Profile life cycle(s)

The material's properties, its inherent hazards (for safety, health and the environment) and exposure are analysed for each of the identified stages of the life cycle. The life cycle profile can be shown as follows:

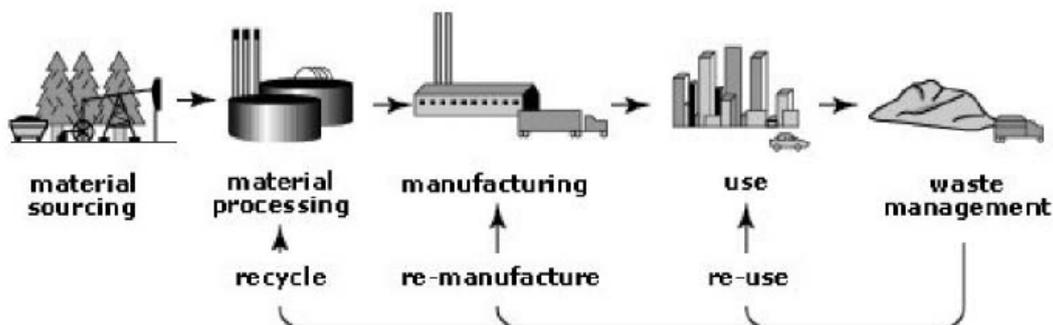


Figure 19: Life cycle diagram

The properties profile identifies and characterises the material's physical and chemical properties. The hazard profile identifies and characterises the potential safety, health and environmental hazards. The exposure profile identifies and characterises the opportunities for potential human and environmental exposure (including exposure through use and accidental exposure/accidental release). These data are organised into sub-groups in two categories: those belonging to all the data that must be provided, and additional data for refining the assessment.

The entire life cycle is taken into account, and the way in which the material's properties, hazards and exposure may change during its life cycle are identified.

### 3) Evaluate risks

The risk assessment framework outlines the main themes of the assessment but does not specify the detailed protocol for achieving a predetermined expression of the result. For each stage of the life cycle, the process below should be followed:

- comparing exposure data with hazard data collected during the previous step in order to determine the targets, exposure routes and environmental compartments, and relevant targets;
- assessment, quantified when possible, of the nature and magnitude of the potential risks identified;
- assessment of the uncertainties in the risk assessment;
- assessment of the probabilities and consequences related to possible variations in the different parameters used (related to the material and its applications);
- identification of gaps in existing knowledge;
- development of a strategy for overcoming the lack of data needed for the assessment, for example by identifying values, assumptions and reasonable worst-case scenarios.

### 4) Assess risk management

In this step, the user assesses the options available for managing the risks identified in step 3 and recommends actions: engineering controls, future equipment, risk communication and product or process modifications.

### 5) Decide, document and act

The user consults with his team to decide in what capacity to continue development and production of the product. The user informs the various stakeholders of the decisions made.

### 6) Review and adapt

The operator verifies that the risk management systems work as intended and adapts them in the face of new information or new conditions, or if deviations are observed.

### ► Advantages/disadvantages

This approach offers a very solid general methodological structure in terms of risk management and comprehensively integrates a large number of parameters that are relevant for risk assessment.

However it is not clear how these data are compiled for assessing or quantifying the risk. Indeed, for greater flexibility in addressing the multiple situations and possible uses, the authors have chosen to provide a general framework for risk assessment and not a "ready-to-use" algorithm. The type of data expected is not specified, the quantitative aspects and the way the data is processed are left to the user's discretion. It is therefore a tool for specialists in risk assessment applied to nanomaterials.

## 8. Life cycle assessment (LCA)

Life cycle assessment (LCA) is a tool for evaluating the environmental impact of a system or a product, from extraction of raw materials through to processing and waste disposal. This definition from the ISO 14040 and ISO 14044 standards (1997, 2006) positions LCA as an environmental management tool conducted in four steps:

- 1) definition of the study objectives and scope;
- 2) analysis of the inventory of data;
- 3) impact assessment (also called life cycle impact assessment - LCIA);
- 4) interpretation of results.

### ► Purpose

LCA has many varied purposes that differ depending on the object studied.

An LCA can be related to a product: in this case its goal is to conduct an input-output assessment of all the materials and energy required to manufacture and use this product, and to deal with it at the end of its life. The LCA will therefore stop at the inventory stage. If the impacts associated with this product's life cycle are to be studied, the pollutants generated at every step of the life cycle will be analysed and their contribution to a corresponding impact class will be calculated (e.g. contribution of  $\text{TiO}_2$  to aquatic ecotoxicity, or contribution of Al to human toxicity, etc.).

An LCA can be comparative, and thus compare two products with the same function or two industrial processes.

### ► Scope

As stated in the definition, LCA assesses and quantifies the impacts of a product, system (process) or service. The scope is therefore vast.

### ► Operating principles

The LCA methodology is based on four steps:

1) The definition of the study objectives and scope justifies the need for an LCA. It sets the spatio-temporal limits of the system and the functional unit (the unit that defines the function of the system and to which all data in the inventory will be related).

2) The analysis of the inventory is a set of data collections and calculation procedures for quantifying flows coming into and out of the system. It is a long and complicated step based on data analysis and quality. In practical terms, the data can come from specific databases for LCA models, direct measurements from the study site, or from the literature. Often several types of data are compiled together. These data are related to a reference unit: the functional unit that is determined according to the system studied (for example a functional unit may be  $1\text{m}^2$  of wall covered by paint made of  $\text{TiO}_2$  nanos, or 1 litre of soup containing silica in nano form).

3) Life cycle impact assessment

Converting the results from the inventory into corresponding impacts is made possible by the characterisation factor CF (Eq.1):

$$CI = CF_{x,i} \times M_x \quad (1)$$

where CI is the category indicator;  $M_x$  is the mass of substance x emitted or extracted and forming part of the results of the inventory, and  $CF_{x,i}$  is the characterisation factor of substance x contributing to the impact category i. The category indicator allows the aggregation of the results from the inventory analysis in common units in each impact category as an impact score, for which the general expression for several substances and environmental compartments is (Eq. 2):

$$S_i = \sum_{e=1}^{e=m} \sum_{x=1}^{x=n} CF_{i,x,e} \times M_{x,e} \quad (2)$$

where  $S_i$  is the impact score for category i,  $M_{x,e}$  is the mass of substances emitted into e, and  $CF_{i,x,e}$  is the characterisation factor for impact category i for substance x which is due to an emission in compartment e; with n and m being the number of substances and compartments respectively.

The characterisation factor is developed by simple or complex mathematical models that differ depending on the LCA methods. It is the product of two factors (Eq.3), of which one is the fate factor (FF) of the pollutant, representing the fraction of the substance transferred from the emission compartment to the recipient compartment, as well as its residence time in the latter; and the other is the effect factor (EF), expressing the substance's effect on organisms by exposure concentration.

$$CF = FF \times EF \quad (3)$$

The analysis can continue as far as damage to the final target (human health, ecosystem quality, climate change, etc.). The impact categories are then weighted by damage factors and grouped into corresponding damage categories. The damage scores, SD, for the category d, are obtained by multiplying the aggregated scores of the impact categories  $S_i$ , by their respective damage factors  $FD_{i,d}$  according to the following equation (Eq. 4):

$$SD_d = \sum_i FD_{i,d} \times S_i \quad (4)$$

There are numerous mathematical assessment methods currently in use: Impact 2002+, ReCiPe, Usetox. They focus on the transfer of pollutants from one medium to another and on impact categories such as global warming, degradation of the ozone layer, ecotoxicity and human health (carcinogenic and non-carcinogenic effects, respiratory effects). They apply mainly to overall data (global, European and national databases) but more recently work has been conducted at the local or site-specific scale with data collected *in situ*. Currently, modelling the impacts using specific characterisation factors is still an interesting line of research for improving methods of assessing the impacts of the life cycle of substances.

Modelling enables the effect and fate of pollutants to be best quantified. These two factors are vital for understanding the behaviour of all pollutants and especially those about which little is known. This is the case with nanoparticles and their behaviour in the environment or their effects on human health.

