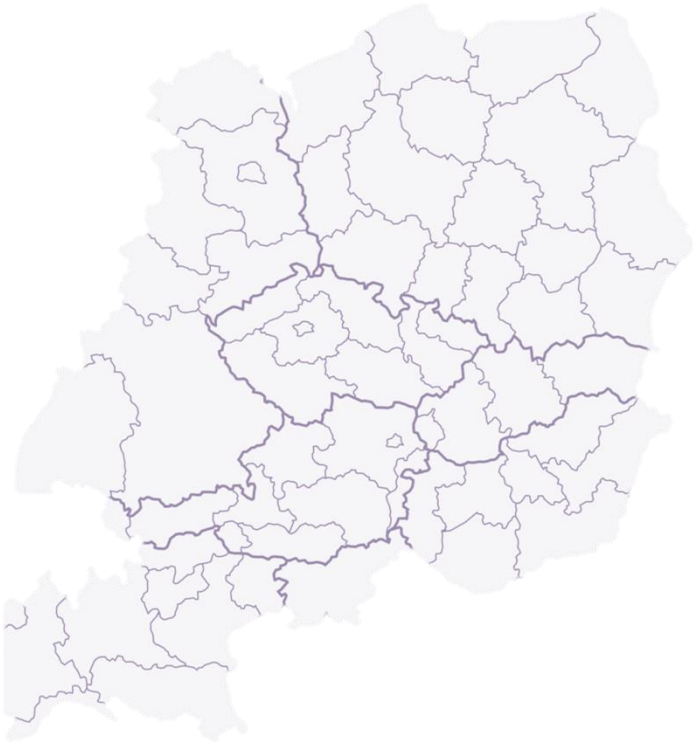
This project is implemented through the CENTRAL EUROPE Programme co-financed by the ERDF.

"NANOFORCE"
Nanotechnology for Chemical Enterprises -how to link scientific knowledge to the business in the Central Europe space



Book of recommendations for the European Commission - Excerpt

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CONTENT

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Executive Summary

The European regulatory framework is growing slowly but steadily, however available regulations and guidance documents miss some details regarding nanotechnological aspects. An established legal advisory board for chemical enterprises starting in nanotechnology provides information on existing safety and nanotech related regulations within the European region including information and guidelines on endpoint measurements for nanomaterials. Within the aim of the Central Europe project NANOFORCE the general objective is to connect public and private organizations, to carry out collaborative and interdisciplinary research on nanomaterials, and to turn the most promising laboratory results into innovative applications or products.

NANOFORCE is providing a set of recommendations collected for the European Commission in order to evaluate the applicability of the available regulations in the European Union. In the light of the revision of the REACH regulation in 2014 the NANOFORCE recommendations should support the European Commission in order to evaluate the current state of guidance for research and industry on nanotechnology implementation. NANOFORCE therefore has collected samples of three nanomaterials of great interest for marketability and provided a data sets of lab analysis which will lead to safety data sheets showing how the tested nanomaterials can be produced and professionally used conforming to safety and users guidelines. Furthermore recommendations are being given on how to adapt a regular bulk material safety data sheet to the special requirements of a nano-product focusing on the correct implementation of nano-derived products and market placement.

Results are generated by standardized in vitro testing methods for human toxicity and ecotoxicological testing of nanomaterials like nano-silver, zinc oxide and titanium dioxide. A set of recommendations provided will focus on nano specific information for adaption of safety data sheets. Additionally to these recommendations the safety data sheet and exposure scenarios are examples published to show how to classify material, provide data for in depth life cycle analysis relevant for the end user and additionally provide a draft of qualitative exposure scenarios.

