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

Warsaw, 10.05.2013

Newsletter of Nanoforce Project

Institute of High Pressure Physics (IHPP), also known as "UNIPRESS", it is a member of the Polish Academy of Sciences. The activity of IHPP includes modern research areas such as: optoelectronic (e.g. semi-and superconductors), bio-physics, nanotechnology. Institute specializes in high-pressure (HP) techniques, used for research purpose as well as for manufacturing of advanced materials. Obtained results present unique values, as exemplified by blue/violet laser diodes, nanostructured bone implants, oxygen sensor, superconducting wires MgB2.

Laboratory of Nanostructures for Photonic and Nanomedicine of IHPP is one of the leading Polish groups in nanotechnology area. Laboratory specializes in synthesis of high quality nanopowders as exemplified by resorbable nano-Hydroxyapatite for bone implants, ZrO_2 for optical oxygen nano-sensors, ZnO for germicidal products. The second, complementary specialization is nanometrology. Laboratory of Nanostructures is equipped with modern devices, essential for nanomaterials characterization, among others for detection of nanoparticles size and size distribution, for measurement of Specific Surface Area, density and Zeta-potential. Experience of the team and laboratory equipment were essential for realization of experimental part of NANOFORCE project.

The crucial task of NANOFORCE project is elaboration of Material Safety Data Sheets and Exposure Scenarios for three types of nanopowders: Ag and ZnO and TiO_2. In IHPP there are investigated two of them: nano-Ag manufactured by polish producer and nano-ZnO synthesized in Laboratory of Nanostructures. Both materials are available on the market as a commercial products.

Comprehensive structural investigations of nanopowders are realized in Laboratory of Nanostructures. Moreover, extensive literature study concerning toxicological and eco-toxicological properties of Ag and ZnO in nano-forms should be also done. Although that applied tests (listed above) are not standardized for materials in their nano-form, but all of them are recommended by EC or OECD for nanomaterials characterization.

1. Nanoparticle size and size distribution - tested using two techniques: Dynamic Light Scattering (DLA) and Nanoparticle Tracking Analysis (NTA).
2. Specific Surface Area (total surface area of nanoparticles per unit of mass) - determined using Brenauer-Emmett-Teller (N2-BET) adsorption method.
3. Density of nanopowders (total mas of nanoparticles per unit of volume) - investigated with use of Helium pycnometer (Volumetric measurement)
4. Crystal structure and crystal size of nanopowders - determined from X-ray diffraction (XRD) patterns.
5. Zeta potential (charge on a particle at the shear plane, related to the stability of dispersions) - established using Laser Doppler Electrophoresis (LDE).
6. Size and morphology of the constituent nano-elements - defined using Transmission Electron Microscopy (TEM) observations.
7. Scanning Electron Microscopy (SEM) investigations - realized as a complementary technique to TEM.

Recommendations based on experiences and literature data

On the base of characterization, taking into account cross-impact of using characterization methods and toxicological tests performed by others experienced in this field project partners we will prepare very carefully Safety Data Sheets for mentioned above nano-powders and also Exposure Scenarios will be done for them. All this is the task to perform in case the nanomaterials would be required to register one day. Right now there is no such regulation but in near future we can expect such request. The most important issue it is to know all features of used nanostructures and its possible hazardous interactions with environment as well as with human health. That is why in project NANOFORCE we learned about Good Laboratory Practice and we encouraged researchers to very careful and responsible use of nanotechnology. Continuing this direction we are obliged to work out the specific Recommendation for Nanotechnology Regulations. We should advise very careful assessment and judgment of nanomaterials taking into account its very specific abilities. We know that depending of reaction precursors and methods of synthesis we can occurred slightly different nanostructures, which will have identical chemical composition with bulk forms but will behave differently and also will differ from nanostructures of others parameters, for example morphology, crystals size, specific surface area, chemical reactivity etc.

