# Towards a method for detecting the potential genotoxicity of nanomaterials


## NANOGENOTOX Stakeholders consultation

Report

Synthesis report on stakeholders consultation
June 2011

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NANOGENOTOX **Grant Agreement n° 2009 21 01**

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# WP 2 : Dissemination of the Joint Action

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| **Workflow** | |
|---|---|
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**Summary**

This consultation took place between end of October 2010 and early January 2011.

19 persons from 5 categories of stakeholders (EU risk assessors and policy makers, scientific community, professional federations representing companies, non-governmental organisations and trade unions) participated in this consultation by answering a questionnaire (during a phone interview or by providing written answers).

The main general conclusions arising from this first phase consultation are the following:

- Regarding the understanding of the project and this consultation initiative
    - All respondents welcomed this consultation and the accompanying information process at the launch of the Joint action,
    - The aim of the Joint Action (JA) is of much interest and raises great expectations,
    - The list of Manufactured Nanomaterials (MNs) selected by the JA and the coordination with OECD WPMN are perceived in a positive manner,
    - More scientific and technical details should be made public when available, for a better understanding of the aim of the JA,
    - Translation of the results into policy orientations is of high concern.
- Regarding some issues which might need clarification or specific attention for the JA to be successful
    - Consideration must be paid to the validity of protocols and testing approaches to be used, both for *in vivo* and *in vitro* testing,
    - Clarification might be needed about trade-off between the use of best knowledge available regarding nanogenotoxicity and the need for harmonisation of protocols,
    - Guidance for the use of the expected protocols and for data analysis is expected,
    - Strengthened coordination with other EU projects related to MN is expected,
    - Dissemination of the results for policy making purposes is of importance.

A draft report of this first phase consultation was presented and discussed among the partners during the General Assembly (GA) of the Joint Action, held in Nancy, France in April 2011.

The present synthesis report incorporates feedback from the partners of the JA after this last GA meeting.



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# 1. OVERVIEW OF THE CONSULTATION PROCESS

## Aim of the consultation

The purpose of this first round of consultation was threefold: first, identifying at an early stage of the JA some key concerns regarding the aim and output of the project which may be of interest for the partners and may enrich the implementation of the project; second, bringing the project to the attention of key concerned actors/stakeholders; and third, establishing contacts in order to facilitate and orient further dissemination activities.

## Description of the Consultation process

Five categories of stakeholders were identified: EU risk assessors and policy makers, scientific community, professional federations representing companies, non-governmental organisations (NGOs) and trade unions (see list of interviewees in annex 1).

These stakeholders were selected using a combination of desk research and communication with various partners. Selection criteria were the following: they had to be implicated at an EU (or international) level, recognised in their domain and exercising a certain influence, recognised for their ability to relay information, having technical and scientific knowledge regarding nanotoxicology and willingness to engage in technical discussion about nanotoxicology.

The list was finalized after presentation and discussion during the NANOGENOTOX General Assembly meeting in September 2010.

From October 2010 to January 2011, more than 45 persons were contacted by e-mail asking for their participation in this consultation. An interview questionnaire (see annex 2) was sent as a support for the interview as well a short presentation of the project and some perceived FAQs (before their release on the project web site). Participants were interviewed by phone or they had the possibility to send back their written answers to the questionnaire.



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A total of 19 organisations responded positively to the consultation. Ten interviews were conducted by phone[1] involving a real two-way interaction. These interviews lasted about 1 hour, and the transcript was subsequently sent to each participant for validation. Nine written contributions were received.

It was agreed that the views expressed by the participants in this consultation do not seek to represent the "official" views of their organisations and that the synthesis report to be published will not allow the identification of individuals in the text. In some few cases the issue of the consultation was raised at a Scientific Committee (or equivalent) of the organisation and persons were nominated to answer the questionnaire formally in the name of the organisation.

## General comments

Given the large number of persons contacted for the consultation, it was an opportunity to share information by presenting in detail the Joint Action and sometimes to remove some misunderstanding. Of course the impact of the dialogue was limited because this first consultation was made at the beginning of the action and no results could yet be given to the interviewees. Nevertheless persons contacted all appreciated that the stakeholders have been consulted at the very beginning of the project.

All the persons interviewed understood the purpose of this first consultation and recognised that 20 interviews is a good number to obtain a rough overview of the opinions at the EU level.