A long version of the recommendation for the European Commission including information on the specific test for the development of Safety Data Sheets and Exposure Scenarios is available on www.nanoforceproject.eu and by the corresponding author: office@bionanonet.at

1 Considerations and Responsible Conclusions

We are living in the nanotechnology era. The total marked of nano-enabled products is in the range of the GNP of Germany and will further dynamically grow. For many countries (e.g. South Korea) nanotechnology is the main driving factor of the economy. Frontiers of research are now shifting to the sub-nanometer (picotechnology) dimensions control range, and to organized nano-structures of high order. Nanometrology, nanosafety and nano-regulatory issues are an important topic. This is typical for a technology that spreads widely in industry. It is also important for policy makers, since public acceptance is crucial for wide applications and for support of research in this area.

The arguments to support nanotechnology development are very strong. It is creating jobs. It contributes to balanced national budgets, what is especially important in the time of economic slowdown. Robust health care systems are possible only in countries with a strong economy, and this is not possible without nanotechnology. Further, nanotechnology offers important solutions for key societal needs. Wellbeing and improved products is very important for an attractive industry offer. Low cost solar energy and green transport will contribute to a clean environment and improved health of citizens of the planet. Improved antibacterial nano-technologies will reduce risk in hospitals, swimming pools, etc., and reduce risks with food consumption or reduce food waste. New cancer therapies, diagnostics tools, and nano-tech based regenerative medicine will radically improve health care, reduce its costs, and spread medical care to developing countries. Nanotechnology based water filters may help water supply in countries with water shortage. Thus slowing down nanotechnology development brings about tremendous risks for citizen's safety and health.
On the other hand, implementing new technologies brings about inevitable risks. History teaches us about many cases health and safety of citizens' was impaired due to new technologies. Sometimes the negative side effects had been discovered many years after the technology was introduced. Thus there are strong requests to slow down introduction of new technologies until possible safety risks are recognized. In the case of nanotechnology, such requests are strengthened because nanotechnology is difficult to understand, and also was a used to attract audience in many horror books or films. Thus nanotechnology public perception is an important issue. There may be strong political pressures from various lobbies in favor of one or other kind of decisions. Regulatory risk is a factor negatively affecting economic growth based on nanotechnology.

Even though the fundamental question is not new, nanotechnology imposes unknown before challenges on regulatory institutions. This is due to the very nature of nanotechnology. In the past, regulations were mainly dealing with materials, or chemical substances. In the case of nano-materials, it is the shape and size that determines their properties. For instance, a gold nanomaterials 100 nm in diameter, 50 nm in diameter, 10 nm in diameter, in form of nano-sized star or triangle or film or sphere, may have completely different properties. Further, the properties will depend on the chemical composition of one mono – layer of molecules attached to the surface of the particles. The existing regulations are not prepared to handle such a situation. Furthermore, it is not any more the total production weight per year that is important, but perhaps the total specific surface or chemical activity measured by various methods, which is important. Testing the structure of nano-objects requires expensive equipment and experienced staff. Thus regulatory organizations are under unknown before pressure from two sides: how to avoid having negative impact on economy and citizens health by introducing too stringent regulations, and at the same time how to satisfy society the need for safety of products on the market or technologies in the working place.
What is the solution for the above dilemma: invest in nanotechnology to improve human health and safety and use "precautionary principle" in the sense not to stop an activity that can bring big benefits" , or slow down nanotechnology development until potential negative side effects are recognized, and use "precautionary principle" in the sense "stop a new technology since it may bring health and safety risk?"