Impacts on a global scale can be modelled using LCA software (Umberto, Gabi, SIMAPRO).

4) Interpretation of the results involves drawing conclusions from the results obtained and making recommendations to policy makers or development stakeholders.

#### ► Advantages / disadvantages

The benefits of the LCA methodology are based on the fact that the product is studied in its entirety from the extraction of raw materials through to processing and waste disposal. An overall view of the impacts is therefore possible. It is possible to go from the impact assessment to the damage assessment for damage to human health, ecosystem quality, climate change and resources.

Modelling the life cycle impact analysis for nanomaterials is a challenge in the sense that it entails first studying their mobility and transfer into soil and water, before quantifying the factor relating to their effect and fate and then calculating a characterisation factor for terrestrial and aquatic ecotoxicity, and human health.

Disadvantages: LCA is not intended to be a risk assessment tool. LCA modelling could become more similar to risk modelling if a factor for exposure was added. But for now these are only avenues of research.

### 9. Summary of the advantages – disadvantages

Among the assessment or management support tools discussed in the preceding paragraphs, the advantages and disadvantages of those considered most relevant to the objectives of this work are summarised in Table 10.

**Table 10: Summary of advantages and disadvantages of selected risk assessment methods and management tools adapted to nanomaterials**

Assessment method	Advantages	Disadvantages
<b><i>A prudent approach to Nanotech Environmental, Health, and Safety Risks</i></b> <u>Lux Research (2005)</u>	<ul style="list-style-type: none"> <li>• Synthetic view, communication.</li> <li>• Applicable to nanoproducts</li> </ul>	<ul style="list-style-type: none"> <li>• Major methodological problems in estimating toxic potential (ratings set by "families" of nanomaterials, does not take into account the life cycle).</li> <li>• Little explanation of the rating system.</li> <li>• No option to update the parameters used.</li> <li>• No assessment of toxicity or risk at each stage of the life cycle.</li> <li>• No assessment of uncertainties</li> </ul>
<b><i>Nano Risk Framework</i></b> <u>DuPont (2007)</u>	<ul style="list-style-type: none"> <li>• Identification of the relevant parameters in number and quality for a risk assessment over the life cycle.</li> <li>• Summary support matrix of available data</li> </ul>	<ul style="list-style-type: none"> <li>• Management/assessment reference work and not a structured assessment method</li> </ul>
<b><i>Precautionary matrix for Synthetic Nanomaterials</i></b> <u>FOPH-FOEN (2011)</u>	<ul style="list-style-type: none"> <li>• Structured risk rating method (questionnaire with closed responses).</li> <li>• Method of rating uncertainties.</li> <li>• Complementary consideration of "worst case" data.</li> <li>• Method already tested and updated accordingly.</li> <li>• The unavailability of data is not an obstacle (max increase)</li> </ul>	<ul style="list-style-type: none"> <li>• Different purposes: tool more suited to the issue of nanomaterials than nanoproducts and does not allow ranking (only 2 levels of risk).</li> <li>• Insufficient number of criteria, especially for the hazard level (no toxicological data).</li> <li>• No distinction between exposure routes/environmental compartments.</li> <li>• Doubts over relevance of the proposed thresholds</li> </ul>
<b><i>Control banding tool for risk level assessment and control of nanoparticle exposures</i></b> <u>Paik (2008)</u>	<ul style="list-style-type: none"> <li>• Simple (rating of parameters) and structured approach.</li> <li>• Hazard level: integration of robust data (toxicological data on the nanomaterial) and other alternative data (data on the "parent" material and physico-chemical properties of the nanomaterial).</li> <li>• The unavailability of data is not an obstacle (¾ increase)</li> </ul>	<ul style="list-style-type: none"> <li>• Different purposes: tool intended for controlling risks in the occupational environment and more suited to the issue of nanomaterials than nanoproducts.</li> <li>• Proportion of user subjectivity introduced in assigning ratings to various parameters.</li> <li>• Criteria are insufficient for the exposure band (no consideration of the nanomaterial matrix or the processes).</li> <li>• No distinction between exposure routes/environmental compartments.</li> <li>• No assessment of uncertainties</li> </ul>

<p><b><i>Graduated health risk management (control banding)</i></b> <u>ANSES (2011)</u></p>	<ul style="list-style-type: none"> <li>• Simple and structured approach.</li> <li>• Hazard level: integration of robust data (toxicological data on the nanomaterial) and other alternative data (data on the "parent" material and physico-chemical properties of the nanomaterial)</li> </ul>	<ul style="list-style-type: none"> <li>• Different purposes: tool intended for controlling risks in the occupational environment and more suited to the issue of nanomaterials than nanoproducts.</li> <li>• Proportion of user subjectivity introduced in via the physico-chemical parameters (solubility and reactivity of the nanomaterial).</li> <li>• Criteria are insufficient for the exposure band (no consideration of the nanomaterial matrix or the processes).</li> <li>• No distinction between exposure routes/environmental compartments.</li> <li>• No assessment of uncertainties</li> </ul>
<p><b><i>NanoRiskCat</i></b> <u>Hansen (2012)</u></p>	<ul style="list-style-type: none"> <li>• Convergent objectives: specific method for assessing nanoproducts.</li> <li>• Simple and structured approach.</li> <li>• Summary possible and traceability of results.</li> <li>• Link with REACH categories.</li> <li>• The unavailability of data is not an obstacle (the level of hazard/exposure is incremented)</li> </ul>	<ul style="list-style-type: none"> <li>• Expression of levels of potential hazard/exposure, not risk.</li> <li>• No distinction between exposure routes/environmental compartments.</li> <li>• Doubts over appropriate REACH categories.</li> <li>• No assessment of uncertainties</li> </ul>
<p><b><i>GreenScreen™ v1.2</i></b> <u>Clean Production Action (2013)</u></p>	<ul style="list-style-type: none"> <li>• Quasi-exhaustive list of effects to be considered for assessing a hazard level together with sources to consult</li> <li>• Simple process</li> <li>• Presentation of results facilitates comparison of hazards between substances</li> </ul>	<ul style="list-style-type: none"> <li>• Different purposes: method intended for comparing intrinsic hazards of chemicals for the same use</li> <li>• Not always a distinction between exposure routes/environmental compartments</li> <li>• Data restricted to those published by health agencies</li> <li>• No consideration of the life cycle</li> <li>• Uncertainties taken into account but no real assessment</li> </ul>

## Annex 9: Assessment of exposure

### 1. Situations of occupational exposure

Every stage of production, from receipt and storage of raw materials to packaging and shipping of finished products, and including the possible transfer of intermediate products, can expose workers to nanomaterials. Similarly, use of nanomaterials, or their incorporation into various matrices and machining of composites containing them, constitute additional sources of exposure, along with cleaning and maintenance of premises and equipment, and waste treatment. A few examples of occupational exposure to manufactured nanomaterials are given below:

- transfer, sampling, weighing, placing in suspension and incorporating nanopowders into a matrix (aerosol formation);
- pouring, stirring, mixing and drying a liquid suspension containing nanomaterials (droplet formation);
- loading or emptying a reactor;
- machining nanocomposites: cutting, polishing, drilling, etc.;
- preparation, packaging, storage and transport of products;
- cleaning equipment and premises: cleaning a reactor, glove box, work bench, etc.;
- servicing and maintenance of equipment and premises: dismantling a reactor, changing used filters, etc.;
- collection, packaging, storage and transport of waste;
- degraded functioning or incidents: leakage of a reactor or a contained system.

The state in which nanomaterials are handled (powder, liquid suspension, gel, composite, etc.) and the ability of products to emit aerosols into the air when handled are factors that influence the level of exposure.

### 2. Characterisation of occupational exposure

Characterising emissions and potential exposure in the workplace during operations using nanomaterials is a difficult task.

