Executive Summary:

NANOIMPACTNET has been a successful and productive multidisciplinary European network on the health and environmental impact of nanomaterials. The project, that ran from 2008 to 2012 brought together scientists from different disciplines as well as facilitated two-way communication to ensure efficient dissemination of information to stakeholders and the European Commission, while at the same time obtaining input from the stakeholders about their needs and concerns relating to the health and safety implications of nanomaterials. In addition to the 24 leading European research groups behind NANOIMPACTNET, the larger network who had signed up to receive information consisted of over 3500 people active in the field of nanosafety. NANOIMPACTNET has organized 4 international conferences, 19 workshops and 8 training schools. In addition to the many reports and articles published in the grey and peer-reviewed literature generated as part of the NANOIMPACTNET activities, the project has also collected research protocols from the various FP6 and FP7 projects, ensured the accessibility of common, agreed terminology for nanotechnologies (in collaboration with ISO) and NANOIMPACTNET members have participated in the NanoSafety Cluster and the US-EU workshops and emerging Communities of Research at the request of the EU.

Since NANOIMPACTNET has come to an end part of the activities are now being continued within the QNano research infrastructure project and via the NanoSafety Cluster. It is recommended that the EU will ensure that the connections forged over the past 4 years will be durable, not only within the nanosafety and risk area but also towards the material scientists and (end) users of nanomaterials. This will be needed as long as major uncertainties exist on the health and safety aspects of nanomaterials and until scientists fully understand what are the key factors that may cause adverse health and environmental problems and how developers of nanomaterials can benefit from this knowledge to design safer products.

Project Context and Objectives:

Summary description of the project context and the main objectives

1. Status of the field before NANOIMPACTNET started

At the start of the NANOIMPACTNET, several national and European projects aimed at investigating the risks associated with nanomaterials were already running. However, many of these programs were running in isolation, and experiences and findings that could benefit the research community at large were not being effectively shared. There was insufficient cross-talk between these initiatives at the scientific level. That made it difficult for European researchers and stakeholders (industries, public interest groups, and policy makers) to access the knowledge created by these projects. There was a clear need for a unified platform where all of this information is collected, compiled and prepared for the direct use by all interested groups.

An additional complicating factor is the tendency in science to only publish so-called positive outcomes or striking results, which in the case of nanotoxicology, implies results where nanomaterials induce a toxic response upon interaction with living organisms. A more balanced view on the hazards of nanomaterials could lead to a shift in thinking away from nanotoxicology to nanosafety or indeed to nanointeractions, encompassing interactions that may have a beneficial outcome (e.g. nanomedicine) as well as those that may not (e.g. nanosafety). Starting a project like NANOIMPACTNET was very timely and provided an excellent opportunity to display both the positive and negative findings from all sorts of toxicological studies ranging from the molecular sciences to ecology, and in particular to integrate the data from nanomedical research. As a consequence of these discussions, leading journal in the field such as "Nanotoxicology" and "Particle and Fibre Toxicology" now accept also studies reporting negative findings.

Another factor that contributed to the confusion and debate about the safety of nanotechnology was the lack of agreement on standards for experimental and measurement work. This makes comparison of results obtained by different groups almost impossible, and hampers progress. Yet there were no platforms available to discuss and to share detailed protocols used in both exposure and hazard assessment. In addition, exchange of the lessons learned during experiments and guidance on for example control for impurities on manufactured nanomaterials was absent. It was therefore important to establish a common methodology and approach to determine the impact of nanomaterials on cells and organisms, and to assess human exposure. In addition it was time to develop standardized protocols to determine the fate and behavior of nanomaterials in environmental compartments and to assess the hazard for a range of organisms and ecosystems. The development of standardized guidelines and protocols was urgently needed to ensure that the research performed in the individual groups (whether funded by other EU programs or nationally) would be of the highest quality and to ensure that the results from different programs and studies will be comparable thanks to harmonized standards and internal checks.

There was also no European-wide nor world-wide consensus on the relevant nanomaterial characterisation metrics, the methods to determine exposure, the likely mechanisms by which nanomaterials can affect cellular functioning, whether the toxicology tests were sufficient to detect the subtleties of nanomaterial-induced cellular damage, or how risk / impact

assessment methodologies needed to be adapted to appropriately address nano-scale related issues.

Many industries assess the risks associated with their products, e.g. the medical device, food, pharmaceutical, cosmetics and chemical industries. It was reasonable to assume that those industries that were developing (and continue to do so) nanotechnology-enabled products are conducting safety evaluations, despite the fact that current regulations do not oblige them to do so for materials of equivalent chemical composition to ones that are already approved in bulk-scale. However, these data generally do not get published in the scientific literature, and thus are not available to the wider scientific community. However, it would be very helpful if information about nanomaterials that industry has found to be safe or "unsafe" were made more widely available to the scientific community.

There was no platform that would bring together leading European groups in the arena of nanosafety, nanorisk assessment and nanotoxicology. As a result there were no clear opportunities to reach consensus in many of these unsolved issues.

2. The aims and objectives of NANOIMPACTNET

The objective of the NANOIMPACTNET co-ordination action was to create a widely supported scientific basis to ensure the safe and responsible development of engineered nanoparticles and nanotechnology-based materials and products, and to support the definition of regulatory measures and implementation of legislation in Europe. This framework included a strong two-way communication to ensure efficient dissemination of information to the various stakeholder groups (notably the European Commission, industry and SMEs, and the general public) while at the same time obtaining input from these groups about their needs and questions.

By combining excellence in research with open communication, NANOIMPACTNET aimed to contribute in the following domains:

- Develop a framework for the (intermediate and final) critical evaluation of methods, protocols and results of research supported by the FP5, and FP6 and FP7 programmes, as well as national funding agencies.

- Guide the development of best practice to ensure that studies are comparable in terms of basic parameters such as particle type, cell types, dispersion and characterisation protocols and appropriate testing methodologies.

- Enhance scientific output through better cross-talk and coordination between European scientists and research projects, identifying knowledge gaps and research strategies to address them.

- Develop strategies to improve access of researchers to the health data that presumably exists within industry.

- Provide active communication of the findings of the researchers to and with major stakeholders, including policy makers, industries and SMEs, and the public.

- Draw policy-relevant recommendations from the activities mentioned, especially in terms of the occupational and public health and environmental impact potentially associated with the findings of the studies.

In consequence, NANOIMPACTNET put in place a series of networking and collaboration-stimulating actions to address the above-mentioned bottlenecks, which were seen as hampering the development of a framework for assessing safety, health and environmental issues associated with nanomaterials.

Project Results:

Description of the main S & T results/foregrounds

NANOIMPACTNET advanced the field in the domains of exposure, hazards and life-cycle analysis, by contributing to the development of a framework for the critical evaluation of data generated in the field.

1. Exposure

In the traditional risk framework, risk management decisions concerning occupational safety and health rely on site-specific risk assessment and information about the effectiveness of available measures to mitigate exposure. In its turn, risk assessment builds on hazard and exposure assessments (Murashov et al., 2009). Although there is mounting evidence that some manufactured nanomaterials may impose a health hazard to humans the target organs and endpoints, the specific dose-response relationships are not clearly delineated. In view of the uncertainty regarding the hazard of manufactured nanomaterials the assessment and control of the potential exposure of workers become crucial in occupational health and safety in order to minimize the risk of the workers.

The current method of assessing worker exposure to airborne particles in the workplace involves measurement of the mass concentration of health-related fractions of particles in the worker's breathing zone. The main exceptions to this methodology are particle-number-based metrics for exposure for fibres and for microorganisms (ISO/TR 12885, 2008; ISO 13794, 1999). However nanoparticles carry only very small masses and therefore generally contribute negligibly to the integral mass concentration of the inhalable or respirable dust fraction. Furthermore, there is evidence that other metrics such as particle number concentration or surface area may be better descriptors for the biological effects of nanoparticles. The issue of exposure metrics has extensively been addressed by Maynard and Aitken (2007). Reflecting the state of the art they conclude that effective approaches to measuring exposure to a wide range of manufactured nanomaterials/nano-objects will require methods for measuring aerosol number, surface area and mass concentration.