They all welcomed the aim of the NANOGENOTOX Joint Action mentioning that there is a great need for this kind of initiative, although the objectives might appear as very ambitious. They all recognised that the evaluation of the impact of MNs on human health must be reinforced and that there is an absence of evidence demonstrating the safety of certain nanotechnology products. They have great expectations regarding the decisions to be made for the risk assessment and risk management of the MNs. Therefore, they underline the need of a complete transparency of the work performed.

They consider that the JA demonstrates a good alignment on the OECD WPMN and ISO TC229. NANOGENOTOX seems also to take into consideration existing data and ongoing projects on the EU and international level.

[1] Interviews were conducted by A. Cadène, F. Etoré, N. Thieriet and B. Vergriette (WP1 and WP2, ANSES, France)



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## 2. SPECIFIC COMMENTS AND EXPECTATIONS

**Regarding nanomaterials tested and techniques used**:

Although perceived as not sufficiently explained in the documentation that was made available, the process of selection of MNs to be tested was mainly considered as satisfactory considering the aim of NANOGENOTOX. The link with the OECD sponsorship programme through the selection of the OECD reference material was clearly appreciated. Nevertheless some participants suggested to test more or other materials (*i.e.* food or cosmetic application). A larger catalogue of MNs tested might have been interesting in order to strengthen extrapolation of the methods/results to other MNs.

A majority of interviewees recognized that it is important to have a clear definition of what is a nanomaterial (or a nanoparticle) in order to highlight what is the rationale behind the size of the nanomaterials tested in the JA.

An emphasis was put on the need for an appropriate characterisation of the MNs tested (so as to allow the correct interpretation of the results) as well as characterisation of the different stages of solubilisation, particularly in water or in physiological media.

Questions were raised about how the previous research are taken into account in NANOGENOTOX and some general experimental difficulties related to MNs were also highlighted:

- How should the test material be suspended in the test medium (e.g. after ultrasonic treatment, use of detergents)? Humans may be exposed to aerosols which then may have a different degree of agglomeration than in water systems.
- Usually relatively high particle concentrations have to be used to increase the chance of uptake in the cell. Many cell types have limited capacity of phagocytosis of solid particles.
- Inflammatory reactions play a substantial role in inducing genotoxic effects in cells via ROS (Reactive Oxygen Species) production.

It was mentioned that even if MNs act *via* oxidative stress, an increase of DNA breaks as measured by the comet assay are not always observed. It might be also questionable if the classical micronucleus test *in vivo* in bone marrow is a suitable test method.

It was also highlighted the need to implement early in the process a harmonized data recording format and that all partners agree on materials and methods to be used.



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In that respect, the data base from BAuA (Federal Institute for Occupational Safety and Health Germany) developed for the research on the carcinogenicity of nanoparticles and other dusts was mentioned.

It was recognised that the "round robin" test is really an important phase of the project. The definition of the positive and negative controls is an important and difficult step in order to validate the developed method.

It was agreed that the supply of the materials being from the JRC European repository of nanomaterials was a guaranty of a full traceability.

**Regarding nanotoxicology concerns:**

It is well understood that genotoxicity is of high concern but a similar initiative might be needed for other issues than genotoxicity that are coming from the ultrafine particles, for example ecotoxicity, reprotoxicity or cardiovascular toxicity.

There is a general concern, out of scope of the JA, about the development of environmental issues, like environmental exposures and effect of the exposure over the years. Stakeholders are interested in biological/degradable nanomaterials, like micelles for example. A lot of them wanted to have more information on the interactions of the MNs with other products.

Life cycle analysis is also very interesting for nanomaterials included in products, it can illustrate the risks but also the benefits of the MNs' uses.

Some participants highlighted the need to have more information on the effect of size, shape and surface properties of MNs on the toxicity of the MNs. What is the importance of physico-chemical parameters which allow nanomaterials to migrate in the body and accumulate in specific tissues?

With the final objective of achieving some predictive risk assessment and preventing countless toxicological assays, nanotoxicology should focus on systematic mechanistic work to scrutinize critical nanomaterial's parameters, determine relevant exposure routes, identify tissues at risk and characterise molecular events involved in any pathogenic effects resulting from MNs exposure.



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Interactions with other environmental agents should also be considered where appropriate, e.g. in the case of titanium dioxide and ultraviolet radiation.