#### Indicators to be considered

Occupational exposure is typically characterised in quantitative terms by measuring the time-weighted average mass concentration of the inhalable, thoracic and respirable fractions of the aerosol. It is preferably carried out using personal samplers placed in the worker's breathing zone. In the case of fibres, exposure is given in number of fibres per unit volume of air. This approach, justified by the existence of correlations between these indicators and health effects, is usually applied to all chemical agents in aerosol form, regardless of the size of the constituent particles.

This conventional approach is called into question for aerosols composed of nanomaterials. Given the existing data on their health effects, it seems increasingly clear that for nanomaterials consisting of insoluble or poorly soluble substances, exposure cannot be assessed just on the basis of the mass and the chemical composition. The mass concentration ( $\text{mg}/\text{m}^3$ ) however, is still a useful measurement, insofar as the particle size is selected, and ensures continuity with exposure data produced in the past. The concentration in total surface area of particles ( $\mu\text{m}^2/\text{m}^3$ ) also seems an appropriate measurement in many circumstances, although it cannot be generalised to all situations. Finally, the number concentration ( $\text{number}/\text{cm}^3$ ) appears to be an

adequate measurement when it is not the surface area of the particles that determines their toxicity. It also enables the finest fraction of a polydispersed aerosol to be identified.

Given that in addition to free nano-objects, agglomerated and aggregated forms must be considered, that nano-objects can diffuse by heterogeneous coagulation on particles of submicron and micron size in the ambient aerosol, and that machining nanocomposites can emit particles of a size corresponding to the respirable fraction, it appears that the particle size range of the aerosol to be considered extends from several nm to about 10 µm.

Chemical composition remains an essential characteristic to be determined, as well as morphology when nano-objects are poorly soluble or insoluble and have a high aspect ratio (length/diameter) – this is the case with nanotubes, nanofibres, etc. – or an irregular or fragmented shape. Other characteristics may also be relevant in some cases, such as crystalline structure, surface reactivity, state of electrostatic charge, solubility, etc.

It is now agreed that all aerosol sampling for analysis of chemical composition should at the least be conducted based on the respirable fraction, and that particle deposition in the respiratory tract should be considered when interpreting results (for example, using a model such as that of the International Commission on Radiological Protection).

### Measurement strategy

The strategies recently published at national and international level are characterised by an approach involving successive phases. In France, the overall approach suggested follows a five-phase path. The first three phases aim to determine whether the targeted operation is likely to emit aerosols of nano-objects and to confirm the need for and feasibility of a measurement campaign. The fourth phase is the measurement campaign itself, with two levels of intervention: basic characterisation and/or expert characterisation enabling more in-depth investigations. The final phase involves analysis of the results.

The measurement campaign aims to identify and characterise the aerosol at the emission source and at various points further away, to enable the assessment of potential exposure during the operation in question. The criteria for choosing between the two levels of intervention take into account the skills and experience in measuring aerosols of nano-objects and interpreting results, the availability of instruments and methods, the conditions of access to the work station, the suitability of instruments in the work station environment (ATEX zone, etc.) and the existence of earlier measurement results for this work station.

If there is uncertainty about the need to conduct a measurement campaign, or if this will prove difficult (multiple sources, access to the process difficult, specific zoning such as ATEX, etc.), conducting specific laboratory tests may be considered in order to assess potential emissions during the operation in question. These tests may involve the emission of an aerosol from nanomaterials in powder form (several approaches, known as "dustiness tests" are currently under development) or the emission of aerosols of composites or products containing nanoparticles undergoing various physical stresses (sanding, drilling, abrasion etc.) or effects (thermal, UV, etc.) simulating an operation or aging.

### Measurement methods and instruments

The first-level characterisation relies on the use of measurement techniques:

- real-time, for concentrations of particles in the air (portable condensation nuclei counters (CNC) and portable optical particle counters for measuring the number concentration; portable optical particle counters and portable laser photometers for measuring the mass concentration),
- integrated, for collecting aerosol samples for analysis of elementary particles or overall chemical composition of the sample collected (observation by transmission or scanning electron microscopy, which may be combined with microanalysis or spectroscopic techniques for studying morphology, and analysis of the elementary particle; fixed-station

sampling of the respiratory fraction with chemical analysis by mass spectrometry for the chemical composition of the aerosol).

The techniques used for the second (expert) level of intervention have better performance (improved lower limit of detection of nanoparticles or upper number concentration limit for CNCs). Techniques for measuring the particle number distribution in real time or the mass distribution as a function of the size (impactors) can also be included, as well as specific techniques for measuring concentrations in surface area ( $\mu\text{m}^2/\text{cm}^3$ ), and possibly sampling devices for integrated real-time measurement in the breathing zone, with the aim of analysing either elementary particles or the overall chemical composition of the sample.

A major problem encountered during real-time measurement comes from the confounding factor represented by the background aerosol, i.e., the ambient aerosol present in the studied premises before any operation actually takes place. It is generally omnipresent, variable in time and space depending on its various sources (combustion, other processes) and on its mode of transfer in the measuring zone due to natural or forced ventilation. It is made up of particles in the nanometer to micron size range, and the concentration levels reached can easily mask the target aerosol emitted by the operation in question. As real-time measurement instruments (e.g. CNC) are not specific to the nature of the particles they observe, it is not always easy to distinguish this background aerosol. However, this distinction is crucial because a number concentration (for example) due to the background aerosol should not be combined with that of the target aerosol, which may be lower by several orders of magnitude. The same is true of the particle size of the target aerosol.

Insofar as the situation study and the preparatory visit have been able to identify the location of a point with possible emissions, for example the transfer of a nanopowder from a container to a beaker, a point closest to the source will be selected for real-time and integrated measurements. Other measurement points should be carefully chosen in the near and/or far field, taking into account the target operation, operator, environment of the work station, design of the room and building, local and general aerualics, etc. In general, it is better to position the integrated measurement points at the height of the worker's respiratory tract.

These recommendations can be applied to all existing work environments (research laboratories, industrial sites, etc.) during the various phases of nanomaterial production and use, during cleaning and maintenance of equipment, etc., and in normal or degraded operating conditions of the process and of protective equipment.

Developments are expected in the field of assessing exposure to nanomaterials, particularly in terms of instruments, measurement criteria and interpretation of results.

Moreover, in the absence of suitable measurement instruments and methods, a qualitative assessment of exposure to nanomaterials can be conducted.

## References

*Préconisations en matière de caractérisation des potentiels d'émission et d'exposition professionnelle aux aérosols lors d'opérations mettant en œuvre des nanomatériaux* [Recommendations in terms of characterisation of the potential for emission of and occupational exposure to aerosols during operations using nanomaterials], INRS, ND 2355 (2012)

*Les nanomatériaux. Définitions, risques toxicologiques, caractérisation de l'exposition professionnelle et mesures de prévention* [Nanomaterials, Definitions, toxic risks, characterisation of occupational exposure and preventive measures], INRS, ED 6050 (2012)

*Nanomatériaux. Prévention des risques dans les laboratoires* [Nanomaterials, Prevention of risks in laboratories], INRS, ED 6115 (2012)

Nanomaterials. Occupational safety, AFSSET, 2008

Nanomaterials. Effects on human health and the environment, AFSSET, 2006

O. WITSCHGER, *Métrie et exposition professionnelle aux aérosols de nanoparticules (nanoaérosols)*, *Les Nanosciences - 4. Nanotechnologie et nanoéthique*. M. Lahmani, F. Marano, P. Houdy. Editions Belin 2010, pp 194-229 (2010)

International Commission on Radiological Protection, ICRP Publication 66: *Human respiratory tract model for radiological protection*, Oxford, Pergamon, 24, n° 1-3, 482 p (1994)

## Annex 10: Medical surveillance of workers

Given the current medical uncertainties about the effects of nanomaterials on health, there is no consensus to date about the content and procedures for medical surveillance of workers potentially exposed to nanomaterials.

At the individual level, surveillance should be adapted to the circumstances of the medical consultations, with the main aims being to determine the worker's suitability for the work station and to inform workers about the risks and protective equipment. Because of a lack of validation in the context of occupational exposure to nanomaterials, individual interpretation of the results from some additional tests remains limited. These tests, which are determined by the occupational physician, are nevertheless useful as they constitute a reference report for recruitment, an aid to determining worker suitability for jobs requiring constraining personal protective equipment to be worn, and as part of longitudinal follow-up of individual health parameters.