The most widespread method that evolved to determine airborne submicron particle number concentrations as a function of particle size, i.e., particle number size distributions, is based on electrical mobility analysis of the particles (Asbach et al, 2009). These techniques usually comprise three main components: (1) a particle charger to predictably charge particles depending on their size; (2) a mobility analyzer which classifies the particles of one polarity according to their electrical mobility; and (3) a particle counter that determines the number concentration of the mobility-classified particles. These three techniques are usually employed in what has become the workhorse for occupational exposure measurements the Scanning Mobility Particle Sizer (SMPS). ISO/TR 27628 (2007) further describes the available methods to measure the above mentioned metrics of nano-objects.

However, for the SMPS no standard method has been agreed on to produce reference particle number concentration (Asbach, 2009). Furthermore these instruments are bulky, expensive and complicated to use and therefore usually only operated by research groups and not by practitioners in SMEs for example. The SMPS also gives no information on the chemical identity of the counted particles.

The latter is also true for the available small portable devices like condensation particle counter (CPC) or surface area monitors. Though they give no information of the size distribution they can be used for assessing sources of nano-objects or the effectiveness of control measures. The state of the art method to get information about size and shape as well as chemical identity and state of agglomeration is the subsequent analyse by electron microscopy of a taken sample. But no standards for sampling or analyse exist yet.

One major finding of most current studies is that during production and handling of nanoparticles the workplace particle number concentration of particles below 100 nm is close to the background concentration in companies. This background aerosol consists of ubiquitous ambient particles and of ultrafine particles from sources in or outside a company e.g. particles emitted from diesel engines by trucks or forklifts, welding fumes or even vacuum cleaners with electric motors close to the process. During the event of a leakage at the bin filling station in the production line of nano titanium dioxide up to 130 000 particles /cm³ have been encountered. The chemical identity of the nanoparticles was verified by transmission electron microscopy (M. Berges, personal communication). This was one rare occasion that primary particles have been encountered at the workplace. The common finding is that aggregates or agglomerates above 100 nm in size were quite often detected at the workplace and correlated with the operations. This is in line with theoretical calculations indicating that most of the particles emitted from processes are agglomerated when reaching the exposed person (see outcome from EU Nanotransport project in Seipenbusch et al (2008).

This adds to the problem of background distinction what can be called a Trojan horse effect (Fasano 1998). Possibly emitted nano-objects may be loosely attached to bigger agglomerates and therefore may not be detected by the measurement devices in the size range below 100 nm. However, as this agglomerates are still respirable they reach may upon inhalation the alveoli in the lung where they may me released in their primary size range after contact with lung surfactant for example.

In summing up the lack of analytical methods particular to distinguish between engineered nano-objects and so called background particles has to be compensated as far as possible by the development different measurement strategies depending on the purpose of workplace measurements. This also includes the development of harmonizes approaches for collecting contextual information at the workplace and reporting of them and the measurement data.

2. Hazard

When NANOIMPACTNET started, a first wave of publications emerged that reported on cellular effects of nanomaterials. However, those early publications lacked comparable cell lines, testing organisms and media, handling protocols and experimental protocols so that data from different groups were poorly comparable and often seemingly in conflict, which contributed to the considerable confusion in the field. It was (and still is) difficult and expensive to measure every single physico-chemical property of a nanomaterial, especially as many of the properties are context dependent and change as the surrounding (solution) conditions change. Very few laboratories or small companies had access to all the different techniques required. Even worse, there was no agreement (neither in Europe nor elsewhere) on a strategy to harmonize and standardize experimental approaches. One important

recognized need was to determine the minimum number of metrics needed to characterise nanoparticles in physiologically relevant media (e.g. cell culture media, plasma, lung surfactants etc.) and how this can be mapped onto the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) models. In order for the considerable amount of research that was already being funded in nanorisk and safety to produce a meaningful body of data, there was a need to standardize the nanomaterials and choices of controls used by the researchers within the network: same source, same batch, and also same nanomaterials from different sources to determine differences between chemically identical nanomaterials manufactured by different suppliers. Similar procedures were needed for the dispersion procedures prior to introduction to cells, and methods whereby the nanomaterials are introduced to the cells, as each affects the nature of the nanomaterials finally presented to cells. Thus, NANOIMPACTNET focused in the beginning strongly on these harmonisation issues and contributed reports, some of which were published in the form of peer-review articles regarding human hazard assessment (Bouwmeester et al. 2011) and environmental characterization (Stone et al. 2010).

Large numbers of publications are now emerging in the literature assessing the hazards of nanomaterials in cells and animals. However, it is becoming increasingly apparent that nanomaterials can interfere with test methods, leading to false positives or negatives, and inconclusive results. Approaches need to be established and validated that are adapted to nanomaterials. Thus, NANOIMPACTNET also led many discussions about best practices and contributed several reports, some published in the scientific literature (Handy et. al. 2012).

A limitation on determining the health and safety of nanomaterials is the lack of information about the health of already exposed and thus potentially affected populations. There was (and still is) no European system to register occupational health related to nanomaterial exposure. In consequence, Occupational Health reporting strategies were discussed at several occasions, the ethical, legal and social limitations of such reporting were considered, and a report and journal article were prepared (Gibson et al. 2010, Riediker et al,. 2012).

When NANOIMPACTNET started in 2008 the environmental health and safety assessment of nanotechnology was still at its early stages. Several modeling studies for nanomaterials were already available in the scientific literature. Some of these studies evaluated the release of nanomaterials to the environment from some selected nanomaterial-containing products during the consumption phase (Boxall et al., 2007; Blaser et al., 2008). Another study used a life cycle based modeling and included the whole life cycle of products that contain certain nanomaterials (see below) (Mueller and Nowack, 2008). This study provided the first comprehensive assessment of the potential concentrations and the associated environmental risks of nano-titanium dioxide (TiO2), nano-silver (Ag) and carbon nanotubes (CNTs). These studies estimated concentration levels of selected nanomaterials in different systems (aquatic, terrestrial, wasterwater systems) based on available knowledge of total usage of products containing nanomaterials. The estimates and approaches carried out were rather tentative but they were a great attempt at starting the whole process of estimating exposure. Since that time the whole area has progressed immensely and this was due to the work of NANOIMPACTNET scientists involved in the early stages and acting as catalysts in this process. At the start of NANOIMPACTNET, assessment of the environmental hazards of engineered or manufactured NM was in its infancy. The knowledge on environmental hazards of NM on aquatic organisms was more advanced, although many gaps still existed (Klaine et al 2008). Most work was focused on a few freshwater species (mostly daphnids, some fish

species and single celled pelagic algae) and some microbial systems. Since that time the work has expanded to cover most phyla, although with variable incidence, and a wider range of endpoints. Very few papers existed at the time using terrestrial organisms as test species, and this was particularly acute in what concerned plants. Discussions on the importance of characterisation and the properties of NM in environmental studies were also in very early stages. Environmental scientists were learning rapidly from human toxicologists and understanding the importance and complexities of particulate exposure studies. When this work started to develop further it became clear that addressing exposure characterisation in the soil matrix was not going to be a simple matter. In fact those methods are starting to be developed just now. This early stage of the field, in the areas of exposure assessment, predictive modeling, characterisation and effects assessment were acknowledged at the NANOIMPACTNET-workshops and considerable progress was made since then, led by NANOIMPACTNET scientists.