**Regarding the outcomes of the Joint Action:**

It is expected that the Joint Action will make significant contribution to the current exploratory phase of testing and it can play an important role towards the validation of test methodologies.

The outcome of the JA should be the establishment of a method that can be applicable and used widely and for a broad range of products. It is a way to avoid the "case-by-case" approach. For the establishment of such a harmonized method intended for a broad application, a wider agreement (*i.e.* outside the project partners) will be needed. This method has to be validated and recognised on a global level (*i.e.* OECD).

For establishing a parallel between *in vitro* and *in vivo* experiments, the JA is supposed to define a "gold standard" for *in vivo* studies to which the *in vitro* methods will be correlated. In the end, the results of the JA should help in finding alternative methods in order to reduce the use of animals in testing.

A knowledge base and a data base will be created by this JA which is also supposed to build a network of laboratories. This is very positive as it can help harmonising experimental procedures across Europe and thus improve standards of research on genotoxicity. It can also help to screen MNs and select their properties and establish links between properties and effects. This is needed in order to help the decision makers regarding the risk assessment through the development of testing guidelines (to be used for regulatory submission, for example). This JA is a first step before the start of real testing; subsequent complementary studies will surely be needed to reach this objective

**Dissemination of the action:**

Considering the outcomes expected from NANOGENOTOX, stakeholders clearly asked for a total transparency of the performed work. The dissemination action plan as proposed is globally appreciated, nevertheless some specific expectations have been expressed.

There is a need for a scientific peer review of the results of the Joint Action and publications in well recognized scientific journals. At the same time these publications should be available to a large public, as early as possible and availability of the results should not be unduly postponed because of academic publication requirements.



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The JA should be able to get scientific recognition outside of the project with the involvement of outside laboratories (from the EU member states but also outside Europe), particularly for the final conference. It is essential that the work of the JA is combined with the work of the scientific community, as well as EU risk assessment and risk management bodies such as the EFSA and DG SANCO.

Communication must be strengthened all along the project, including as far as possible its work plan and details on methodologies applied. A mailing list should be created to disseminate important findings during the JA. Presentation/communication of intermediate data but also of full detailed protocols and "gold standard" methodology as well as guidance on data analysis will be needed. Attention must also be paid to the first results in particular if they are negative.

The general public should be a target, even if it is difficult to communicate scientific results to non specialists. During the dissemination process it is necessary to pay attention to the mass media. Results should be communicated but also the difficulties encountered; the gap between science and society has to be reduced.

It is really important to ensure the follow up the Joint Action in order to be sure that the method will be used, especially in case there should be a need for modifying the methods already used or some guidelines, like the OECD guidelines. A crucial issue is the policy use of the results of the Joint Action. Interviewees recommended to involve the Member States, for example at the final conference, and to invite persons from the health side but also from the environment side. According to the nature of the results coming from this JA, it must be ensured that decisions to be taken regarding risk management will not suffer any delay.

## 3. SYNTHESIS OF COMMENTS ACCORDING TO DIFFERENT CATEGORIES OF STAKEHOLDERS

Although many interviewees shared viewpoints and expressed similar concerns independently from their professional belonging, this section is an attempt to highlight main concerns expressed by the different categories of stakeholders.

<u>EU risk assessors and policy makers:</u>

It is recognised that there is a lack of information and projects regarding the human health and environmental impact of MNs for short-term but mainly for long term



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exposure. The general public is really concerned by the uncertainty related to the use of MNs. Life cycle analysis was also discussed.

They insisted on the strong interactions needed with REACH, SCENHIR and OECD MN sponsorship programme. They recognised the need for a method applicable for many MNs and that can be used all over Europe. More MNs tested in the action would have been interesting.

The establishment of a strong data base is also crucial, in order to produce open access publications on "gold standard" methodology and detailed critical assessments of important factors that can affect genotoxicity testing of MNs both *in vitro* and *in vivo*. These should include (a) guidelines for preparing and characterising MNs used in exposure tests, (b) detailed treatment protocols to ensure results are obtained under conditions that mimic as closely as possible human exposure conditions, (c) sensitive, reproducible and MNs-compatible genotoxicity testing protocols that are documented in sufficient detail to enable their replication by interested laboratories, (d) guidance on genotoxicity data analysis and interpretation and (e) establishment of quality assurance procedures.