Recommendation for nanomaterials safe exploatation :

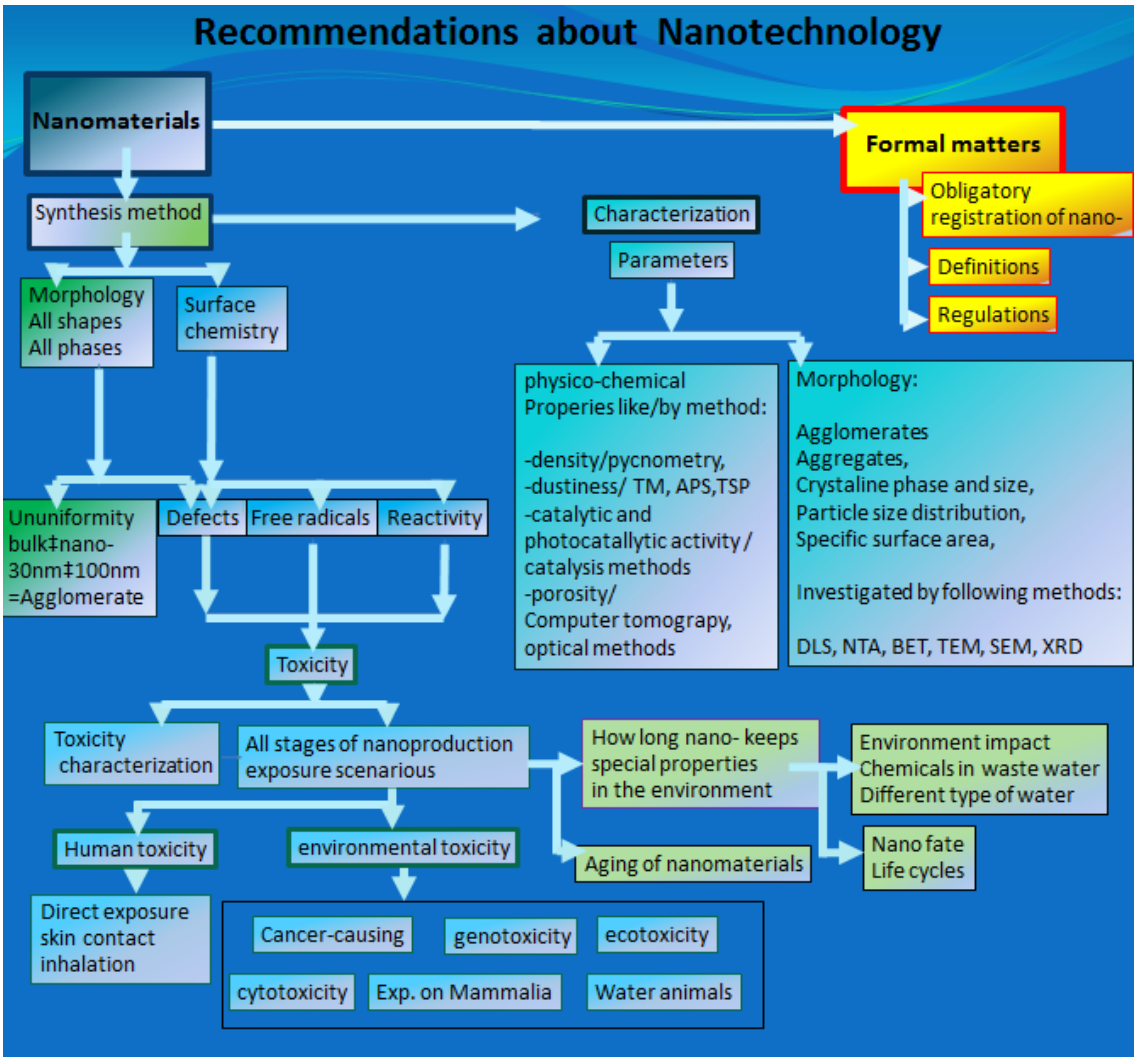
1. **Proper definition** of nanomaterials including their classification with all types and stages definition as well [1-3].
2. **Clarification of the rules for requirements of nanomaterials testing and registration besides their tonnage and properties of bulk form.**
3. **Specific physicochemical properties and characterization of nanomaterials:**
   - Agglomeration/aggregation [2,4]
   - Crystalline phase [2]
   - Dustiness [5]
   - Crystallite size [1-3,6]
   - Representative TEM picture/s
   - Particle size distribution [3]
   - Specific surface area [1,3,6]
   - Surface chemistry [2,6]

- Shape [4,7]
- Catalytic or photo-catalytic activity [2,6]
- Pour density
- Porosity