3. Life cycle analysis

The past two decades represent exponential increases in the applications of nanotechnology and bursts of nanotoxicology research with the widespread manufacture and use of NM. Many knowledge gaps with respect to health and environmental impact assessment of NM were identified that need to be filled. In parallel with industry rapid drive to take advantage of the new opportunities offered by nanotechnologies, it is imperative that these developments have to take place in a safe and sustainable manner. The increasing use of NM in consumer products has raised certain concerns over their safety to human health and the environment. There were (and still are) a number of major uncertainties and knowledge gaps with regards to the behavior of NM in the environment, their toxicological properties and potential exposure scenarios. To close these knowledge gaps we will need to generate of new basic knowledge, but it is unlikely that these uncertainties will be resolved in the immediate future.

These knowledge gaps are not only of academic interest but have also implications for industries, investors and policy makers. Though existing regulatory frameworks in principle cover all important aspects of production and products, NM often display different chemical, physical, and biological characteristics than the bulk form of the same substance, highlighting the need for specific nano-policies (Baun and Hansen, 2008). At the time when NANOIMPACTNET started, there were no clear regulatory guidelines specific to NM for testing medicines, cosmetics, food additives or other consumer products. The regulations and guidelines were the same as those for other products. However, there was a concern that any novel properties of NM that undermine basic assumptions in biological responses could result in a hazard being missed, or overestimation of hazard e.g. in a medicine that is actually safe (false positive).

An approach proposed at that time was to take the complete life cycle of nanoproducts into consideration when assessing the risks of NM. This systematic investigation of the product life cycle stages (i.e. production, transport, use, disposal/recycling) would provide a holistic perspective on risks and on opportunities of nanoproducts (e.g. less energy consumption, less hazardous by-products compared to conventional materials). In this way, less formalized and rather qualitative life cycle concepts may uncover prospective knowledge and knowledge gaps for human and environmental exposure to NM throughout the life cycle of nanoproducts

and reveal systematically other emerging risks such as cross-product contamination. Last but not least, life cycle assessment (LCA) and in particular life cycle impact assessment (LCIA) both of which are formalized concepts for evaluating product and materials life cycles - may be used to e.g. assess the relative environmental performance (e.g. material and energy consumption) of nanoproducts in comparison with their conventional equivalents. The complementary use of these different life cycle concepts with the current knowledge on risk assessment may provide a sound basis for informed decision making by the industry and regulators.

NANOIMPACTNET has played an important role in setting the scene for the application of life-cycle concepts in the area of NM by organizing two events devoted to this issue.

4. The framework for critical assessment of data

The beneficiaries of the NANOIMPACTNET consortium (= the NANOIMPACTNET core-team) were committed to apply their individual expertise to the generation of European critical mass, and a cohesive and coherent European approach to the development of a nanomaterial risk and safety assessment framework, based on the highest quality science, the development of consensus approaches, and the development and implementation of standard protocols for all stages. The NANOIMPACTNET co-ordination action has facilitated interactions and cooperative working practices amongst these key players on a European level (= core-team + members) to create a multidisciplinary network that includes scientists and stakeholders from all European countries.

NANOIMPACTNET created a range of consensus reports on best practice in nanotoxicology, nanoecotoxicology, health and exposure assessment, environmental dispersion, and impact assessment. These include a set of standardized nanomaterials and dispersion guidelines, which ensure that (a) the quality of the nano-safety data generated in Europe is the highest possible, and (b) the results from the various National and EU projects currently funded and to be funded in future rounds of FP7 and other programs are comparable, meaningful and relevant. The protocols, consensus reports, data compilations and other outputs have been centralised into a website and at the end of this project will be handed over to QNano. In addition, NANOIMPACTNET has succeeded in generating a generalised framework to interpret the findings from all of the ongoing and future studies on nanomaterial impacts on health and the environment in a policy-relevant way. This has been achieved by linking findings from exposure assessment and toxicology to risk and health impact assessment.

NANOIMPACTNET was a coordination and networking action, not a research project - as such it contributed by:

- Facilitating collaboration between projects including support for the NanoSafety Cluster and the preparation of the Compendium

- Communicating results to stakeholders and their needs back to researchers

- Helping to implement the EU's Action Plan for Nanotechnology

Looking back on the four years of the project's lifetime a cautionary remark appears to be appropriate. While the value of building a community and getting people together cannot be overestimated to progress the field, going beyond the exchange of information into harmonisation of methods and regulatory issues proved to be difficult. Every stakeholder acts within its framework of own interests and a slightly different legal playground that may effectively limit any harmonisation in a networking activity. This may be highlighted by the long discussions between stakeholders and the EU Commission on the "simple" task of defining a "nanomaterial". In addition regulatory bodies set up their own projects independently to get experts together, e.g. RiP-ON, NanoREG. Also other top-down initiated networking activities were drawing resources from the community, and approaches to centralise on-going activities are still needed. The critical framework for data assessment provided via NANOIMPACTNET is further described in the final integrative report of NANOIMPACTNET (D5.1).

5. Nanomaterial characterisation and hazard assessment (toxicity and ecotoxicity)

It is clear that knowledge in the area of environmental health and safety of NM has come a long way since NANOIMPACTNET started. Members of WP1 and WP2, scientists at the bench, working with industry, regulators and other stakeholders, identified priority areas for research and worked on them during the implementation of NANOIMPACTNET. It is clear that much has been achieved by NANOIMPACTNET partners, with members of WP1 and WP2 being active contributors.

5.1 Nanomaterial hazard for humans

An important first statement is that while there remains considerable uncertainty in the literature regarding the impacts and potential impacts of nanomaterials, at the end of the four years of NANOIMPACTNET we can conclude that a nanomaterial cannot be declared as toxic or non-toxic simply because it is a material at the nanoscale. The acute toxicity seems to be influenced strongly by the chemical composition of its surface; but also factors such as scale, shape, overall composition and complex ageing processes of the material in the environment and body seem to influence the toxicity. Furthermore, attention in now shifting to the need for appropriate in vitro and other methods to study effects with longer latency or from longer term exposure to nanomaterials, and to issues such as the potential for bioaccumulation, biodegradation in situ and subtle changes in cellular signaling as a result of exposure to nanomaterials.

A very key question that remains to be resolved, and which explains much of the continued uncertainty in the scientific nanosafety literature, is the need to address issues around nanomaterial batch-to-batch variability, and to couple this with a full understanding of the role of the synthesis route on the surface composition of nominally identical nanomaterials. For example, silica nanoparticles can be prepared by at least five different routes, utilising different catalysts etc., with consequence for subtle differences in the surface composition. Currently, researchers (and industry) do not distinguish between these as potentially different nanomaterials, with potentially different uptake and impacts (as a result of their slightly different surface compositions), and as such it is difficult to see how the data in the literature

can converge. Thus, a naming system for nanomaterials that accounts for synthesis route and potentially for surface description, is needed to allow identification of differences in the base nanomaterials that could be correlated with differences in their dispersion characteristics in the exposure medium and thus differences in their uptake and consequently differences in the observed dose-response characteristics.

The role and importance of characterising nanomaterials not just in their pristine, as-synthesised state, but also as they exist in situ in complex biological matrices, such as cell culture media for in vitro studies, has become more fully appreciated, but unfortunately such characterisation is still missing from many published articles. This is in part due to the fact that consensus has not yet been reached as to the minimal characterisation requirements for nanomaterials (as powders, in simple dispersions and in situ as presented to living systems). The first NANOIMPACTNET workshop proposed that a pragmatic way forward might be to fully characterize the NM at production, and to then further explore a very limited set of parameters before use (or exposure), in order to take into account the effects of storage and sterilization and how different environments/conditions affect the physicochemical characteristics of NM (Bouwmeester, 2011).