NANOGENOTOX should be used to harmonise the procedures across Europe. It is also the first step before further testing.Results must be submitted to strong peer review process. The "round robin" test is a key step and this JA will build a network of EU laboratories. Exchanges with representatives from health and environment sectors (Ministries at EU level, Agencies etc...) should be sought.

Scientific Community:

According to the interviewed persons representing the scientific community, NANOGENOTOX is of great interest as it should significantly improve knowledge in nano-toxicity. Genotoxicity is assumed to be one of the classical relevant axes of effects that can result from exposure to MNs. A rigorous review of the results and method should be done. They all consider this Joint Action as a further step in this field; however they do not expect all issues related to risk assessment to be solved. Some expect inconclusive datasets because of the time frame dedicated to data analysis which they consider as being too short.

To follow this work, scientists would like some complementary investigations related to the possible mode of toxicological action of nanomaterials with the objective of distinguishing a parameter's influence on the toxicological profile. It has been proposed for instance to try to make a distinction between effects of the nanoform (size, shape, surface properties, etc.) and those attributed to solutes released by solubilisation. Vectorisation for substances adsorbed in its surface is also a well known toxicological mode of action for particulate matter.



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As the Joint Action focuses only on the toxicological effect of the pristine particles, the relevance of studying the interactions of particle core / pristine particles with adsorbed substances has also been pointed out in the framework of risk assessment.

Professional federations representing companies:

Manufacturers think that NANOGENOTOX may fill the gap of knowledge regarding toxicity of MNs. They insist that NANOGENOTOX should complement and support ongoing work at OECD. The good alignment on OECD WPMN, ISO and other ongoing projects is a good point. Thus NANOGENOTOX can play an important role towards the validation of the test methodologies and help to develop a harmonized testing policy/guidance. However, in order to be used by the industries the developed method will have to be validated and recognised at the international level (i.e. OECD).

All interviewees asked for a rapid dissemination of the results when available (raw data and summarized results), in order to provoke discussions on the topics during scientific conferences. This communication has to be conducted with a maximum of transparency. They insist on how the method will be taken up by laboratories and how it will be translated into guidelines.

They recognise that an important aim of this action is to link *in vitro* and *in vivo* methods as well as to try to find an alternative method to the use of animals.

Manufacturers wonder if with 3 groups of MNs a generalisation is possible and they think that a larger catalogue of material would have been interesting.

Life cycle analysis is an important point for manufacturers. They are concerned by the difficulties to make distinction between the contribution of man made and naturally occurring nanoparticles in the toxicity assessment.

Non Governmental Organisations:

NGO representatives are concerned about how the results will be used for the assessment of the safety of the final consumer product: there is a need for better knowledge on the presence of nanoparticles in the final products. The development of a harmonized approach can help the safety assessment. It will be important to link the developed methodology with other methodologies in order to have a harmonized approach and also to reach a wider agreement from people outside of the project. NGOs also wanted to know if a complete characterisation of the products will be conducted or if the JA will use the characteristics given by the manufacturers.



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The general public is an important target for such a project and effort should be made for results communication in a simple manner.

Some were very concerned by the experimental difficulties that will be encountered with the nanomaterials compared to classical chemicals; they highlighted the need to develop inside the JA a harmonized data recording system. They consider that the JA helps in harmonising experimental procedures across Europe and thus improve standards of research on genotoxicity.

They insist on the policy use of the results of the action and the need for on time / early regulation considering hazard signals already existing.

Trade unions:

Data on workers exposure are very poor for the moment and it is recommended to make more efforts for the measurement of workers exposure and for the protection of workers in workplaces. EU legislation is also weak both on reprotoxic issues and on nanomaterials and the impact of NANOGENOTOX on the activities of ECHA would also be useful.

They added that it is interesting to obtain validated data not only produced by the manufacturers but also by public institutes. There is strong concern on how the *in vitro* and *in vivo* testing approaches and generated data relate to human exposure and risk.

Regarding the practical issues related to risk assessment for consumers and general population, as mentioned by NGO representatives, they wondered about the relevance of a method dedicated to genotoxicity with regard to MNs with risk assessment of products containing MNs. For the moment, the Joint Action is focused on the substances, not on the final consumer products. With this issue in mind, they wanted to bring to the attention of the Joint Action at the future needs for linking the outcome of the work performed in NANOGENOTOX to final consumer product. Therefore, as it is also important to get a method which is able to compare or make a link with the final consumer product at different steps of the life cycle of the product.