4. **Method should depends on planned use. Methods should be well described and documented [1].**
5. Using **different methods of measurements** we should take into consideration that nanoparticles interact with measuring environment and can change their properties [2].(Examples of different measurement methods are diffusion velocity in liquids, electrophoretic mobility in gases and dynamic light scattering of particles or the BET surface area of particle system. The method should be very well **documented and described**, including measurement's statistic data.
6. **Transfer of nanoparticles from one medium to another** can also affect nanoparticles properties and influence it's toxicity.(examples: different types of waters in NANOFATE experiments or deposition of nanoparticles from liquid, air or powder to a surface for exposure to a vacuum environment for electron microscopy)
7. **Different morphology** for this same stoichiometric nanomaterial can cause slightly different effects on humans and environment [4,7].
8. **Different particle size** of nano- materials can cause slightly different effects. It is known that very small particles can penetrate inside the body and cross the barriers which are unreachable for bigger ones. Sometimes only one size from the nanometric particle range has specific properties [8] (example: only 30nm Fe_2O_3 among different iron oxides is not toxic and it is used as contrast in MRI [9]).
9. It is not certain how **nanomaterial can differs from its bulk form [2,7]**. Is it reasonable to use some measures for bulk form to physical and chemical characterization of nanomaterials? (example: parameters of bulk form of a substance should not be automatically applied for its nano counterparts)
10. Another question it is about **nanomaterials fate at the environment** and inside human body, are they still nanomaterials after agglomeration and ionization in the liquid medium [7]? Do they keep their properties [1,3]?

11. Life cycle of nanoparticles can influence danger of workers exposure. Each physical form (powder, embedded in a solid matrix or attached to a substrate) has its own exposure pattern and should be characterized throughout the product lifecycle.
12. The question is also **how long nanomaterials keep their properties while storage**? The question of aging, what kind of storage is preferable to avoid access of moist [10,11]?
13. Specific methods for **toxicological assessment** (best would be screening tests in vitro which could be helpful for judgment of in vivo effects) [7],
14. There are also evidences that addition of **nano- can provide better properties** than bulk form (Pt used as nanomaterial for cancer treatment or better conditions for growth after application of nanoZnO – University of Nova Gorica - NANOFORCE)

Table 1. (below) Nanotechnology Recommendations Tree. From current stage to proper legal regulations concerning nanostructures, including all definitions, request for registration all nano-product and exposure scenarios for all stages of nanoproducts manufacturing processes.



Recommendation Road Map

Recommendation in the time horizon of three- four years until 2016, when most of the question marks would be solved, regulations and nanoparticles registration principles would be worked out. according to recent scientific founding's.

All the project 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.

2013 Current stage of knowledge:

- Basic research investigation in progress
- Searching for best standard of characterization methods
- Searching for best toxicological tests
- Working on legislation matter