It is fundamental to document all information collected on health events, the results of additional tests and exposure. This should be kept in individual employee medical records, ideally in a standardised format, to allow later exploitation of the data for epidemiological research.

France has a specific occupational health system that could play a key role in setting up this scheme. Creating registers for exposure and establishing prospective cohorts are the necessary prerequisites for conducting epidemiological studies. These studies will help to improve knowledge about the effects of nanomaterials on human health and about populations at risk requiring special measures. Finally, they will provide an opportunity to assess the various additional tests that can be conducted in the context of occupational health, which include the determination of biomarkers of early effects.

The individual and collective approaches of the medical surveillance scheme are complementary and require concerted action in which occupational physicians could play a central role.

### References

*Surveillance médicale des travailleurs exposés à des nanomatériaux. Les enseignements du congrès de Keystone* [Medical surveillance of workers exposed to nanomaterials. Lessons learned from the Keystone convention], DMT 124, TP11, 2010.

## Annex 11: French proposal for the European NANoREG project

The figure below describes the interconnections between hazard and exposure when conducting a risk assessment for one nanomaterial and one use/step of its life cycle.

*Figure 1: Exposure/hazard interconnections*

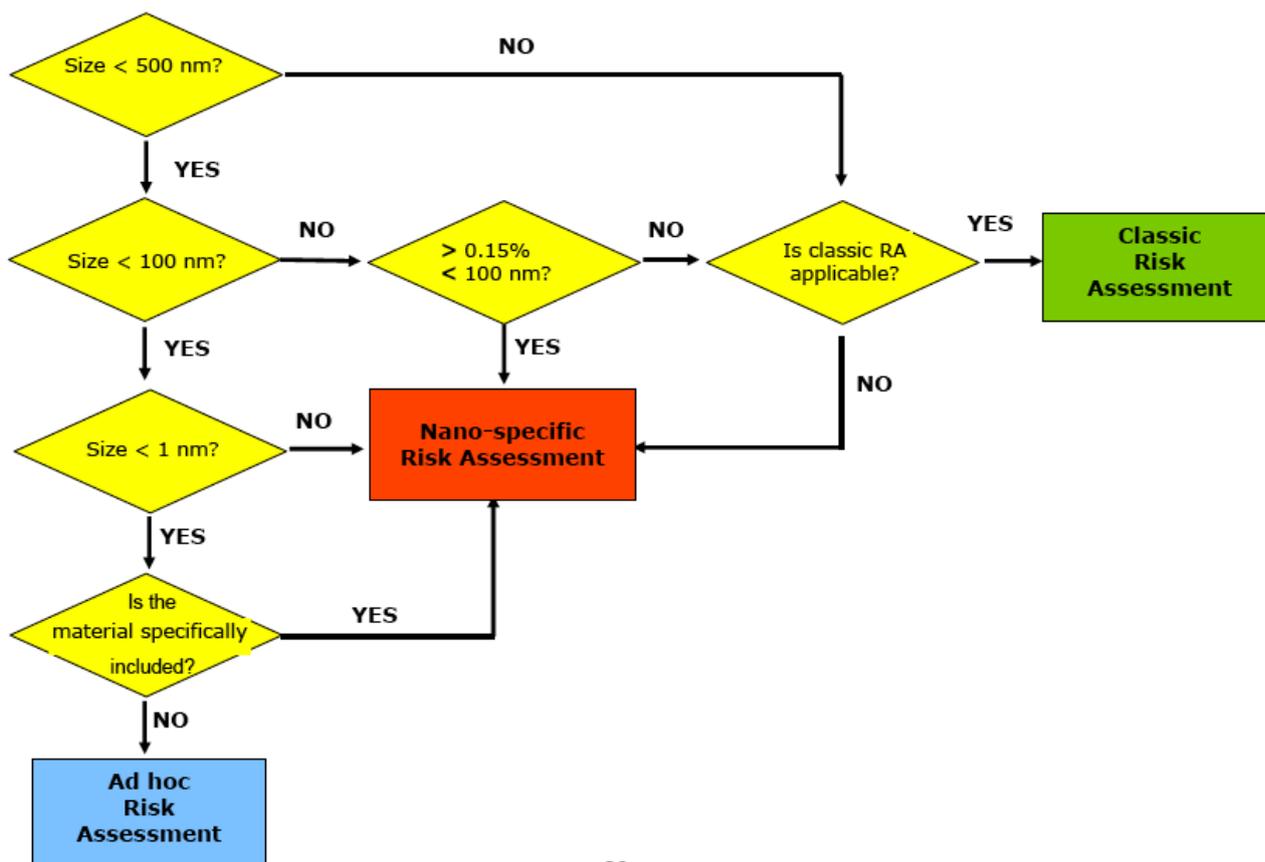
### ***a. Exposure through life cycle (WP3, Figure 1)***

The scope of the exposure assessment will consider all stages of the life cycle of the nanomaterials resulting from the manufacturing process, the identified uses and waste management. Human exposure should address worker exposure, consumer exposure (including via food or drink) and exposure via the environment. The challenge of this WP is to create a new state of the art and to develop an integrated approach to assess the potential risks of MNPs under more realistic conditions (e.g. low doses, chronic exposure, complex aqueous environment, and trophic links).

#### **Identification**

The first task in WP3 is to determine whether the product considered is a nanomaterial or not.

For this purpose, the conclusions of the Dec-2010 SCENIHR report could be used. The decision tree can be applied to many materials and it is a first step for moving from a case-by-case basis to a more general approach.



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Figure 2: The use of size to identify an approach for the risk assessment of nanomaterials (SCENIHR©)

There is also an alternative or complementary view of the first stage of the procedure which is based on the categorisation by Hansen *et al.* (2007)<sup>120</sup>. This categorisation could be of great help by adding some nuance to the SCENHIR decision tree.

<sup>120</sup> Hansen, S. F., B. H. Larsen, S. I. Olsen, A. Baun, (2007), "Categorization framework to aid hazard identification of nanomaterials". *Nanotoxicology* 1(3):243-U369.