Significant emphasis was placed on developing and disseminating best practice with regards to nanomaterials dispersion and characterisation and for in vitro and in vivo experimentation with nanomaterials. To develop robust procedures with a high degree of credibility it was recommended that cell lines which are readily available and traceable, i.e. same source and commercially available, should be used rather than specifically isolated / modified cell lines, and that cell line characterization is crucial and should be reported and included in any data for publication (Lynch, 2009). This recommendation has not been implemented in practice yet, and QNano will make a concerted effort to pick up this issue again and highlights its importance to the research community. The focus of this first training school was thus on protocols for handling nanomaterials and protocols for toxicology testing, such that the importance of ensuring a controlled dose (understanding of aggregation of nanoparticles in the presence of biological fluids), controlled presentation of nanoparticles to the test system, and development of appropriate testing strategies taking into account the novel aspects of nanomaterials which can influence the testing, were passed onto younger researchers.

The next workshop specifically addressed aspects where nanomaterials themselves could interfere with the test assays themselves, and the establishment of appropriate controls for such interference from the presence of nanomaterials. The conclusions of the meeting were that the novel characteristics of nanomaterials are such that special consideration needs for potential artefacts from the presence of nanomaterials must be given to all experimental tests and protocols being adapted for use with nanoparticles (Byrne, 2010). As for all chemicals, it is essential that protocols be standardised and models validated. Relevant in vivo doses need to be accurately translated to ex vivo and in vitro doses. The lack of ENP standards as controls was identified as a severe limitation in this respect, and this remains the case to date, since the few reference nanomaterials that exist are certified size standards and the certification applied only to the dispersion medium in which they are shipped (typically water), making them of limited use for in situ characterisation and for biological studies.

The second WP1 training event addressed higher level issues of best practice for safe handling of nanomaterials to ensure that NANOIMPACTNET students and postdoctoral researchers working with nanomaterials were up-to-date with international best practice, and

that this was being implemented in their laboratories. It also began the process of ensuring that the next generation of researchers would be equipment to work easily and seamlessly with other stakeholders, such as regulatory authorities, and this address the topic 'closing the gap between research and regulation' by identifying the needs of the regulatory agencies and looking at developing strategies to ensure that NANOIMPACTNET research and outputs are designed to fulfill the needs of the regulatory community. This is clearly an issue of ongoing importance, especially in light of the EC definition of a nanomaterial for regulatory purposes (http://ec.europa.eu/environment/chemicals/nanotech/pdf/commission_recommendation.pdf), and the persistent lack of agreed and validated methods and approaches for assessing the safety of nanomaterials.

The third Training School in the NANOIMPACTNET series on "Handling protocols and toxicological testing strategies" focused on methods of ensuring reproducible presentation of nanoparticles to cells and on methods to quantify the uptake of nanoparticles into cells, taking account of issues such as nanomaterials dispersion in the exposure media (which affects the available dose of nanomaterials) and the potential for labels to leak out of nanoparticles under biological conditions, which can confound the interpretation of the uptake studies data. Practical approaches to ensure quantitatively reproducible and reliable studies were presented, and students had the opportunity to analyse real images and to count nanoparticles in cells.

The second main theme of WP1 was development of strategies to assess occupational exposure to nanomaterials, and that is covered in detail in a later section. However, as part of the integrating efforts, the last workshop from WP1 attempted to bridge the traditional gap between exposure studies and impact / hazard assessment studies, via a workshop to design an experimental strategy that would cover all aspects from release, through exposure through impact assessment, and identify strategies for simplification of experimental design whilst still capturing the major transformations undergone by nanomaterials during these steps. An important outcome from the workshop was that while there is an inherent temptation to recommend a release and exposure model and a toxicity model, this would result in continued separation of the concepts of exposure and hazard assessment, whereas what is needed is an approach that integrates these. Thus, it is vital triage approaches in a way that allows researchers to design experiments that mimic more realistically the fate of nanomaterials as they undergo their lifecycle from release, through exposure, to uptake and impact in biological systems (Lynch, 2012).

5.2 Nanomaterial hazards to the environment

Reflecting on the last four years it is clear that NANOIMPACTNET meetings were central to the international development of EHS of Nanotechnology. The multidisciplinarity of the partnership facilitated knowledge exchange in an area that requires information from so many fields (physics, chemistry, biology, and sub-disciplines). For example, nano-research in (soil) ecotoxicology is very complex, especially in relation to the characterisation of nanomaterials in soil/sediment and biological tissues. For this a multi-partner approach is essential. Even simple assays can generally not completely be addressed by a single partner. NANOIMPACTNET -meetings allowed the expansion of knowledge on methods and approaches to be used, but also on who may be able to carry out such approaches. NANOIMPACTNET was also an excellent platform to create and sustain the necessary

networks and contacts to address the issues mentioned above. This included updates on the latest developments, but also on a personal level being able to meet and discuss issues face-to-face. The workshops and training schools offered excellent opportunities for practical discussions in varied areas, including characterisation of nanomaterials, design and use of reference materials, design of (eco-)tox studies, validity of standard tests and generation of exposure scenarios.

For example, at the start of NANOIMPACTNET, assessment of risks of NM on soil organisms was in its infancy. The science on risks of nanoparticles on aquatic organisms was much more advanced, although many gaps still existed (Klaine et al 2008). In 2008, the methods for exposure characterisation in the soil matrix were totally non-existent. This was acknowledged at the NANOIMPACTNET -workshop held at September 2008 in Zurich, as described above. It was highlighted that in aqueous solutions some NM properties could be addressed, but in a more complex matrix, like soil or sediment, this was not possible. The paper resulted from this workshop (Stone et al 2010), clearly illustrated the need for development of characterisation methods for soil and other more complex matrices. In 2009, a specific NANOIMPACTNET -workshop was focused on the environmental fate/behavior of NM in the environment, and a large part of the meeting was devoted to the methods for characterisation of NMs. At the NANOIMPACTNET-workshop in 2010 in Dublin, this issue was raised again. This workshop was dedicated to obtain an overview of standard operating procedures for ecotoxicity testing on NM. This included a review on methods for nanoparticles characterisation in complex matrices, like soil (Handy et al 2012). At this workshop, it was clearly illustrated that much progress was made, techniques like SEM-EDX, x-ray spectroscopy using Synchotron radiation, but also SP-ICP-MS and F-FFF-ICP-MS. The development of these techniques took place during NANOIMPACTNET, by NANOIMPACTNET members (and others) and the work of NANOIMPACTNET helped to shape and direct that development. NANOIMPACTNET -meetings and workshop were excellent platforms to share this knowledge, and to scrutinise the applicability of the methods for the different types of matrices. The above mentioned NANOIMPACTNET -meetings boosted the urgency and need for development of characterisation methods and exposure assessment.

Results from NANOIMPACTNET's work have been described in refereed publications and resulted in direct information to advisory bodies, such as the OECD. NANOIMPACTNET has also resulted in several opportunities for consortium development for project proposals, at national and international levels (e.g. FP7 project MARINA).

This work is continuing, of course, with emerging areas being addressed. Key development areas are highlighted below.

From the view point of environmental protection it is recommended that general concerns for the environment should not be a barrier to the responsible innovation and development of new nanotechnology. The current strategies for the environmental hazard and risk assessment of new substances are generally fit for purpose with respect to nanomaterials. There are currently data gaps on environmental effects of nanomaterials. However, this would be the case with any new substance(s), and it is a matter of routine work to collect more data and the reiterative process of reducing uncertainties as more knowledge is obtained that will close these gaps. The knowledge gaps are accounted for in uncertainty factors in risk assessment, and the overall situation is reasonable but precautionary.