2014 - 2015 The ways how to do: Gap Analyses, Strategies for Gap Closure

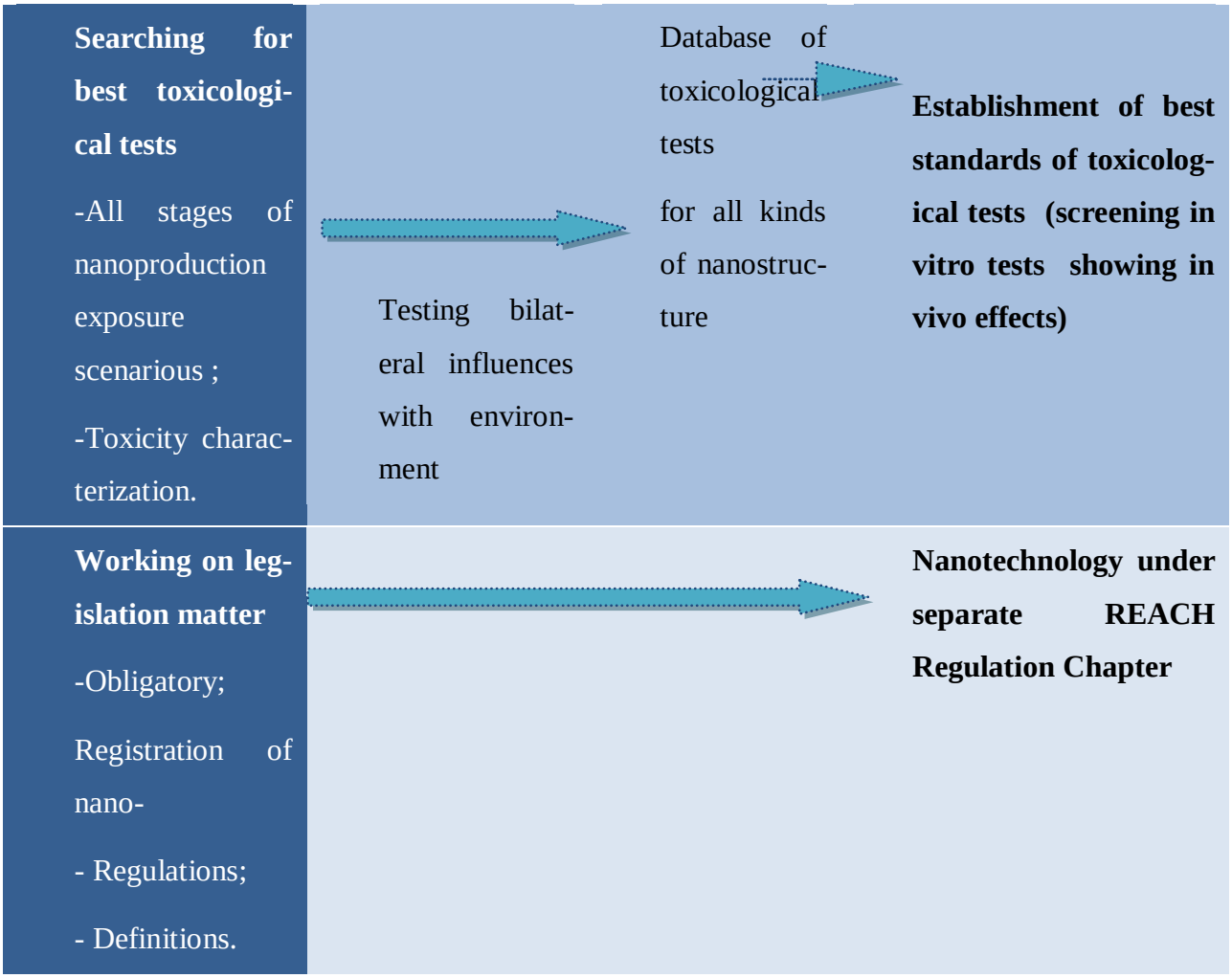
- Different methods of characterization cross – impact
- Database of results for different nanostructures with exact methods of synthesis SDS, ES
- Database of toxicological tests for all kinds of nanostructure

2016 The goals to be achieved:

- Regulation (definitions, tonnage, nanoproducts registration requirements},
- Best standards of characterizations
- Best standards of toxicological tests (screening in vitro tests showing in vivo effects)
- Request to make exposure scenarios on all stages of nanoproducts production

Table 2. (below) Nanotechnology Road map in time horizon 2013-2016. Pending on legislation changing process, experimental works in laboratories, standard methods of characterizations and toxicology for specific nanostructures abilities and tailored to it regulations should be completed by the end of 2016.

Recommendation Road Map			
Current state	**Gap Analyses**	**Gap Closure Strategy**	**Formal matters**
2013	**2014**	**2015**	**2016**
Basic research investigation -influence on morphology, surface chemistry .	Different methods of characterization cross – impacts		**Elaboration of legal regulations specific for nano structure abilities**
Searching for best standard of characterization methods: - Physico-chemical properties ; - morphology .	Database of results for different nanostructures with exact methods of synthesis SDS, ES		**Establishment of best standards of characterizations** **Request to make exposure scenarios on all stages of nanoproducts**



We were also asked to write a chapter to the book: "The Nano-Micro Interface II - Bridging the Micro and Nano Worlds" (Ed. H. Fecht) Wiley-VCH where the title is : Guidelines for Safe Operation with Nanomaterials. Since progress in development of the legal framework for use of nanomaterials in market products is slow, there is considerable “regulatory risk” for enterprises wanting to introduce nanomaterials and products based on them to the market. There is a considerable risk that a developed with big efforts product will not be permitted to enter the market, or will require extensive additional tests before market entry, because of new regulations introduced during the product development time. Further, marketing of nanotechnology-improved products is a challenge for industry due to poor understanding by the wide public what nanotechnology is, and what risks and benefits it brings. Thus companies may be reluctant to advertise, or even admit that they are using nanomaterials in their products. Other enterprises are refraining from investing into nanotechnology due to regulatory risks.