In our opinion new regulations should be based on a deep knowledge of nanotechnology. Nanotechnology needs nano-science based regulations.
It is clear that, if particle dimensions are less than 100 nm there may be a need for special regulations. Additional factors: surface layer chemical composition, real average size, size distribution, specific surface, shape, gradients of chemical composition inside the particles become perhaps more important than the material the nanomaterials is build off. Thus, for nanomaterials less than 100 nm, there may be a need for special regulations before market entry is allowed.
Direct implementation of the above recommendation will however paralyse the nanotechnology market, since methods to analyse nanostructures in an industrial scale are at their infancy, are quite expensive, or not possible yet at all. Application of standard protocols used in the community of environment and health protection experts, is not suitable for nanotechnology, because the nano-aspects of the investigated objects will not be taken into account. A standard protocol is to subject some living organisms to a dose of a substance, and investigate its effect on life time or reproduction ability of the organism. A nanotechnology based approach will take into account the nanomaterials size, size distribution, shape and surface capping layer on toxicity. However, it will take first of all into account the specific characteristic of nanomaterials, which is their extraordinary physico-chemical activity, which depends on their specific surface area (total area of one gram the nanomaterials) and surface capping layer. Thus taking into account factor alone the that the particle size is less than 100 nm is a big error, since beyond that shape there is a myriad of nanostructures, which may have a beneficial or harmful health effect. Further, the attraction force between nanomaterials is tremendous. Two nanomaterials may attract each other with a force per unit area in the range of 1 GPa. This leads to strong agglomeration. For instance in water encountered in typical environmental conditions (soil water, see water, river water), ZnO nanomaterials 20 nm in diameter will agglomerate and form micron sized agglomerates. Thus any living organism will interact with micron sized particles, not any nano-particles. Further, if the nanomaterials are embedded in a matrix (e.g. polymer) and are chemically bonded with the polymer, removing them from the matrix would require pressures also in the range of GPa, that means extremely strong forces. Finally, nanomaterials are thermodynamically unstable. Thy will tend to reduce energy by reducing specific surface (e.g. bond to a matrix or dissolve and precipitate as large particles). Finally, the nanomaterials will dissolve, and concern will be with the toxicity of the ions released in the environment and not with the nanomaterials themselves. Thus, our knowledge of nano-science leads us to following conclusions:

- Standard tests for toxicity of materials or chemical substances are not suitable for nano materials. New test have to be developed
- Nano-science will help to develop new tests for nano-products
- Nano-products environmental and health impacts must be evaluated case by case.
- Present knowledge of nano-science tells us that in most cases nanomaterials will agglomerate and or dissolve, and not act on humans or animals as nano-objects.
- Precautionary principle is to be regarded from the point of view of the possible negative impact of new regulations in the field of nanotechnology on citizens' health and safety , since they may prevent to implement important new technologies

2 Recommendations

2.1 Key messages

One key message is that the approach to toxicity studies is currently particle-based; it needs to be shifted towards a product-based approach. The results include safety data sheets of the products and are supported by a comprehensive list of references and review of relevant literature. Guidelines provided by different research institutions such as ITS Nano or ISO and OECD as well as guidance provided by REACH could help to determine the most appropriate testing methods, appropriate safety measures and appropriate concentrations to be applied in each experiment. Additionally they could help to design experiments in more appropriate way to enable further comparisons of research results.

The size of the nanomaterials can have a major influence on their interactions with nano/bio interfaces - very small particles can easily penetrate through boundaries inside the body, while big agglomerates can have toxicological impact because of their surface chemistry.

To ensure that complete and real effects of nanomaterials and nanomaterials in the environment are well taken into consideration, several effects of nanomaterials should be tested:

- their effects on bacteria, different cell types, arthropods (especially detritivores), mammals, algae and plants. Lethal as well as sublethal effects should be evaluated (including effects on reproduction);

- all experiments should draw clear distinctions between different dispersion protocols and different media (deionized water, physiological solution, water with peptides, marine water…);

- the effects and the fate of nanomaterials on/in the cells;

- the fate of nanomaterials in the soil and in the water bodies (distribution between water and bottom, possibilities for further resuspension, effects on organisms in and on the mud).

For all nanomaterials that are used in the form of dust, the amount of leaking into the surrounding air and impact area needs to be considered. For these nanomaterials, the effects on respiratory, and olfactory and digestive systems, as well as on skin and eyes are extremely important.

For all nanomaterials that are used in the form of solutions, it is important to evaluate the toxicity effects of the (pure) solutions. Moreover, their effect on freshwater and marine ecosystems should be evaluated, together with their effects and fate in cells (tissue cultures).

For nanomaterials which are used as coatings, paints or new solid materials, it is essential to evaluate the degree of leakage of these nanomaterials into the environment in different conditions (water, temperature, sun radiation, acid/alkaline environment, ageing, etc.), the fate of these leached nanomaterials in the environment and their toxic effect on different non-target organisms.