Regarding the dissemination plan of the results of the JA, it is recommended that the general public should be reached even it might be a challenging issue.



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## 4. FOLLOW UP OF THE CONSULTATION PROCESS

This section intends to provide clarification and answers from JA partners about some questions raised by stakeholders during this first consultation phase.

**Regarding coordination and dissemination activities**

At the initiative of WP2, various initiatives were undertaken and will be further developed in order to ensure appropriate dissemination and consultation activities.

The website will be regularly updated in order to provide all information deemed necessary regarding implementation of the Joint Action. For instance, new items have been created concerning relations and links between JA partners and other EU projects (see the following link consortium), and CVs of the Work Package leaders will be available. Dispersion Protocol for MNs and exposure media that were adopted by the partners will be available on the website, as well as other public reports after validation by the Steering Committee and the EAHC.

A mailing list has been set up in order to ensure proper dissemination of the first Newsletter, and next issues of this bi-annual newsletter will highlight recent development of the JA.

Concerning next phases of stakeholder consultation, it is planned to organize a workshop in April 2012 in Brussels in the remit of the General Assembly meeting, gathering stakeholder representatives and partners of the JA. This event will be an opportunity to present and discuss work progress and the way forward. Early 2013, during the final JA conference, it is also planned in coordination with WP3 (in charge of the evaluation process), to organize a specific session with stakeholders to prepare and discuss proposal for policy recommendations (see next point).

Ad hoc communication leaflets will be elaborated at the end of the JA in order to provide information accessible to various categories of stakeholders.

**Regarding evaluation and peer review process**

Under the leadership of WP3, an internal Evaluation Team has been set up, gathering one representative from each WP, with the aim of ensuring the follow up of the scientific quality of the work done and of the deliverables produced. In addition, an external



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academic reviewer panel will be created, composed of persons with proven expertise (publication in peer-reviewed scientific journals in nano-analytics and nanogenotoxicology). They will join the evaluation team after signing a confidentiality agreement. An Evaluation Plan was adopted by all the partners and the internal review process of the data delivered was initiated. At WP meetings, workshops, and at General Assemblies methodology is discussed within the consortium. The consortium involves members from laboratories with state-of-the-art bearing equipment and highly skilled personnel who are involved in other EU "nano-projects". The results will be presented in scientific journals with peer-review process. All the teams involved in the JA are planning to publish their results in international scientific journals.

The Evaluation Team will produce interim evaluation reports and a final evaluation report with policy recommendations. Coordination with the stakeholder consultation process is planned in order to discuss and prepare proposals for policy recommendations. The final project conference will include a specific session devoted to proposals for recommendations. These recommendations will be then integrated in the JA final publishable report.

**Regarding methodologies used within the JA**

The web-site was updated in order to take into consideration some questions raised by the stakeholders consulted and FAQs were created. Some of these questions are reported in Annexe 3.



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# *Annex 1: List of interviewees*

| Categories | Organisation | Name | Position | Response* |
|---|---|---|---|---|
| EU risk assessors and policy makers | European Food Safety Authority - EFSA | D. Carlander | Scientific officer,<br>Scientific Committee and Advisory Forum Unit | PI |
| | Health Protection Agency UK - HPA | K. Rothkamm | Head, Cytogenetics & Biomarkers Group | WA |
| | European Agency for Safety and Health at Work<br>EU-OSHA | E. Brun | Project officer | PI |
| | EU environmental health action plan coordinator<br>DG SANCO | J. Gallo | Unit Health Determinants | PI |
| Scientific Community | NanoImpactNet | M. Riediker | Coordinator, NanoImpactNet | WA |
| | European centre for validation of alternative methods ECVAM | J. Kreysa | Head of unit, in vitro method | WA |
| | World Health Organisation – WHO<br>Regional Office for Europe | M. Martuzzi | European Centre for Environment and Health (Rome Office) | PI |
| | International Union of Pure and Applied Chemistry IUPAC | J. H. Duffus | Chairman subcommittee on toxicology and risk assessment | WA |
| Professional federations representing companies | Nanotechnology Industries Association - NIA | S. Friedrichs | Director General | WA |
| | European Chemical Industry Council - CEFIC | S. Gallet<br>J. Holmqvist | Long Range Research Initiative Programme Manager;<br>Issue manager for nanomaterials | PI |
| | EU food and drink industries - CIAA | B. Kettlitz | Director Food Policy, Science and R&D | PI |
| | European Centre for Ecotoxicity and Toxicology of chemicals - ECETOC | E. M. Donner<br>M. Schulz | Senior Research Toxicologist, Genetic Toxicology at DuPont<br>Head of Laboratory for genotoxicity at BASF | WA<br>WA |