Regarding regulatory aspects there is a need to reconsider the appropriateness of current approaches and consider some of the technical modifications to hazard assessment (Malkiewicz et al 2011). There is a need to move forward now with firm action and implementation on how to manage the myriad of different nano structures and chemistries in the regulatory process. All parties (companies with nano products to register, the scientific community, and the regulatory experts) are in general agreement that the approach of treating every nanomaterial as a new substance is not logistically feasible (the regulatory agencies and test houses simply do not have the capacity to implement such an approach), would be far too expensive, and not scientifically desirable as new versions of materials will continue to be produced in the future. Instead, action is need on implementing a precise decision trees on how to manage groups of materials through the regulatory process based on shared physico-chemical properties and types of probable biological effects.

Funding should be directed at specific knowledge gaps that can be filled in order to reduce the uncertainty in hazard and risk assessments for both the environment and human health. Some of the critical knowledge gaps are caused by a lack of suitable analytical methods at the bench to collect the required data. One of the highest priorities must be funding to develop routine methods for the measurement of nanomaterials in tissues. For example, this information on tissue concentrations would be used to link cause and effect, used to calculate key parameters in environmental risk assessments like bioconcentration factors (see Handy et al., 2012 for concerns on these tests), or be needed for the pharmacokinetics that are an absolute requirement for the registrations of new medicines. These are potential bottle necks that can prevent a product being registered, and therefore prevent innovation. Solving some of these technical issues will unlock the economic potential of new materials and would therefore be money worth investing in research. The funding agencies must also accept that a bigger proportion than usual of this technical research on methodology will be high risk, and there must be a mechanism in the funding schemes to fund high risk (and potentially high reward) proposals that would not be funded in current grant schemes. Along similar arguments, methods for detection of nanomaterials in soils (current no viable methods) will be absolutely essential to enable national environmental monitoring schemes for contamination. Tissue and soil methods could also be used to monitor contamination in the human food chain (food safety agencies).

It is important that industry works in close collaboration with research in this field. This is taking place at present but it is important that the regulatory agencies continue to support and stimulate this collaboration. In this context it is important that information on uses, and releases in available so that emission and exposure scenarios can be adequately calculated.

The principle of protecting workers by preventing exposure (and therefore negligible risk) also applies to nanomaterials. Safe systems of work such as those exemplified to the Control of Substances Hazardous to Health (COSHH) seem appropriate for nanomaterials. The evidence so far is that routine protective measures such as rubber gloves, the use of dust masks suitable for ultrafine materials, and safety glasses also work for nanomaterials when modest volumes are used (such as in the research laboratory). So, safety managers should not be unduly concerned that strategies or normal procedures will not work. However, the information in the public domain used for these risk assessments needs updating for nanomaterials. The information on material safety data sheets (MSDS) should be specific to the material, and not just generic information for an existing chemical formula of the same substance. For example, a MSDS needs to be specific for carbon nanotubes, not for

carbonaceous materials in general. Many workers undergo annual health surveillance as part of management strategies. However, a mechanism needs to be in place so that the doctors conducting these assessments are kept up to date with adverse effects that are nano-specific. For example, the focus on lung function tests for dusts might distract attention away from other risks such as immunological health, or cancer risks.

It is important to obtain data on the leach rate or decay rate of nanoproducts (products containing nanomaterials) in realistic landfill conditions. We already know that some free nanomaterials can penetrate clay (typically used as a liner on landfill) and move into ground water, so the expectation is some long term slow release at the end of a products life. Of course, at this early stage, if product manufacturers can design for re-use and not recycling then perhaps the amount going to landfill can be reduced in the first place.

The current REACH guidelines do not have specific inclusions for nanomaterials. A pre-requisite to applying a nano-specific sub-set in the reach strategy will only be logical if a unique sub-set of nano-specific biological effects are also identified. So far the latter has been elusive with the toxic mechanisms for nanomaterials (e.g., genotoxicity, respiratory toxicity, inflammation from oxidative stress, etc.) also being well known for traditional chemicals. They may be uncertainties in some of the assumption behind calculations in REACH for nanomaterials, for example, not knowing if a release is linear over time or not in an exposure model. However, these sorts of problems apply to other chemicals, and they are not a new issue per se for nano.

6. Occupational exposure monitoring

6.1 Strategies to measure, analyze and share data regarding worker exposure to nanoparticles

The main techniques currently employed are either microscopic methods for information on particle morphology, state of aggregation and chemical composition, or methods discriminating particles by size in relevant media (water, air, etc.).). The latter methods sometimes allow subsequent separate analysis for chemical composition. Examples are an Aerosol Mass Spectrometer (Sullivan and Prather, 2005) or the Field Flow Fractionation technique coupled with a mass spectrometer for liquids. Both methods provide information on particles-size dependent chemistry.

The major obstacle in studying NM release, transformation and exposure is the identification of the particles themselves. Discrimination of particles by type (e.g. engineered vs natural vs process originated), is of importance when assessing exposures, and in subsequent for analyses that interface with health studies. This problem increases as the NM becomes ever more removed from the actual source (both in time and space). For examples, in a workplace environment specific nano-objects are expected and release / exposure can be targeted using specific search criteria and protocols, and hence it is possible to limit resources to parameters exactly fitting the purpose. Some strategies and techniques have been developed and tested in workplaces (reviewed in Kuhlbusch et al., 2011). However, severe limitations exist even for research purposes and the existing techniques cannot be employed in routine workplace measurements. In the other extreme case, the environment, it becomes very difficult to develop an appropriate and feasible method as nanomaterials may undergo modifications e.g. aging processes.

Another limitation is that currently there are very few measurement techniques that simulate aspiration efficiency and the deposition in the trachea-bronchial and alveolar regions resulting in a mismatch between the concentration measured, the concentration inhaled, and the estimate of the deposited dose. To obtain health related exposure information, modeling techniques have to be applied to the data. This lack of health related exposure data, among other factors, makes the establishment of occupational exposure limits difficult.

6.2 Release:

While NM release is a prerequisite of downstream exposure, little has been done so far to systematically approach this area. General processes and areas of possible release of nano-objects and nanomaterials are:

- Production

- Handling and use of nanomaterials

- Aging processes of nanomaterials

- End of Life (EoL) activities such as e.g. recycling, disposal

Possible release during production may occur through leaks to water and air in closed systems or open production processes. These have been studied in several European and national studies such as NANOSH, CarboSafe, nanoGEM. The other two areas handling and use and aging summarise a huge area. Handling and use covers e.g. handling of powders, diffuse emission from production plants, mechanical treatment of nanomaterials, e.g. sanding, drilling,abrasion during use, spraying of nano-sprays, while aging covers all processes taking place in the environment such as selective degradation, wash-out, increased brittleness of material.

EoL activities include activities related to:

1) re-use or recycling, such as disassembling, and mechanical or thermal processes like crushing, melting, torch cutting,

2) waste treatment, e.g. incineration, and

) disposal, e.g. landfill. Especially during high energy process release of nano-objects may not be excluded.

Research and development activities aimed at understanding processes relating to release of NM. This research and development activities are likely to increase in the near future since it allows a) detailed studies of processes, b) standardised testing for certain possibly relevant release mechanisms, c) international harmonisation, d) deriving quantitative information of possible release rates, and e) good characterisation of the physical chemical characteristics of the released material.

6.3 Transport and Transformation:

It is well accepted that nanomaterials may undergo various changes during subsequent processing activities and upon release, but also during environmental transport. Transformation processes taking place in air as well as in liquid environmental media. Examples of such changes include loss of coatings, change in coating composition, development of a corona which depends on the particle surface properties, and dissolution in liquid media. These changes are fast and current technologies are not sufficiently well-developed to provide rapid assessments in a coherent manner. Comprehensive research activities to develop some predictive models on how the material will interact with its surroundings, and how that may influence subsequent transport, accumulation and reactivity are on their way and we can expect a huge increase in the knowledge base relating to NM transport and transformation - including predictive modeling - to take place within the next 10 years.