In order to produce a meaningful SDS, it is necessary to have the specific information for the product. One of the benefits of nanomaterials is their wide applicability within different fields of application. Following the sections of an SDS, NANOFORCE suggests that the uses to be reported are realistic uses and are linked to the actual use of the product, and not all the possible uses than can be foreseen.

As far as classifications and labeling are concerned, it could be possible to use the same classification as that used for of the bulk substance. Concerning all points mentioned to be taken into consideration for a nano safety data sheet, the preparations of a nano exposure scenario has to relay on the data gained within the process of testing a nanomaterial.

Concerning the Physical-Chemical characteristics, the last IUCLID5 version includes also the 13 nano-specific endpoints recommended by OECD. Those endpoints should also be specifically measured for the product in question and included in the SDS. The endpoints are: Agglomeration/aggregation; Crystalline phase; Crystallite and grain size; Aspect ratio / shape; Specific surface area; Zeta potential; Surface chemistry; Dustiness; Porosity; Pour density; Photo catalytic activity; Radical formation potential; Catalytic activity. Not all parameters are relevant to all materials. In our opinion, the minimum set of parameters that should be reported for all materials are size distribution, aspect ratio/shape, surface area, surface chemistry, dustiness (for powder), crystalline phase (for nanomaterials with different crystalline phases). Especially important for nanomaterials is the waste management and the disposal phase. This part should be carefully evaluated, collecting scientific evidences to suggest the best way to dispose of the wastes and of the final product.
In order to produce a useful ES, the actual exposure in realistic conditions should be measured. This is because the available exposure models are not specific for nanomaterials, and the final result will be not precise, and overestimated most of the time for the precautionary principle application (e.g. release of nanosilver from a treated surface assumed 100%). This assumption can greatly reduce the applicability of nanomaterials.

2.2 Recommendations based on the NANOFORCE testing results

Suggestions for thorough revisions of REACH have been presented by NGOs (CIEL 2012), (EEB 2012) and by several Governments and Agencies (KEMI 2013), (UBA 2013). These suggestions and first experiences from the REACH registrations (Aschberger 2013) were discussed at a workshop in Brussels in Spring 2013. In summary, NANOFORCE has revised the positions of the Stakeholders and furthermore commented the major issues with respect to possible knowledge gaps:

a) Uniform definition
b) “Case by Case” approach for nanomaterials
c) Standardized methods and reference material (particles in relevant media) as well as reports on safety and exposure assessment for registered nanomaterials
d) Life-Cycle approach in consideration of the precautionary principle

Within the project NANOFORCE research groups have been carrying out characterization and toxicity tests on three nanomaterials (TiO_2, nanoAg, nanoZnO) within the aim to evaluate possible knowledge gaps in risk assessment.

a) Uniform definition

There is the need of a proper definition of nanomaterials including their classification and differentiation into natural, incidental and engineered nanomaterials. Additionally a definition should demand on revealing information on size and characteristics of nanomaterials especially for further registration and labelling purposes (Bleeker et al., 2013).

b) 'Case by Case' approach for nanomaterials

Despite the considerable amount of work and resources in the nanosafety domain, there are still many unanswered questions that have to be addressed to achieve a safe and sustainable development of nanotechnology in commercial applications. The "Second Regulatory Review on Nanomaterials" suggests that the risk assessment of nanomaterials should still be performed on a case-by-case basis; however, while this approach still is crucial to fill several knowledge gaps that are still existing in the whole nano-risk assessment procedure, a strategy to achieve the possibility of a read-across between results achieved into different projects has to be promoted (European Commission, 2012).

This strategy starts from the better use of existing results. Several initiatives, represented for instance by FP6 and FP7 projects, or the OEDC WPMN sponsorship programme, have provided significant amount of data on different classes of nanomaterials. This data represent a valuable source of information, and should be deeply analysed to understand and identify the real knowledge gaps to be addressed in the future research and to develop a science based regulation. Another possible outcome of the analysis of these data is the generation of rational grouping and modelling approaches, which should be encouraged with the aim of focussing the testing requirements for companies producing and developing nanomaterials.