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| Categories | Organisation | Name | Position | Response* |
|---|---|---|---|---|
| NGOs | European consumer voice in standardisation<br><br>ANEC | C. Giovannini | Research and Innovation Manager | PI |
| | The Center for International Environmental Law - CIEL | D. Azoulay | Managing Attorney | PI |
| | Friend of the Earth (Germany) - BUND | H. Muhle | Working Group on Environmental Chemicals / Toxicology | WA |
| | The European Environmental Bureau - EEB | L. Duprez | Policy Officer | PI |
| | The International Council on Animal Protection in OECD Programmes - ICAPO | G. Buckland | Science Policy Officer – Research & Toxicology Department (Human Society International) | PI |
| Trade unions | European trade Union Institute - ETUI | A.P. del Castillo<br><br>L. Vogel | Researcher in nanotechnologies<br><br>Director of health and safety Dpt. | PI |
| | International Union of Food, Agricultural, Hotel, Restaurant, Catering, Tobacco and Allied Workers' Associations - IUF | P. Rossman | Communications Director | WA |

* Response: PI: phone interview, WA: written answer

Individuals from the following organisations were contacted but did not answer: European Chemicals Agency (ECHA), European Cosmetics association (COLIPA), Health and Environment Alliance (HEAL), European Federation of Building and Woodworkers (EFBH), European Mine, Chemical and Energy Workers' Federation (EMCEF). The EU parliament Committee on the environment, public health, food safety (ENVI) and the Food and Agriculture Organisation (FAO), answered that they were not in a position to participate in this consultation apparently focused on methodological and scientific issues.

The approach for conducting the consultation as well as the questionnaire were developed with the help of Myriam Ricaud and Eric Drais (WP2 partners, INRS) and Brice Laurent (Sociologist, Mines ParisTech, France). The questionnaire was "tested" with the participation of Pierre-Yves Montéleon (Occupational Health Manager, CFTC-Trade Union, France), Francelyne Marano (Professor, cytophysiology and cellular toxicology, Paris 7 University, France) and Alain Kaufmann (Sociologist, Lausanne University, Switzerland).



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## *Annex 2: Questionnaire*

# Questionnaire for stakeholder consultation

| | |
|---|---|
| **Respondent profile** | |
| Name: | |
| Position: | |
| Organization's name: | |
| E-mail: | |
| Country: | |
| **Interview** | |
| Date | |
| Person conducting the interview | |
| Interview | By phone ☐<br>Face to face ☐<br>Web conference ☐<br>Written contribution ☐ |

1. What are your reactions with regard to the NANOGENOTOX Joint Action? Do you have any specific questions or observations regarding this initiative (aim, objectives, partners, methodology, etc. )?

2. What are your expectations regarding the outcome of this Joint Action?



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3. What do you think is the contribution that the JA can make towards gaining a better understanding of the potential toxicity of nanomaterials?

4. Do you have particular expectations and/or suggestions regarding the consultation/dissemination strategy of the Joint Action?

5. Which activities related to nanotoxicology in general would you like to see reinforced, expanded or developed?

6. Other suggestions/comments



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# *Annex 3: FAQs*

- *Q1: The validation of the method must be done with a good choice of positive and negative controls ($TiO_2$, carbon black ...). The choice is difficult, data from exposed human or animal are poor.*
  A1: The vehicle used for the negative controls will include all the components used for dispersion except the NMs. For the positive controls, several genotoxic chemicals (depending on the test performed, for instance mitomycin C is used in the *in vitro* micronucleus assay) will be included which would anyway be done for the validation of the method. There are only few data available on possible nanoparticle positive controls in the different tests. Partners have chosen nanoparticle controls to be included in all experimental series, based on preliminary positive data available in some of the participating laboratories. ZnO will be used in the *in vitro* studies, and carbon black in the *in vivo* studies. The tests responding positively shall be identified with these candidate nanomaterials.