6.4 Workplace Exposure:

The workplace is the best characterised exposure scenario due to the expected highest exposure probability and concentrations for humans. Still most of the studies conducted were mainly exposure related or even more release related. No specific personal exposure measurements at various workplaces have been conducted so far, leading to a robust exposure assessment via inhalation or oral uptake. Dermal exposure has virtually not been studied with the exception of Van Duuren-Stuurman et al. (2010) using a shortened version of the observational dermal exposure assessment (DREAM) to estimate the likelihood for exposure. However, the relevance of uptake via the intact skin has been demonstrated (Grosera et al., 2009). With exception of intend use of nanomaterials in food, possible oral uptake following inhalation exposure has not been studied yet.

All studies conducted so far were only related to short term exposures. No concept of exposure monitoring or long term exposure assessments have been conducted to the knowledge of the authors.

Another topic to be addressed and with mayor advances to be expected is related to the type of workplaces and work processes. The current workplace investigations focus on areas where nanomaterials are produced. Knowledge on use and processes with nanomaterials in the second or later stage can currently not be assessed due to lack of knowledge on their use. Labeling, which is also needed to identify possible exposure via consumer goods is one way to address this safety research topic.

The exposure related studies have shown mainly larger agglomerates and aggregates of nano-objects are released. Only a few studies clearly showed release and possible exposure to nano-objects that have at least one dimension below 100nm in size.

The next steps to pursue with regard to workplace exposure are the development and testing of personal devices delivering reliable results to reduction of nano-object exposure. Focus should be set to personal real time instruments that simulate uptake, e.g. deposition in the different areas of the respiratory tract. The development of realistic exposure scenarios is needed for comparative assessments of different tasks and processes. They should be based on an extensive data set on workplace exposure, generated in an as much as possible harmonized way (Brouwer et al., 2012). The included data should come along with auxiliary

contextual information that is required to interpret the measurement results for risk assessment and mediation purposes. The exposure scenarios are also needed to derive information on uptake for combined assessments of hazard and exposure potential.

6.5 Exposure via consumer products:

The use of a wide range of consumer product types may result in different exposure scenarios, e.g. personal care products, cleaning, coating products etc. So far, no real test scenarios of release and exposure to nano-objects exist. Current knowledge is limited to selected tests conducted for a few spray and cream formulations. In addition, we lack information on NP content in consumer's products despite the large use. Information on their use and application is needed to permit a better evaluation of possible exposure pathways. One of the main obstacles in studying consumer exposure is the reliable measurement of particles in the different matrices of consumer goods.

Strategies to overcome this limitation have to be developed. These may be based on testing of different release processes and realistic exposure scenarios for consumers to allow the use of nano-object specific measurement techniques and strategies.

During consumer usage, nanomaterials are subjected to mechanical, thermal and environmental stress situations. Studies based on the characterisation of airborne particles release due to individual processes can roughly be classified by the investigated nanomaterial used for coating and nanomaterials used for composites. Coatings could be understood being a thin layer of composite material, as the engineered nanoparticles are intentionally embedded in a matrix material. However, for exposure studies composites and coating cannot be compared and have to be analysed in different ways. The relatively long duration of the current aerosol measurement restricted the intensity of abrasion. This means that with higher abrasion intensity the coating would be worn off before the measurement finishes. Therefore just a limited simulation of exposure is possible. To assess the real potential impact of nanomaterial on the environment and the human health, it is therefore necessary to characterize, with feasible techniques, the particles once released into the environment.

6.6 Exposure via the environment:

Environmental exposure to NM represents potentially the most widespread mechanism for exposure, and therefore is of relevance for the whole population as well as animals and plants (which may act as intermediaries in NM transport/transformation). However, the complexity of studying interactions and distributions of nanomaterials in the wider environment, coupled with relatively low concentrations presents a challenge. Environmental studies have so far been limited to release related studies such as Kaegi et al. (2008) studying the TiO2 wash-off from facades. The main way currently assessing possible environmental concentration is using emission based approaches and models such as pursued by Gottschalk et al. (2010). Here they use information on production rates, release fractions, assumed or based on measurement (e.g. sewage plant studies), and environmental transport to model environmental concentrations. These concentrations may be compared with environmental no effect levels

for plants, animals and humans to assess a possible risk. Still the application of the model is limited on a priori information which would be good to overcome in the future.

Currently no environmental monitoring technology is in place to allow for the monitoring of the expected increased environmental concentrations of persistent nanomaterials. This is certainly one point of future research which is currently not seen to be an easy task.

6.7 Sample preparation for hazard assessment:

Many nanomaterials are supplied as powders and need proper dispersion in vehicle media to allow the assessment of their human and environmental hazards. However, addition of nanoparticles to media could change their physico-chemical characteristics, leading for instance to aggregation or formation of a protein corona changing their surface reactivity and size. Thus the addition of serum, albumin of detergents which allow a better and more stable dispersion could also modify the biological responses induced (Val et al 2009). A good dispersion without modification of nanoparticle characteristics, especially surface reactivity due to coating with proteins, is necessary to accurately mimic real life exposures. This preparation of nanomaterials should take into account the exposure routes and target organs to be studied as for instance during inhalation, nanoparticles will get into contact with biological lining fluids mainly composed of lipids whereas ingested nanoparticles will encounter the acidic gastrointestinal fluids. Standardized dispersion protocols are established or under development to harmonize preparation methods (OECD guidelines) but their impacts on biological responses still need further investigations.

7 Human and environmental Impact assessment

The term life cycle assessment (LCA) stands for a clearly defined methodological framework that has been developed in the early 1990's as reported e.g. in the ISO 14040/14044 standards. However, it is often misunderstood by experts from other domains as equivalent to life cycle concepts. For example, the expectation was expressed by toxicologists that LCA will structure experimental toxicological results in order to assess the potential toxicological hazard of NM. The focus of an LCA is, however not to structure toxicological results. On the contrary LCA-experts rely on characterization factors for NM elaborated by toxicologists in order to be able to differentiate bulk material from nanoscale material during the LCIA step. Furthermore, LCA experts expressed their need for knowledge on NM emissions during all product life cycle stages. The improved understanding of these concepts and the interdependences of the various fields were described and published in the form of a manuscript (Som et al. 2010). The main conclusion from this paper is that life cycle methods could answer some basic questions for decision making in the innovation and regulation processes and help to identify and prioritize important areas for further research related to environment, health and safety (EHS) aspects, and other risks. Combining recent knowledge on risk assessment with the knowledge generated using life cycle concepts will allow an early and holistic view of the potential risks of NM and a comprehensive map of the current state-of-knowledge and the uncertainties. It helps to bring systematically together experiences and findings from often isolated research disciplines. This strategy will generate essential information for an informed decision making by industry, research institutions and regulatory

agencies to develop and foster safe and sustainable development of the new technology. LCA, however, is the appropriate method to assess the expected and advertised environmental benefits of nanoproducts compared to conventional products without NM.

To be useful in hazard assessment, validated and optimal test procedures for in vitro assays are needed, including relevant exposure conditions, appropriate exposure scenarios and appropriate dispersion protocols. Before in vitro data can be used to assess the possibility of read across or extrapolation of toxicological data between similar (nano)materials consensus on the appropriate dose metric and more knowledge on toxicokinetics is needed. Despite the many limitations, in vitro studies were thought to be useful in the risk assessment of manufactured NM by providing information with respect to their relative toxicity, a weight of evidence approach, dose range finding and fundamental knowledge on relevant possible modes of action. Given the exploratory character of the early workshop of NANOIMPACTNET where these issues were discussed, the results were not submitted for publication in a peer reviewed open access paper, but published as a NANOIMPACTNET - report (Dekkers et al., 2010).