Explanation of particle properties which are important for the characterisation of nanomaterials can be found in the table below (table 1). The NANOFORCE project group has selected a set of parameters to test the nanomaterials based on the selection provided by several research and working groups (such as OECD WPMN).

Table 1 Specific physiochemical properties and characterization of nanomaterials – Literature Study and experience on recommendations for parameter-selection

Parameter	Sources
Agglomeration/aggregation	(OECD, 2010) (SCENIHR, 2009) (Pettitt & Lead, 2013) (Tiede et al., 2008) (Pronk et al., 2009)
Crystaline phase	(OECD, 2010) (SCENIHR, 2009) (Pettitt & Lead, 2013)
Crystallite size	(OECD, 2010) (SCENIHR, 2009) (Bleeker et al., 2013) (Pettitt & Lead, 2013)
Dustiness	(OECD, 2010)
Representative TEM picture/s	(OECD, 2010) (Tiede et al., 2008)
Particle size distribution	(OECD, 2010) (SCENIHR, 2009) (Bleeker et al., 2013) (Tiede et al., 2008) (Pronk et al., 2009)
Specific surface area	(OECD, 2010) (SCENIHR, 2009) (Bleeker et al., 2013) (Tiede et al., 2008) (Pronk et al., 2009)
Surface chemistry	(OECD, 2010) (SCENIHR, 2009) (Pettitt & Lead, 2013) (Tiede et al., 2008) (Pronk et al., 2009)
Shape	(SCENIHR, 2009) (Tiede et al., 2008)(Pettitt & Lead, 2013) (Pronk et al., 2009)
Catalytic or photocatalytic activity	(OECD, 2010) (SCENIHR, 2009) (Pettitt & Lead, 2013) (Pronk et al., 2009)
Pour density	(OECD, 2010) (SCENIHR, 2009)
Porosity	(OECD, 2010)

In addition the method selection should be depending on the planed use of the nanomaterials and the methods used should be well described documented and preferably standardized. It is necessary to test nanomaterials in different media due to changes in physio-chemical properties and toxicity

(Tiede et al., 2008). Furthermore tests have to be performed on nanomaterials concerning their life cycle (environmental fate, storage, etc.) (Bleeker et al., 2013).

Using different methods of measurements we should take into consideration that nanomaterials interact with environment and can change their properties (Pettitt & Lead, 2013).

c) Standardized methods and reference material (particles in relevant media) as well as reports on safety and exposure assessment for registered nanomaterials

- Toxicity assessment of nanomaterials should include the same steps as toxicity assessment of other substances. Special attention has to be taken to test effects of nanomaterials on different organisms that feed on organic matter in/at the bottom of the water bodies and in/on the soil (impacts on their metabolism, reproduction, accumulation etc.). This is especially important in studies of toxicity in the sea and in other waters with considerable ion content.
- Non standardized test, including in vitro test and toxicogenomics approaches should be considered when validated protocols are available, according to Reg EC 1907/2006 Annex 11
- Determination of toxicity of nanomaterials themself (not aggregates) is important in studies of transportation through membranes and accumulation within cells (study of cell-lines). Here we propose the use of sonication with the addition of a protein stabilizer. In the majority of other cases we recommend tests which will study conditions, which appear in the nature (without sonification etc. of the final test solution).
- For photo-active substances it is needed to include studies of toxicity under/after irradiation.
- For substances including nanomaterials it is important to test their release from these materials under different conditions they will face with during their life-time. For example paints should be tested on leaching of their substances under rain and immersion, include effects of UV radiation).