- *Q2: Why WP5 and WP7 are separated? Why the in vitro and in vivo tests are not carried out in parallel?*
  A2: Tests are not conducted in parallel because most of the partners are involved in two or three WP (WP5, WP6 and WP7) and it is difficult to perform the assays concomitantly. Moreover, WP6 will not investigate all the CNTs tested in WP5 and the results from WP5 and WP7 will help in selecting 4 out of the 7 CNTs for investigation in WP6.

- *Q3: Why only pulmonary, digestive and cutaneous cell lines are used? We know that the liver and the brain can be a good target.*
  *A3:* Given the limited budget, the JA had to concentrate on primary target tissues of MN exposure by inhalation, oral, and skin routes. The liver and the kidneys will eventually be exposed at least in oral exposure and the brain at least by inhalation. However, MN doses in these organs will be lower than in the organs selected in the JA, and a sound study would probably require a long-term exposure to reach toxicologically adequate organ doses. Developing genotoxicity assays for the secondary target organs would be a great challenge requiring basic research and cannot be the scope of a Joint Action (but rather of research projects).

- *Q4: The JA should establish a harmonized data recording format early in the project. The data base should be accessible during and after the JA.*
  Harmonized data recording is progressively put in place according to progress of work undertaken by the different work-packages. The Steering Committee is exploring different tools for future comparison of data sets and their accessibility at the end of the JA (e.g NANOhub developed by JRC).

- *Q5: In vitro method for genotoxicity can only be used to evaluate a hazard and not a risk (which includes the knowledge of a dose-response curve).*
  A5 : Due to many uncertainties resulting from methodological and ethical reasons, extrapolation from *in vitro* to *in vivo* target organ dose is difficult. This project does only qualitative comparison of the hazards detected *in vitro* versus *in vivo*. However, at the level of chemicals safety testing, *in vitro* methods are routinely



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used to identify the genotoxic hazard. A genotoxic concern may trigger further investigations.

- *Q6: It could be interesting to investigate the effects of MNs via ROS because inflammatory reactions play a substantial role in genotoxic effects.*
  A6: Identification of oxidative DNA damage is included in the comet assay protocol of some participating laboratories, thus new data will be available on this question, too. Although ROS production and inflammation are considered potential mechanisms for MN genotoxicity, they are not the only potential mechanisms. NANOGENOTOX wanted to assess techniques that could reveal genotoxicity due to various different mechanisms. The association between inflammation and genotoxicity still require a lot of basic research which is not a task of Joint Actions.

- *Q7: Some of the assay (lymphocytes micronucleus assay) provide indirect information about the potential toxicity of MNs.*
  A7: The lymphocyte micronucleus assay was included because it has very much been used in regulatory testing and it is interesting to see how these cells perform in comparison with the cell lines used. Lymphocytes may not be very efficient in taking up MNs as compared with the other cell types used, but the smallest MNs are certainly expected to be internalized by lymphocytes too. Moreover, effects can also be mediated through action on the cellular membrane. It is a general question, concerning also other cell types, if much of the effects of MNs are actually indirect.

- *Q8: Comet assay might not be relevant for MNs*.
  A8: NANOGENOTOX offers an excellent possibility to compare the outcome of the comet assay and the micronucleus assay in a number of cellular systems and tissues.

- *Q9: Is the classical micronucleus test (MNT) in vivo in bone marrow a suitable test method?*
  A9: Up to date, very few studies exist on this assay with MNs. Surprisingly, the *in vivo* MNT assay has given positive results with some MNs, suggesting possible systemic effects. Therefore, it is worth the effort to see how this assay compares with genotoxicity in target organs. NANOGENOTOX will provide new *in vivo* data which will shed more light on this question.

- Q10: *What are the definition of nanomaterial and Manufactured nanomaterial (MN) used in the JA.*
  A10: Nanomaterial : material with any external dimension in the nanoscale or having internal or surface structure in the nanoscale. Note: Generic term covering both nano-object and nanostructured material. (ISO/TS 80004-1: 2010 ).
  Manufactured nanomaterial (MN): nanomaterial intentionally produced to have specific properties or composition (ISO/TS 80004-1: 2010 ).



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