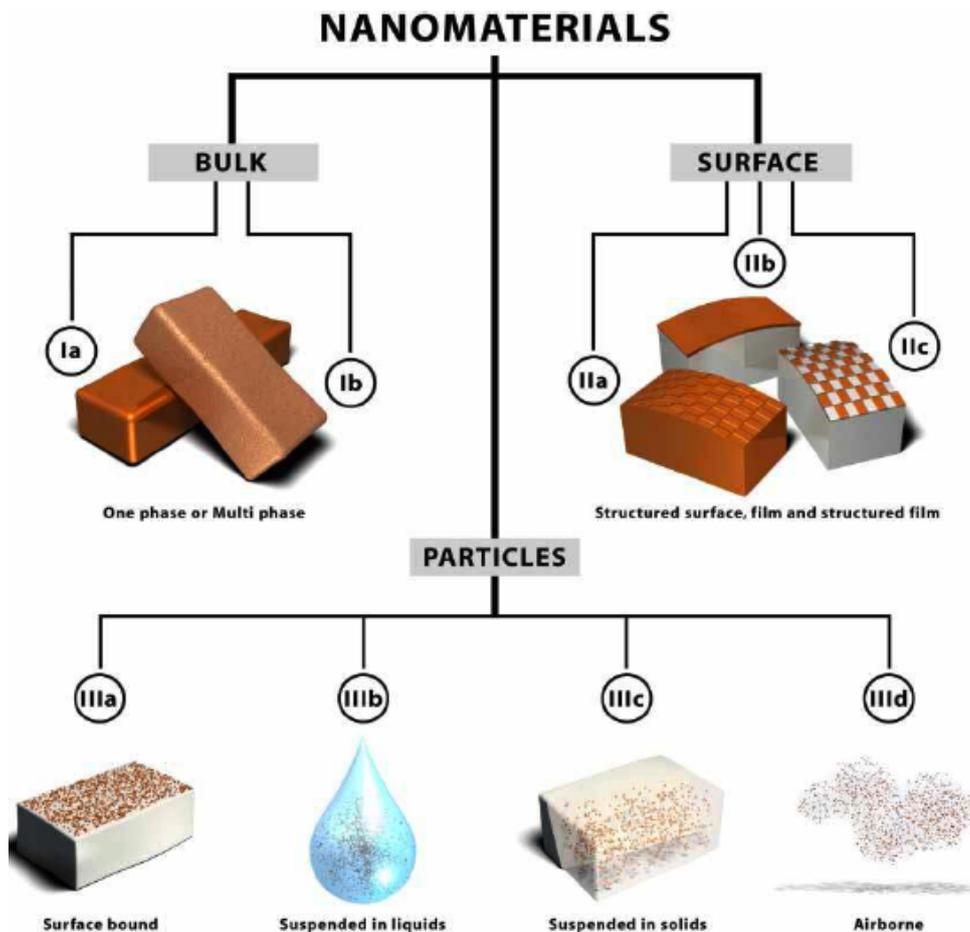


Figure 3: The categorization framework for nanomaterials. The nanomaterials are categorized according to the location of the nanostructure in the material. (Hansen et al, 2007©)

Whatever the chosen alternative, this first screening step has to be carried out using reference methods that can be traced to the International System of units and for which uncertainty values have been reduced and well defined. In this framework, metrological Atomic Force Microscopy (AFM) and SMPS appear to be suitable tools for traceable dimensional measurements at the nanometer scale.

### **Life cycle**

If the previous categorization leads to the conclusion that the product is a nanomaterial, then it is important to define the life cycle of the given product using a simple approach that can be summarized by the following diagram. This would be the second task in WP3.

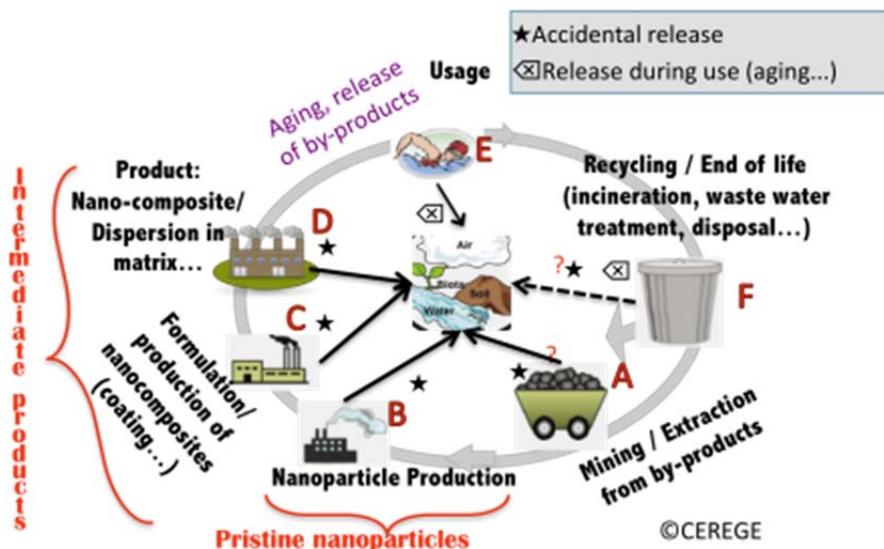


Figure 4: Simplified life cycle analysis of nano-product = TASK 2

According to this approach, a simple LC analysis will directly provide answers to key concerns of regulators such as the possibility of using toxicology and exposure results from pristine nano-TiO<sub>2</sub> particles to determine the risk related to sunscreen incorporating nano-TiO<sub>2</sub>. A simple LC analysis will provide the answer "YES" or "NO" as a function of the location within the life cycle stage:

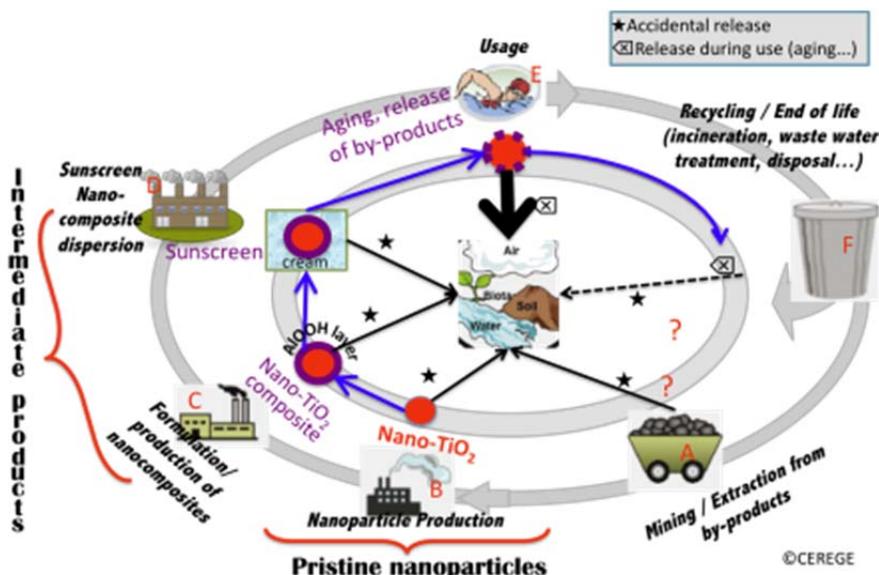


Figure 5: Life cycle of sunscreen incorporating nano-TiO<sub>2</sub>

In this particular case:

- workers will be exposed to bare nano-TiO<sub>2</sub> (mainly through inhalation) in stage B, to the nano-composite in stages C and D (nano-TiO<sub>2</sub> core surrounded by AlOOH, SiO<sub>2</sub> layers and PDMS (silane)) again mainly through inhalation, and finally to the sunscreen in stage D.
- Consumer exposure only occurs during stage E. Direct exposure: dermal application of the cream. Indirect exposure: cream released into water (lake, sea, swimming pool), then aged (possibly chemically altered). There, consumers can be exposed through oral or dermal

contact or as the end point of food chain transfer (aging, mesocosm experiments).

With such a framework we can then guide the risk assessment of products towards specific experiments/tests by defining the subsequent toxicology and exposure tests.

Some important scientific issues at this stage are:

- Full characterization of nanomaterials including uncertainty about the measurement of a specific property of the nanomaterials, for example concerning the estimation of uncertainties of size distribution measurements given by SMPS;
- Difficulties comparing the size distributions measured by different analyzers; a harmonized procedure for evaluating uncertainties on particle size distributions is needed;
- Obtaining reference NP aerosols with well-defined size distributions to be able to determine adequate correlations between properties and observed toxicity;
- The need for a bank of well characterised **Reference Materials**.

### **Exposure decision tree**

Here, the main idea is to develop or use simple standard protocols that take into account selected properties of the nanomaterial that could modify its behaviour, in order to reduce as much as possible the number of tests needed to determine a realistic level of exposure to the nanomaterial.

The following figure gives an example of simple tests and a decision tree for the aquatic ecosystem exposure scenario.

*Figure 6: Exposure decision tree in the case of an aquatic ecosystem*

One possible output of this decision tree could determine whether specific nanotests are needed or whether regular or non-nano tests are adequate for the nanomaterials considered.

Of course such a 'theoretical' framework cannot be applied according to current knowledge, and specific questions need to be answered to help regulation:

- What is a standardized water for testing the chemical stability?
- What incubation times are the most appropriate?
- How can we define slow vs. fast dissolution rates?
- Can we adapt resistance/aging tests developed for regular materials to materials incorporating nanoparticles (outdoor paint resistance tests, polymer crash tests, etc.)?
- Can we develop simple mesocosm experiments (pre-normative research) to cover the only relevant routes of environmental exposure, in order to determine: i) the NP's biological effects on ecosystem functioning, (ii) the distribution and bioavailability of the NPs within the different compartments (e.g. water, sediments, aquatic bacteria and bivalves, benthic or planktonic organisms), (iii) the biotransformation (redox reactions, ROS production) of the ENPs in the compartments where the concentrations are greatest, (iv) the trophic transfer of NPs and v) the effects on the metabolism of the organisms which accumulate the ENPs?
- Limit of detection, and locating MNPs within the complex media of the ecosystems (e.g. water, soil, sediment, plant, biota):
  - Can we develop sensors/detectors to quantify worker exposure?