Since these workshops, there has been more progress with respect to the risk assessment of NM. This involves the use of in vitro data as well as the development of different strategies, approaches, guidelines and frameworks for the risk assessment of NM. Because it is unrealistic and undesirable to perform a full battery of toxicity tests, including in vivo toxicity tests for all NM of different sizes, shapes, chemical compositions, etc., the use of in vitro data is becoming more important in the risk assessment of NM. Several publications have addressed the use of in vitro data in: a) the first tiers of testing strategies, b) extrapolation between similar (nano)materials, or c) the development of classification or grouping approaches, property-activity-relations and computer model systems (Park et al., 2011; Lai et al., 2012). In addition, several (testing) strategies, approaches, guidelines and frameworks for the risk assessment of NM have been developed or described in recent literature (Savolainen et al., 2010; Hristozov et al., 2012; and more). Also, several tools for qualitative or semi-quantitative risk assessment have been developed (Van Duuren-Stuurman et al. 2012; ANSES, 2010; etc.). Many of these approaches and tools are based on overcoming the limited knowledge on the behavior of NM in humans and the environment. Important aspects being addressed are:

- Solubility, biodegradability and dissolution of nanoparticles (NPs) in biological media (the environment and living organisms),

- Interactions of the NM with the environment,

- Structure or (chemical)composition identified as hazardous (e.g. asbestos-like fibres),

- Reactivity (production of reactive species in e.g. acellular systems),

- Uptake by cells and absorption by organisms,

- Comparison with data on the non-nano ("bulk") form of the NM,

- Confinement of the NM within a solid matrix.

Although significant knowledge has been gained in the last couple of years, still more data is needed with respect to the identified knowledge gaps in the risk assessment of NM.

In the discussions of NANOIMPACTNET, the field of nanomedicine was recognized as one where there is a fundamental need to ensure that the products do not cause more harm than good: therapeutic or diagnostic interventions can serve as good example. Nanomedicine was thus chosen as topic for several workshops, discussion sessions and training schools. The vastly developing area of nanomedicine creates new tools, methods and approaches with significant impact on existing conservative practices. Molecules and nanoscale materials are manipulated to produce nanostructures that can interact with human cells offering a range of new solutions for diagnoses and smart therapies. New technology solutions will clearly be beneficial for patients and will have direct impact on health and the quality of life in future societies. Applications of nanomedicine range from drug delivery, imaging, surgery, tissue engineering, regenerative medicine and early diagnosis and therapy of diseases such as cancer, diabetes, Alzheimer‘s, Parkinson‘s and cardiovascular diseases, etc. (Boulaiz et al., 2011; Gupta, 2011). The use of nano in medical applications also brings humans into direct contact with NM most often in the form of nanoparticles (NPs). A balance between therapeutic efficacy and safety with a high ratio of benefits to risks is demanded. In many cases the same methods used for NM efficacy are being used by toxicologist to identify potential health risks. Thus accurate and precise analytical (for characterisation of NM) and biological methods (for efficacy/safety assessment), and certified NPs/NM reference standards (for quality assurance) together with appropriate in vitro and in vivo models need to be selected, developed and validated.

Though existing regulatory frameworks in principle cover all important aspects of production and products, NM often display different chemical, physical, and biological characteristics than the bulk form of the same substance, highlighting the need for specific nano-policies. This is especially true in terms of REACH regulations, where most NM production volumes (by mass) are well below the triggers for data requirements regarding health and safety (Malkiewicz, 2011). The current guidelines for testing medicines from the EU medicines agency do not require any nano-specific changes to protocols. Nanomedicines are currently tested in the same way as other medicines. There is also a concern that nano-specific effects on body systems are not established and we currently do not know if adequate coverage of clinical care is achieved during clinical trials to protect the volunteers and patients. A publication addressing these aspects is under preparation.

7.1 Protocols for assessment of biological hazards

The NANOIMPACTNET Workshop (Deliverable 1.6) on "Protocols for assessment of biological hazards of engineered nanoparticles (ENPs)", held in Lausanne, Switzerland on the 24th March 2009 gathered representatives of the consortium and external experts with the aim of establishing the current state of the art in terms of protocols and procedures for the assessment of biological hazards of nanoparticles to humans, to highlight failings in currently accepted practice, and to make recommendations for the future development of the field. The topic was subdivided into the three areas of in vitro, in vivo and ex vivo testing strategies. Discussions were also restricted to ENPs (thus not all NM).

It was concluded that many deficiencies and challenges remain in all three aspects of ENP assessment, in vitro, ex vivo and in vivo. Much has been learned to date, but the variability of samples themselves, preparation and presentation approaches as well as exposure protocols

renders the available information difficult to interpret. The identified lack of both ENP standards and ENP positive and negative controls is an obvious challenge. Standard protocols for dispersion of nanoparticles should be agreed and differentiation between intrinsic particle responses and realistic exposure scenarios (in which particles are more likely to be present as aggregates) should be made. Although not discussed in terms of hazard evaluation, visualisation and tracking techniques, in vitro and in vivo, need to be improved. Overall, existing protocols are prone to artefacts and these should be well documented and publicised, with all publications including the control tests used to verify the applicability of the chosen assay to the particular ENP being studied. Much can potentially be learned from the pharmaceutical and cosmetics industries, as well as from the current understanding of the biological hazards of ultrafine particles.

It is clear at this stage that few conclusive and definitive recommendations on protocols can be made, and that further consideration should be devoted to refining techniques and methods based on evolving knowledge and experience. Standardisation and validation of techniques and models is essential. Progress can potentially be achieved through a further focused working group of the NANOIMPACTNET WP1 partners, to build on the findings of this workshop.

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Potential Impact:

Potential impact and main dissemination activities and exploitation of results

1. Protocols for nanoimpact / nanosafety assessment

Over the past 4 years, NANOIMPACTNET constructed an online-space (http://www.nanoimpactnet.eu/) for sharing research protocols with other members within NANOIMPACTNET. The aim of this exercise was to enable laboratories throughout the world to easily compare their methods and subsequently develop common protocols and strategies for the testing of nanomaterials in regards to their interaction with biological systems and their numerous physical characteristics. It was intended that this would be achieved by the following process;

- Members of NANOIMPACTNET would submit their protocol to the NANOIMPACTNET protocol database using an approved (within the NANOIMPACTNET management committee) protocol template.

- NANOIMPACTNET members would then be able to view these submitted protocols, test them within their own laboratories and then comment upon the protocol itself. These comments would then be made freely available to all NANOIMPACTNET members to incite an open discussion.

- If NANOIMPACTNET members decided to apply a specific protocol, either in its original format or slightly modified, the protocol would then be upgraded to a protocol recommended by NANOIMPACTNET.

- Following this, the protocol would then be further scrutinized by NANOIMPACTNET members, and then, if adequate, would become an 'officially recommended protocol by NANOIMPACTNET, being highlighted throughout the field and international bodies as a 'recommended protocol' for nanoscience.

Although the aim of this process was clearly evident to all as being beneficial for the entire nanoscience field, difficulties were encountered.

Initially, in order to get the protocol database up and running, only protocols that had been published in peer-reviewed journals were considered. This was ineffective however, with only 8 protocols being submitted and transferred into the NANOIMPACTNET protocol template in the first 2 years of NANOIMPACTNET. It was evident within the first two years of the NANOIMPACTNET project that most within the field were not motivated, or there was not sufficient appetite (despite the constant calls for protocols) for people to submit their protocols to NANOIMPACTNET in order to share them with the nanoscience community. The effort to convince other research groups to adapt a protocol of their laboratories to a prescribed format has also been underestimated. In addition, during this period, the issue of copyright had to be clarified since the protocols used were previously published in many different forms of publications. This process further inhibited the efforts of the protocol database. In an attempt therefore, to increase the number of protocols submitted to the NANOIMPACTNET protocol database that could be easily adapted into the NANOIMPACTNET template, collaboration between previous and existing EU projects (namely ENPRA, NanoInteract and NanoCare) enabled a significant increase in the number

of protocols published on the NANOIMPACTNET protocol database website. Additionally, interaction with institutions in the United States of America (i.e. NIST) facilitated a further increase in the protocols submitted to NANOIMPACTNET. Via these collaborations, the secondary aim of the protocol database (i.e. the sharing of protocols throughout the wider network of the nanoscience field) was achieved. Although the latter part is an advantageous outcome of the NANOIMPACTNET protocol database project, the overarching problem of individuals not submitting original protocols to the NANOIMPACTNET database was an issue that was not able to be overcome within the 3 year period.