Most results are related to toxicity studies of pure nanomaterials based on *in vitro* and *in vivo* experiments. Undoubtedly, this basic research is essential. But at this point the question must be answered, how these results should be treated. Since these are particle-based individual results, they may not be used for general statements and warnings, especially if no practice-relevant exposure conditions were selected. Otherwise, the public may be confused in general. Concerning hazard testing, a shift in the toxicology testing is required. Toxicology testing shall indeed take an increasing advantage of 21st century tools, in particular High-Content and High-Throughput tools, to have a faster and more informative description of the toxicological feature of each material, with also the perspective of reducing the costs.[1]

To ensure the possibility to read across, a crucial step is that data are achieved with common protocols; a recently published document published by the Danish Environmental Protection Agency (The Danish Environmental Protection Agency, 2013) reviews several of the endpoints used for the regulatory registration of chemicals and the applicability of the test methods used to achieve them to nanomaterials. In addition, several ongoing research initiatives in Europe, for instance the MARINA and NANOVALID research projects, are still validating research tests, to develop standard operating procedures (SOPs) to be adopted also for regulatory testing. The use of harmonised protocols is of paramount importance for the achievement of data that is comparable for the development of grouping and modelling approaches. It is important to point out that such grouping approaches can go beyond the traditional grouping according physicochemical characteristics, but should include exposure routes and toxicological behaviours. The goal is to assess the increased or decreased hazard given by the nano implementation during the product life cycle, and find commonalities between different products. This work would allow the performance of life cycle risk assessment (always linked to a product or a group of products) and to allow an appropriate risk management of end of life phases, and an effective risk communication.

[1] http://www.ncbi.nlm.nih.gov/pubmed/23879741

Finally, effective risk communication strategies are to be developed, taking into account safety documents. This aspect is especially relevant for downstream users, consumers, and organisations dealing with recycling and disposal. There is an issue of communication of potential safety problems linked to nanomaterials use. For example, it is important to give all relevant information, and consumers should be advised about the appropriate use of nanoproducts, and disposal requirements. There are projects starting to deal with these issues, but results should be used to implement a nano communication strategy.

d) Life-Cycle approach in consideration of the precautionary principle

It is important, both for research and regulation of nanomaterials, to be more product-oriented. The mean to achieve this is the development of a testing strategy that covers the whole life cycle of the nanomaterial, hence applying a cradle-to-grave approach. As indeed highlighted into several documents and scientific papers, both the usage and wasting conditions, and the activity of environmental stressors, are able to modify the physicochemical characteristics of nanomaterials, hence potentially influencing their environmental fate, and their toxicological behaviours. Therefore, while regulation should address this issue, research in parallel should identify when modifications during the life cycle are enough to trigger the necessity of a novel risk assessment.[2]