- Can we develop sensors/detectors to quantify environmental exposure?
- Can we develop sensors/detectors to quantify food exposure?
- Dustiness (or propensity of a powder to form an aerosol; measurement of agglomeration energies of nanopowders)
- Evaluation of measurement tools and development of harmonized measurement strategies
- Effectiveness of engineering controls and protection factors
- Effectiveness of respiratory protective devices and protection factors
- Modelling of aerosol behaviour
- Exposure monitoring of individuals: development of biomarkers of exposure
- Development of Adjustable Pocket Sized Mesocosms (APSM, 70x20x60 cm)
- Sampling strategies for characterising nano-object emission and release in the environment
- Development of computer simulation models and tools to predict the space-time evolution of nanosized aerosols
- The link between emission potential and real exposure
- Establish a quantitative method to assess common preventive measures in occupational exposure

### ***b. Danger/hazard***

#### **Pre-screening tests**

The life cycle analysis and the exposure characterisation will define realistic exposure routes. Therefore a simple pre-screening test may also be applied to reduce the number of toxico- and ecotoxicological tests to be conducted on the considered nanomaterial (reducing time and costs).

*Figure 7: Pre-screening tests prior to toxicological and ecotoxicological tests*

Several scientific issues must be solved for this pre-screening:

- Determine the chemical stability of the nanomaterial in various media.
- Propose a relevant procedure according the type of nanomaterials, the cell line and the toxicological end-point with standard procedure for dissolution in media of nanomaterials,
- Procedure for detection and quantification of nanomaterials
- Standard procedure for determining aggregation (specifically for ecotox, whether water column or benthic organisms should be given priority)
- Choice of adapted cell lines and standard procedure
- Use of a set of early-warning and sensitive biomarkers (oxidative-stress, genotoxicity, neurotoxicity, behaviour, reprotoxicity).
- Integration of individual biomarker responses into simple indices for ranking the ecotoxicity of NPs.
- Etc.

The underlying idea is to prioritise the tests to be performed according to specific parameters.

### **Toxicological Test**

In order to reduce the time and the number of toxicological tests, some tests will necessarily be performed in the first line, such as toxicokinetic tests in order to determine with the pre-screening test results if further testing is needed. The ranking of the toxicological testing is summarised below.

*Figure 8: Toxicology and ecotoxicology decision tree*

Among French experts involved in this project, the decision whether to leave the *in vitro* cytotoxicity study and *in vitro* genotoxicity study here, or to place them just before or inside the pre-screening 4A test battery has not been taken yet.

Scientific Content:

- Toxicity (acute, subacute (28d), subchronic (90d), and chronic) tests, required under the

REACH Regulation both for the unprioritized and prioritized substances for phased-in and non phased-in substances have to be proposed,

- Unambiguous toxicological test protocols,
- Current modes of delivery of nanoparticles in actual tests designed to reflect relevant exposure scenarios are not reproducible and therefore need further evaluation,
- Develop whole body and nose only *in-vivo* inhalation protocols by inhalation of a NP aerosol formed from a dry powder,
- Develop exposure protocols for the validation of predictive methods (*in-vivo* tests for rapid screening studies),
- Be able to conduct *in-vivo* and *in-vitro* studies for either screening or hazard characterization,
- Evaluate *in vivo* testing of short exposure duration compared to longer exposure duration,
- Relationships between nanomaterial properties and toxicity,
- Characterize "good nanomaterial dispersion" in relation to the reality of human exposition (avoiding agglomeration or not, using artificial dispersants or not, etc.).

### **Ecotoxicological Test**

Need for a decision tree? Yes

Current approaches to assess the ENP's safety are based on classical ecotoxicology approaches, which are not completely suitable in terms of exposure routes, duration or selected species.

In order to select the best ecotoxicological tests (species, exposure medium), pre-screening tests are essential to determine particularly the dispersion/aggregation/settling state of nanomaterials in the exposure medium. If aggregation/sedimentation occurs in the aquatic environment, a minority of ENPs will be bioavailable for direct uptake by planktonic organisms while the major part will interact with benthic organisms after settling.

Scientific content:

- Need to develop standardized ecotoxicity test protocols,
- Need to conduct experiments in realistic environmental concentrations:
  - Low doses as indicated by Predicted Environmental Concentrations of ENPs arising from use in consumer products
  - Chronic exposure: Long-term studies are needed, not only short-term tests. Duration of exposure needs to be reconsidered and include the temporal scales involved in ENP fate.
  - Complex environments (aqueous, sediments, soils)
  - Mesocosm experiments: differences have been observed between laboratory studies and field ecosystem experiments.
- Ecologically relevant *in vivo* models of several different species (e.g. fish, bivalves, crustaceans, worms, etc.) since those playing an important role in the functioning of ecosystems with different biological traits should be considered
- Need to progress with the relationships between nanomaterial physico-chemical properties (aggregation, surface modifications, dissolution mechanisms, redox processes) and ecotoxicological effects
- How are particles changed by organisms after uptake?
- Need to link effects to better exposure characterization. More studies on bioavailability, bioaccumulation, food chain transfer and organism life histories in relation to the routes of exposure (water, sediment, soil, diet).

## Annex 12: List of nanomaterial analysis platforms currently being developed in France

- **NanoID** - Equipex project

Project initiator: LITEN (CEA Grenoble)

Partners: CEREGE (CNRS-Aix-Marseille University); LCP (CNRS-Aix-Marseille University); U 959 (INSERM); IsTERRE (CNRS-J Fourier University); LSA-CIME (ANSES)

<https://www.cerege.fr/spip.php?article138>

- **PFNC Minatec - CEA Grenoble**

<http://www.minatec.org/recherche/developper-nouvelles-competences-en-caracterisation>

- **CARMEN by the LNE**

[https://www.lne.fr/fr/actualites/popup\\_actu/2013/actu.asp?titre=plate-forme-carmen](https://www.lne.fr/fr/actualites/popup_actu/2013/actu.asp?titre=plate-forme-carmen)

- **INRS Nano unit**

<http://www.inrs.fr/accueil/footer/presse/cp-pole-nano.html>

- **Nano Platform: Jean Lamour Institute**

<http://ijl.univ-lorraine.fr/la-recherche/centres-de-competences.html>

- **Ineris Nanosafety Platform**

<http://www.ineris.fr/centredoc/cp-halle-nano-1355847867.pdf>

- **NanoSafety Platform (PNS) - CEA Grenoble**

<http://www.cea.fr/presse/liste-des-communiqués/inauguration-de-la-plate-forme-nanosecurite-a-gr-123729>