Despite the above barriers, approximately 20% of worldwide produced goods will be based on nanotechnology until 2020, raising the global volume for products derived from nanotechnology from €200 billion in 2009 to €2.5 trillion by 2015 [2]. Thus the economic impact of nano-products related regulations could be very big, since regulations may enhance or slow down new products industrialisation. Products entering the European market are subject to a standard authorization procedure guided by the EU directive on general product safety. The European Commission (EC) fosters extensively research for responsible nanotechnology development through a combination of scientific research, with exploring ethical issues [1]. The development of nanotechnology risk assessments and the standardization of the nanomaterial characterization methods are priority tasks. The authors believe that above assessments must take into account on one hand the risks of nanomaterials application, and on the other hand the societal risks of slow economic growth if nanotechnology development moves to other regions of the world, as well as the advantages nanomaterials bring for the society: improved health, reduced energy consumption or green energy implementation, improved civil and military security etc.

The goal of the regulatory efforts is to build up a framework of guidance for industry to ensure the safe use of nanomaterials throughout the product life cycle. This chapter explains the progress and challenges in developing the regulatory framework for nanomaterials handling, how to consciously use nanotechnology and what problems may be encountered. The ordered chapter is mainly based on the results of an assessment carried out within the Central Europe project NANOFORCE and contains terms and definitions in nanotechnology, the development of nanotechnology regulations, the nanomaterial's characterization methods from the point of view of regulations. Additionally in the book chapter we raise an issue of safe working condition for nanotechnology in industry like: nanomaterials safety data sheet, and their labelling.

Bibliography:

[1] Eric A.J. Bleeker, Wim H. de Jong, Robert E. Geertsma, Monique Groenewold, Evelyn H.W. Heugens, Marjorie Koers-Jacquemijns, Dik van de Meent, Jan R. Popma, Anton G. Rietveld, Susan W.P. Wijnhoven, Flemming R. Cassee, Agnes G. Oomen, Considerations on the EU definition of a nanomaterial: Science to support policy making, Regulatory Toxicology and Pharmacology, 65 (2013) 119–125.

[2] Michala E. Pettitt a, Jamie R. Lead, Minimum physicochemical characterisation requirements for nanomaterial regulation Environment International 52 (2013) 41–50.

[3] Eric A.J. Bleeker, Wim H. de Jong, Robert E. Geertsma, Monique Groenewold, Evelyn

H.W. Heugens, Marjorie Koers-Jacquemijns, Dik van de Meent, Jan R. Popma, Anton G. Rietveld, Susan W.P. Wijnhoven, Flemming R. Cassee, Agnes G. Oomen, Regulatory Toxicology and Pharmacology 65 (2013) 119–125.

[4] Karen Tiede, Alistair B.A. Boxall, Steven P. Tear , John Lewis , Helen David & Martin Hassellöv, Detection and characterization of engineered nanoparticles in food and the environment, Food Additives and Contaminants, Vol. 25, No. 7, July 2008, 795–821.

[5] F. Hamelmann and E. Schmidt Methods of Estimating the Dustiness of Industrial Powders – A Review, KONA No.21 (2003) 7-17.

[6] Catalina Marambio-Jones • Eric M. V. Hoek, A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment, J Nanopart Res (2010) 12:1531–1551.

[7] M.E.J. Pronk et al. Nanomaterials under REACH, Nanosilver case study, Report 601780003/2009 RIVM , National Institute for Public Health and the Enviromant.

[8] Adam Briggs, Stéphanie Corde, Sianne Oktaria, Ryan Brown, Anatoly Rosenfeld, Michael Lerch,Konstantin Konstantinov, Moeava Tehei, Cerium oxide nanoparticles: influence of the high-Z component revealed on radioresistant 9L cell survival under X-ray irradiation, Nanomedicine: Nanotechnology, Biology, and Medicine NANO-00734; No of Pages 8.

[9] Shailendra Pratap Singh, M.F. Rahman, U.S.N. Murty, M. Mahboob, Paramjit Grover Comparative study of genotoxicity and tissue distribution of nano and micron sized iron oxide in rats after acute oral treatment, Toxicology and Applied Pharmacology 266 (2013) 56–66.

[10] R.A. Varin,M. Jang, T. Czujko, Z.S. Wronski; The effect of ball milling under hydrogen and argon on the desorption properties of MgH_2 covered with layer of $Mg(OH)_2$. J.Alloys and Compds 493 (2010) L29-L32.

[11] J. Cermak, L. Kral Improvement of hydrogen storage characteristics of Mg/Mg2Ni by alloying: Beneficial effect of In. J. Power Sources 214 (2012) 208-215.

Project NANOFORCE Team:

Iwona Malka, Joanna Sobczyk, Anna Świderska - Środa