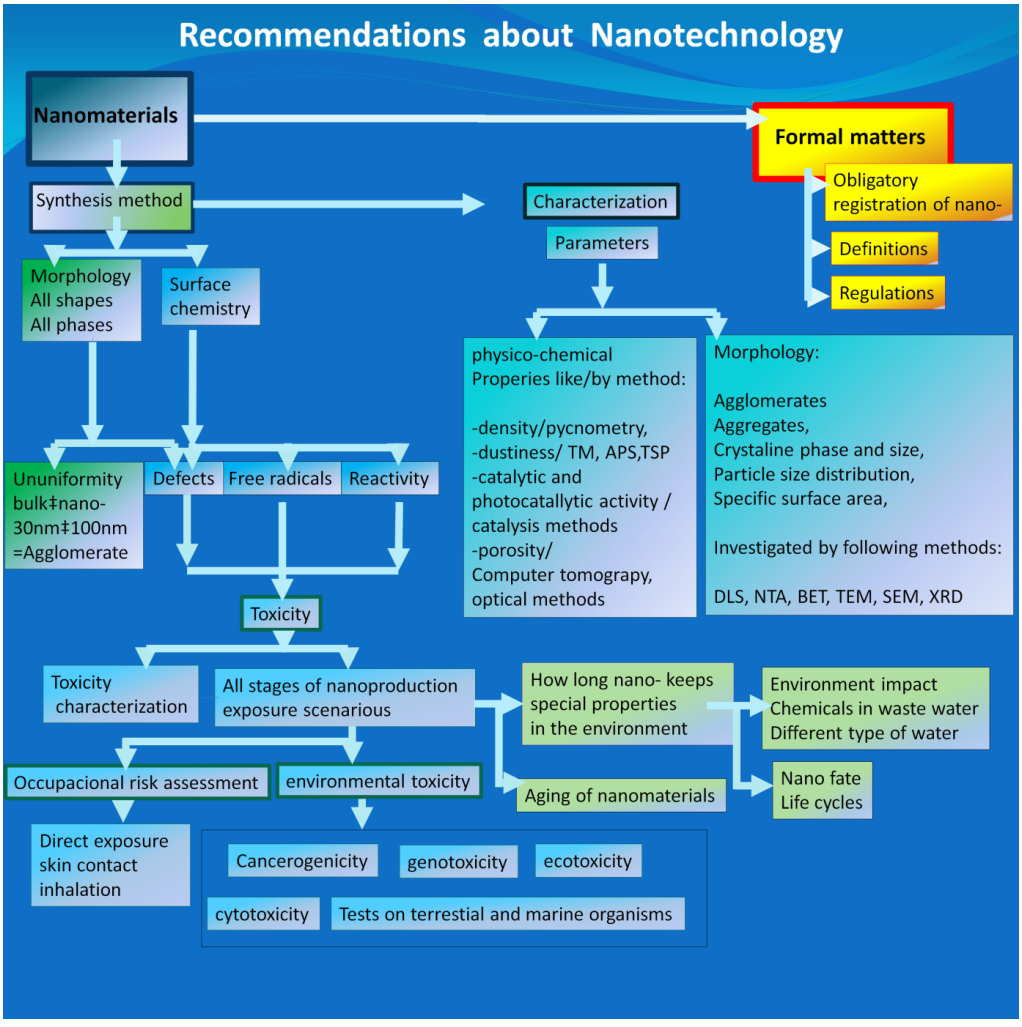

Figure 1 NANOFORCE - Recommendations on future Research Development with respect to current regulations (NANOFORCE project 2011-2014)

[2] http://www.efsa.europa.eu/en/efsajournal/doc/958.pdf

2.3 Recommendations concerning future nano-safety research

Insufficient knowledge on the effects of nanomaterials has led to concern over the environmental, health and safety risks potentially associated with nanotechnology and nanomaterials. They can interact differently with biological systems than their conventional counterparts and exhibit different toxicological, ecotoxicological and exposure features. Whilst a number of general approaches to the risk assessment, safe handling and control of nanomaterials have been published including detailed methods[3, 4, 5, 6] currently, there is no single piece of guidance which can provide a definitive, step-by-step approach. Scientific community as well as international organizations should work on methodologies for assessing risks associated with nanomaterials. Priority has been given to developing horizontal standards for terminology and nomenclature, measurement and characterization, health, safety and the environmental (HSE) and nanomaterial characterization[7].

The future research should focus on exposure assessment, both via experimental activity and modelling, for groups of products, activities, and uses. In this area there is the need to have standardized methods, and different SOPs, to be able to compare the different scenarios. Another issue is the grouping of nanomaterials for testing and eventually forecasting nanomaterials toxicity and properties. This can simplify the CLP activity and the estimation of DNEL and PNEC.

i. Future research projects should be more standardized to improve comparability of results.
ii. The long-term studies of toxicity should be continued.
iii. The possible interaction of different types of nanomaterials should also be examined more closely.

2.4 Outline and Roadmap

Recommendation in the time of Horizon2020 in order to foresee the development within the next three- four years until 2016, when most of the question marks would be solved, regulations and nanomaterials registration principles would be worked out according to recent scientific findings. All the projects under supervision of OECD and European Union will be completed and all legislation work will be finished. Simultaneously the most important research investigations hopefully result in standards of characterization methods, standard of toxicological methods and availability to created databases of SDS, ES, characterization and toxicological tests.

[3] UK, the Control of Substances Hazardous to Health Regulations (COSHH).

[4] BSI PD 6699-2:2007 Nanotechnologies: Guide to safe handling and disposal of manufactured nanomaterials.