- **Raimond Castaing Micro-characterisation Centre - UMS 3623**

<http://ccarcastaing.fr/>

## Annex 13: Review of ISO standards published under the direct responsibility of the ISO/TC 229 (March 2014)

<a href="#">ISO/TR 11360:2010</a>	Nanotechnologies – Methodology for the classification and categorization of nanomaterials
<a href="#">ISO/TS 11308:2011</a>	Nanotechnologies – Characterization of single-wall carbon nanotubes using thermogravimetric analysis
<a href="#">ISO/TS 11251:2010</a>	Nanotechnologies – Characterization of volatile components in single-wall carbon nanotube samples using evolved gas analysis/gas chromatograph-mass spectrometry
<a href="#">ISO/TR 10929:2012</a>	Nanotechnologies – Characterization of multi-wall carbon nanotube (MWCNT) samples
<a href="#">ISO/TS 10868:2011</a>	Nanotechnologies – Characterization of single-wall carbon nanotubes using ultraviolet-visible-near infrared (UV-Vis-NIR) absorption spectroscopy
<a href="#">ISO/TS 80004-8:2013</a>	Nanotechnologies – Vocabulary – Part 8: Nanomanufacturing process
<a href="#">ISO/TS 80004-7:2011</a>	Nanotechnologies – Vocabulary – Part 7: Diagnostics and therapeutics for healthcare
<a href="#">ISO/TS 80004-6:2013</a>	Nanotechnologies – Vocabulary – Part 6: Nano-object characterisation
<a href="#">ISO/TS 80004-5:2011</a>	Nanotechnologies – Vocabulary – Part 5: Nano-bio interface
<a href="#">ISO/TS 80004-4:2011</a>	Nanotechnologies – Vocabulary – Part 4: Nanostructured materials
<a href="#">ISO/TS 80004-3:2010</a>	Nanotechnologies – Vocabulary – Part 3: Carbon nano-objects
<a href="#">ISO/TS 10867:2010</a>	Nanotechnologies – Characterization of single-wall carbon nanotubes using near infrared photoluminescence spectroscopy
<a href="#">ISO/TS 80004-1:2010</a>	Nanotechnologies – Vocabulary – Part 1: Core terms
<a href="#">IEC/TS 62622:2012</a>	Artificial gratings used in nanotechnology – Description and measurement of dimensional quality parameters
<a href="#">ISO 29701:2010</a>	Nanotechnologies – Endotoxin test on nanomaterial samples for in vitro systems – Limulus amoebocyte lysate (LAL) test
<a href="#">ISO/TS 27687:2008</a>	Nanotechnologies – Terminology and definitions for nano-objects – Nanoparticle, nanofibre and nanoplate
<a href="#">ISO 10808:2010</a>	Nanotechnologies – Characterization of nanoparticles in inhalation exposure chambers for inhalation toxicity testing
<a href="#">ISO/TS 17200:2013</a>	Nanotechnologies – Nanoparticles in powder form – Characteristics and measurements
<a href="#">ISO 10801:2010</a>	Nanotechnologies – Generation of metal nanoparticles for inhalation toxicity testing using the evaporation/condensation method

<a href="#">ISO/TS 16195:2013</a>	Nanotechnologies – Guidance for developing representative test materials consisting of nano-objects in dry powder form
<a href="#">ISO/TR 14786:2014</a>	Nanotechnologies – Considerations for the development of chemical nomenclature for selected nano-objects
<a href="#">ISO/TS 14101:2012</a>	Surface characterization of gold nanoparticles for nanomaterial specific toxicity screening: FT-IR method
<a href="#">ISO/TS 13830:2013</a>	Nanotechnologies – Guidance on voluntary labelling for consumer products containing manufactured nano-objects
<a href="#">ISO/TR 13329:2012</a>	Nanomaterials – Preparation of material safety data sheet (MSDS)
<a href="#">ISO/TS 13278:2011</a>	Nanotechnologies – Determination of elemental impurities in samples of carbon nanotubes using inductively coupled plasma mass spectrometry
<a href="#">ISO/TR 13121:2011</a>	Nanotechnologies – Nanomaterial risk evaluation
<a href="#">ISO/TR 13014:2012/Cor 1:2012</a>	
<a href="#">ISO/TR 13014:2012</a>	Nanotechnologies – Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment
<a href="#">ISO/TS 10798:2011</a>	Nanotechnologies – Characterization of single-wall carbon nanotubes using scanning electron microscopy and energy dispersive X-ray spectrometry analysis
<a href="#">ISO/TS 12901-2:2014</a>	Nanotechnologies – Occupational risk management applied to engineered nanomaterials – Part 2: Use of the control banding approach
<a href="#">ISO/TS 12901-1:2012</a>	Nanotechnologies – Occupational risk management applied to engineered nanomaterials – Part 1: Principles and approaches
<a href="#">ISO/TR 12885:2008</a>	Nanotechnologies – Health and safety practices in occupational settings relevant to nanotechnologies
<a href="#">ISO/TS 12805:2011</a>	Nanotechnologies – Materials specifications – Guidance on specifying nano-objects
<a href="#">ISO/TR 12802:2010</a>	Nanotechnologies – Model taxonomic framework for use in developing vocabularies -- Core concepts
<a href="#">ISO/TS 12025:2012</a>	Nanomaterials – Quantification of nano-object release from powders by generation of aerosols
<a href="#">ISO/TS 11937:2012</a>	Nanotechnologies – Nanoscale titanium dioxide in powder form -- Characteristics and measurement
<a href="#">ISO/TS 11931:2012</a>	Nanotechnologies – Nanoscale calcium carbonate in powder form -- Characteristics and measurement
<a href="#">ISO/TS 11888:2011</a>	Nanotechnologies – Characterization of multiwall carbon nanotubes – Mesoscopic shape factors
<a href="#">ISO/TR 11811:2012</a>	Nanotechnologies – Guidance on methods for nano- and

	microtribology measurements
<a href="#">ISO/TS 10797:2012</a>	Nanotechnologies – Characterization of single-wall carbon nanotubes using transmission electron microscopy

## Annex 14: Review of publications available from the OECD (March 2014)

These are publications from the OECD Working Party on Manufactured Nanomaterials (WPMN) whose goal is to share updated knowledge developed within the activities of the WPMN and more broadly of the OECD.

No. 40	<a href="#">ENV/JM/MONO(2014)1</a> <a href="#">ENV/JM/MONO(2014)1/ADD</a>	Ecotoxicology and Environmental Fate of Manufactured Nanomaterials: Test Guidelines
No. 39	<a href="#">ENV/JM/MONO(2013)17</a>	Environmentally Sustainable Use of Manufactured Nanomaterials - Workshop held on 14 September 2011 in Rome, Italy
No. 38	<a href="#">ENV/JM/MONO(2013)18</a>	Co-Operation on Risk Assessment: Prioritisation of Important Issues on Risk Assessment of Manufactured Nanomaterials - Final Report
No. 37	<a href="#">ENV/JM/MONO(2013)2</a>	Current Developments on the Safety of Manufactured Nanomaterials - Tour de Table at the 10th Meeting of the Working Party on Manufactured Nanomaterials
No. 36	<a href="#">ENV/JM/MONO(2012)40</a>	Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials
No. 35	<a href="#">ENV/JM/MONO(2012)14</a>	Inhalation Toxicity Testing: Expert Meeting on Potential Revisions to OECD Test Guidelines and Guidance Document
No. 34	<a href="#">ENV/JM/MONO(2012)13</a>	Current Developments on the Safety of Manufactured Nanomaterials - Tour de Table at the 9th Meeting of the Working Party on Manufactured Nanomaterials
No. 33	<a href="#">ENV/JM/MONO(2012)8</a>	Important Issues on Risk Assessment of Manufactured Nanomaterials
No. 32	<a href="#">ENV/JM/MONO(2011)54</a>	National Activities on Life Cycle Assessment of Nanomaterials
No. 31	<a href="#">ENV/JM/MONO(2011)53</a>	Information Gathering Schemes on Nanomaterials: Lessons Learned and Reported Information
No. 30	<a href="#">ENV/JM/MONO(2011)52</a>	Regulated Nanomaterials: 2006-2009
No. 29	<a href="#">ENV/JM/MONO(2011)12</a>	Current Developments/Activities on the Safety of Manufactured Nanomaterials - Tour de Table at the 8th Meeting of the Working Party on Manufactured Nanomaterials
No. 28	<a href="#">ENV/JM/MONO(2010)47</a>	Compilation and Comparison of Guidelines Related to Exposure to Nanomaterials in Laboratories
No. 27	<a href="#">ENV/JM/MONO(2010)46</a>	List of Manufactured Nanomaterials and List of Endpoints for Phase One of the Sponsorship Programme for the Testing of Manufactured Nanomaterials: Revision
No. 26	<a href="#">ENV/JM/MONO(2010)42</a>	Current Developments/Activities on the Safety of Manufactured Nanomaterials, Tour de Table at the 7th Meeting of the Working Party on Manufactured Nanomaterials
No. 25	<a href="#">ENV/JM/MONO(2009)20/REV</a>	Guidance Manual for the Testing of Manufactured Nanomaterials: OECD Sponsorship Programme: First Revision
No. 24	<a href="#">ENV/JM/MONO(2010)25</a>	Preliminary Guidance Notes on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials
No. 23	<a href="#">ENV/JM/MONO(2010)12</a>	Report of the Questionnaire on Regulatory Regimes for Manufactured Nanomaterials (2010)
No. 22	<a href="#">ENV/JM/MONO(2010)11</a>	OECD Programme on the Safety of Manufactured Nanomaterials 2009-2012 Operational Plans of the Projects
No. 21	<a href="#">ENV/JM/MONO(2010)10</a>	Report of the Workshop on Risk Assessment of