[5] BSI PD 6699-3:2010 Nanotechnologies. Guide to assessing airborne exposure in occupational settings relevant to nanomaterials.

[6] OECD ENV/JM/MONO (2009)16 Emission Assessment for the Identification of Sources and Release of Airborne Manufactured Nanomaterials in the Workplace: Compilation of Existing Guidance.

[7] ISO/TC 229 Business Plan (Date: 12/01/2011), Version: Draft 4.

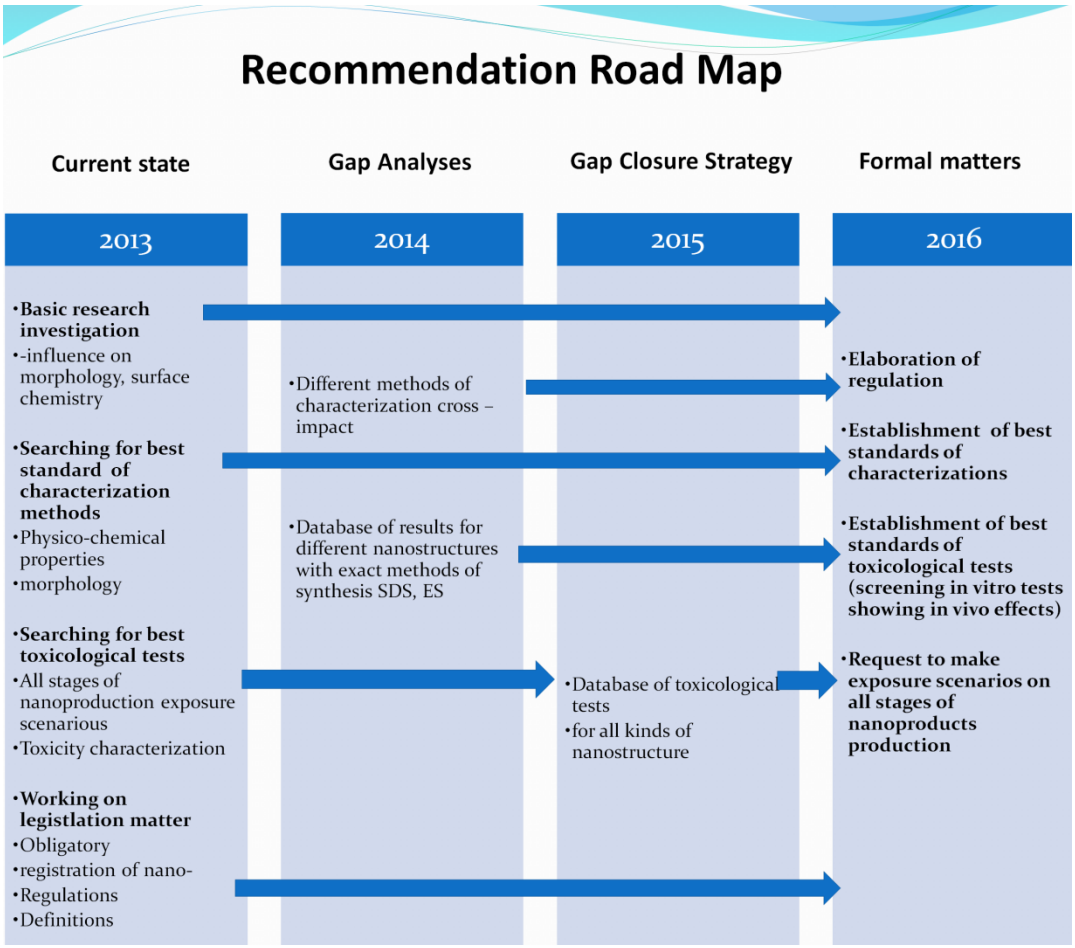

Figure 2 NANOFORCE Recommendation Roadmap for future Nanotechnology-R&D with respect to regulation (NANOFORCE project 2011-2014)

3 List of Literature

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