		Manufactured Nanomaterials in a regulatory context, held on 16-18 September 2009, in Washington D.C. United States.
No. 20	<a href="#">ENV/JM/MONO(2010)4</a>	Current Developments/Activities on the Safety of Manufactured Nanomaterials: Tour de Table at the 6th Meeting of the Working Party on Manufactured Nanomaterials, 28-30 October 2009
No. 19	<a href="#">ENV/JM/MONO(2009)45</a>	Analysis of Information Gathering Initiatives on Manufactured Nanomaterials
No. 18	<a href="#">ENV/JM/MONO(2009)24</a>	Manufactured Nanomaterials: Roadmap for Activities during 2009 and 2010
No. 17	<a href="#">ENV/JM/MONO(2009)23</a>	Current Developments in Delegations and other International Organisations on the Safety of Manufactured Nanomaterials- Tour de Table
No. 16	<a href="#">ENV/JM/MONO(2009)22</a>	Manufactured Nanomaterials: Work Programme 2009-2012
No. 15	<a href="#">ENV/JM/MONO(2009)21</a>	Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials
No. 14	ENV/JM/MONO(2009)20 This document has been <a href="#">updated</a> ENV/JM/MONO(2009)20/rev	Guidance Manual for the Testing of Manufactured Nanomaterials: OECD's Sponsorship Programme
No. 13	<a href="#">ENV/JM/MONO(2009)18</a>	Report of an OECD Workshop on Exposure Assessment and Exposure Mitigation: Manufactured Nanomaterials
No. 12	<a href="#">ENV/JM/MONO(2009)17</a>	Comparison of Guidance on Selection of Skin Protective Equipment and Respirators for Use in the Workplace: Manufactured Nanomaterials
No. 11	<a href="#">ENV/JM/MONO(2009)16</a>	Emission Assessment for Identification of Sources and Release of Airborne Manufactured Nanomaterials in the Workplace: Compilation of Existing Guidance
No. 10	<a href="#">ENV/JM/MONO(2009)15</a>	Identification, Compilation and Analysis of Guidance Information for Exposure Measurement and Exposure Mitigation: Manufactured Nanomaterials
No. 9	<a href="#">ENV/JM/MONO(2009)10</a>	EHS Research Strategies on Manufactured Nanomaterials: Compilation of Outputs
No. 8	<a href="#">ENV/JM/MONO(2009)6</a>	Preliminary Analysis of Exposure Measurement and Exposure Mitigation in Occupational Settings: Manufactured Nanomaterials
No. 7	<a href="#">ENV/JM/MONO(2008)29</a>	Current Developments/Activities on the Safety of Manufactured Nanomaterials: Tour de Table at the 4th Meeting of the Working Party on Manufactured Nanomaterials, 11-13 June 2008
No. 6	ENV/JM/MONO(2008)13/REV This document has been <a href="#">updated</a> ENV/JM/MONO(2010)46	List of Manufactured Nanomaterials and List of Endpoints for Phase One of the OECD Testing Programme
No. 5	<a href="#">ENV/JM/MONO(2008)7</a>	Current Developments/Activities on the Safety of Manufactured Nanomaterials: Tour de Table at the 3rd Meeting of the Working Party on Manufactured Nanomaterials, 28-30 November 2007
No. 4	<a href="#">ENV/JM/MONO(2008)2</a>	Manufactured Nanomaterials: Programme of Work 2006-2008
No. 3	<a href="#">ENV/JM/MONO(2007)16</a>	Current Developments/Activities on the Safety of Manufactured Nanomaterials: Tour de table at the 2nd Meeting of the Working Party on Manufactured Nanomaterials, 25-27 April 2007
No. 2	<a href="#">ENV/JM/MONO(2006)35</a>	Current Developments/Activities on the Safety of Manufactured Nanomaterials: Tour de table at the 1st Meeting of the Working Party on Manufactured

		Nanomaterials, 26-27 October 2006
No.1	<a href="#">ENV/JM/MONO(2006)19</a>	Report of the OECD Workshop on the Safety of Manufactured Nanomaterials: Building Co-operation, Co-ordination and Communication, 7-8 December 2005

## Annex 15: Nanogenotox – review of reports

Nanogenotox is a European joint action. One of its objectives was to develop a robust, sensitive and specific methodology for characterising the genotoxic hazard by studying the *in vitro* and *in vivo* genotoxicity and toxicokinetics of 14 nanoparticles (SiO<sub>2</sub>, TiO<sub>2</sub> and carbon nanotubes), which could be used to determine the genotoxic risk associated with exposure to nanomaterials. All the reports below can be downloaded from the website [www.nanogenotox.eu](http://www.nanogenotox.eu).

DELIVERABLE 2:	<u>Standard operating procedures for characterisation of the selected manufactured nanomaterials types.</u> June 2011, 2168 KB
DELIVERABLE 3:	<u>Final protocol for producing suitable manufactured nanomaterial exposure media.</u> July 2011, 1479 KB
DELIVERABLE 4.1:	<u>Summary report on primary physicochemical properties of manufactured nanomaterials used in NANOGENOTOX.</u> March 2013, 1331 KB
DELIVERABLE 4.2:	<u>Transmission Electron Microscopic characterisation of NANOGENOTOX nanomaterials.</u> March 2013, 3144 KB
DELIVERABLE 4.3:	<u>Crystallite size, mineralogical and chemical purity of NANOGENOTOX nanomaterials.</u> March 2013, 2340 KB
DELIVERABLE 4.4:	<u>Determination of specific surface area of NANOGENOTOX nanomaterials.</u> March 2013, 3644 KB
DELIVERABLE 4.5:	<u>Surface charge, hydrodynamic size and size distribution by zetametry, dynamic light scattering (DLS) and small-angle X-ray scattering (SAXS) in optimized aqueous suspensions for titanium and silicon dioxide.</u> March 2013, 3689 KB
DELIVERABLE 4.6:	<u>Dustiness of NANOGENOTOX nanomaterials using the NRCWE small rotating drum and the INRS Vortex shaker.</u> March 2013, 2028 KB
DELIVERABLE 4.7:	<u>Hydrochemical reactivity, solubility, and biodurability of NANOGENOTOX nanomaterials.</u> March 2013, 2496 KB
DELIVERABLE 5: B	<u><i>In vitro</i> testing strategy for nanomaterials.</u> March 2013, 2794 KB
DELIVERABLE 6:	<u>Characterisation of manufactured nanomaterials for their clastogenic/aneugenic effects or DNA damage potentials and correlation analysis.</u> March 2013, 2734 KB
DELIVERABLE 7:	<u>Identification of target organs and biodistribution including ADME parameters.</u> March 2013, 2959 KB
MILESTONE REPORT 2:	<u>Determination of acute toxicity of TiO<sub>2</sub>, SiO<sub>2</sub>, and CNT nanomaterials of the NANOGENOTOX Joint Action Plan.</u> June 2012, 492 KB
MILESTONE REPORT 2:	<u>Evaluation of the determination of Ti in tissues.</u> April 2013, 1373 KB
	<u>The final NANOGENOTOX publishable report</u> March 2013, 1975 KB



Agence nationale de sécurité sanitaire  
de l'alimentation, de l'environnement et du travail  
27-31 avenue du général Leclerc  
94701 Maisons-Alfort Cedex  
[www.anses.fr](http://www.anses.fr)