In total however, 21 protocols were processed within the period of NANOIMPACTNET and published on its website (access to the protocols was always limited to NANOIMPACTNET members only, although all could see what protocols were present on the website). In addition to this, many protocols were submitted following a final request from NANOIMPACTNET to all its members in March 2012. These protocols will now be transferred as 'unformatted' protocols to QNano in an attempt to continue this protocol database and to encourage the realisation of its original aim.

The lessons learned from this process are many. It is clear that those within the field are motivated to discuss obtaining harmonies for all the different types of protocols used/available within nanoscience. Despite this, the realisation of the amount of work and time that is required for such harmonisation is paramount, and causes constant discussion as to who is the right, or available person/institute to perform this task. Also, due to a constant high volume work-load of many of the individuals involved within such discussions, it is difficult to gain such an outlook quickly and efficiently. This of course could be helped by all within the field, not only the researchers. Peer-review journals could also provide increased support by requesting authors to be absolutely specific in their description of protocols within the materials and methods section, and also not allowing/accepting the simple referencing of previously published protocols that were slightly adapted. Furthermore, funding agencies could begin to request specific protocols by those requesting funds. Additionally, the funding agencies could also start to make a clear outline for the definitive description of protocols in the project final reports, as well as a comparative discussion as to why researchers chose/implemented certain protocols compared to others available within the literature/field.

Whilst constant discussion, and continuation of the protocol database will occur (in the format of the NanoSafety Cluster and QNano), the problem of why protocols will not be submitted could continue. Clear efforts need to be made in order to motivate and provide individual researchers with a reason why to submit protocols. Also, time and effort has to specifically be put into such a project. It is not possible to create an advantageous protocol database that intends to provide a plethora of protocols for all researchers in nanoscience as an add-on within one workpackage of a project. If such aspects are taken into consideration, coupled with a financial backing, then a protocol database for all nanoscience research is certainly possible and feasible within the next few years.

2. Communication

In its objectives NANOIMPACTNET always stressed strong two-way communication between the project and the various stakeholder groups (notably the European Commission,

industry and SMEs, and the general public). This includes both responsiveness to needs of stakeholders and efficient dissemination of information and outputs to them. In terms of communication, and despite the differences in language, scientific cultures and practices and standards, the Project has had a fair degree of success in unifying interested parties, identifying gaps in comparability, standards and harmonisation and took steps in disseminating information and making policy and practical recommendations. An in-house feedback survey of the Project's clients and stakeholders indicated that respondents believe that NANOIMPACTNET did especially well on coordinating the identification and addressing of gaps in knowledge, and that it also did well on training. More could be done in collating and standardising protocols (research methods).

Overall, the Project has succeeded in generating sufficient European communication to form the basis for a nanosafety culture to continue to grow where none existed before. This new culture now provides a platform for the development of the NanoSafety Cluster and QNano, as long as the EC continues to understand the importance of, and funding for, the indispensable communication dimension.

The Project has shown the particular Importance of effective and efficient communication in training schools and problem-based workshops, stakeholder dialogue events, improved database collation and dissemination (e.g. protocols), a pro-active approach to industry, meetings with regulators and international collaborators.

The stakeholder database, dialogue and interactive events of NANOIMPACTNET have shown that a wide range of parties from very diverse areas of social and economic life are very interested in - and concerned about - the health impacts of nanomaterials. This interest and concern will grow as nanotechnologies enter into wider areas of consumer applications, manufacturing and energy-production processes, as well as fields such as medicine and pharmaceuticals. In the field of nanotechnology there is no monopoly of knowledge, priority-identification or problem-resolution; so its development depends on the nurturing and facilitation of communications between stakeholders at various levels with differing expertise and conceptual frameworks.

Resources allowing, greater attention should be paid to specific stakeholders. These include: major industries, SMEs, business consultants, entrepreneurs, venture capitalists, intermediate manufacturers, shareholders and investors, consumers, consumer associations (both governmental and voluntary/independent), non-governmental organisations (NGOs) concerned with health and environment, the media, the science and engineering education sector, the healthcare sector, medical equipment manufacturers, pharmaceutical companies and research laboratories, academic researchers, policy-makers, government funding bodies, insurance companies, regulatory bodies, lawyers and law firms, professional bodies in science, technology and engineering, the security sector, the food and agriculture sector, waste management specialists and many others.

The NanoSafety Cluster provides an opportunity to bring together and consolidate the nanosafety data, concepts and emerging problem areas in thematic hubs to be explored in more focused workshops, publications, policy recommendations and media reports. To some extent the same could be done in QNano to involve some of the more significant second-tier parties - such as educationists, manufacturers with specific problems, production process innovators and instrument designers in particular access arrangements and exchanges.

However, as a research infrastructure, all of QNano's activities are focused on improvement of the research infrastructure for the field.

For crucial recommendations on the development of stakeholder dialogue see the list provided in chapter "Outlook for stakeholder dialogue". Further recommended reading:

4th NanoImpactNet Report on Stakeholders and their Interests in Nanomedicine, Characterisation and Communication, Geoffrey Hunt, Luigi Cazolai, Darren Hart and Juan Riego-Sintes, 09.09.2011, Deliverable 4.1d.

Hunt, G. & Riediker, M (2011) 'Building Expert Consensus on Uncertainty and Complexity in Nanomaterial Safety, Nanotechnology Perceptions, Vol. 7 (July) 82-98.

3. Stakeholder dialogue: achievements, lessons, deployment, further work, recommendations

The stakeholder database, dialogue and interactive events of NANOIMPACTNET have shown that a wide range of parties from very diverse areas of social and economic life are very interested in - and concerned about - the health impacts of nanomaterials. This interest and concern will grow as nanotechnologies enter into wider areas of consumer applications, manufacturing and energy-production processes, as well as fields such as medicine and pharmaceuticals. It certainly cannot be said that it is an esoteric field of minor concern.

Interested stakeholders include major industries, SMEs, business consultants, entrepreneurs, venture capitalists, intermediate manufacturers, shareholders and investors, consumers, consumer associations (both governmental and voluntary/independent), NGOs concerned with health and environment, the media, the science and engineering education sector, the healthcare sector, medical equipment manufacturers, pharmaceutical companies and research laboratories, academic researchers, policy-makers, government funding bodies, insurance companies, regulatory bodies, lawyers and law firms, professional bodies in science, technology and engineering, the security sector, the food and agriculture sector, waste management specialists and many others. This complex diversity is no doubt due to the enabling character of nanoscience and its technologies, which means that it cuts across all scientific disciplines - the physical, chemical, biological, medical, ecological and so on.

NANOIMPACTNET has learned that communication and shared learning in the complex panoply of diverse and converging nanotechnology interests is an issue that needs special attention. A failure to support this issue will hinder technical and industrial development.

For example, regarding nanomedicine, NANOIMPACTNET has learned through a survey that one-fifth of the sampled participants thought it likely that the risks of therapeutic nanomedicine (in areas such as cancer) are too great for it to be allowed to proceed, while four-fifths did not think this was the case. In the same survey the participants were divided about 50:50 on the question whether nanomedicine should be subject to special regulation? (See: 4th NANOIMPACTNET Report on Stakeholders and their Interests in Nanomedicine, Characterisation and Communication, Geoffrey Hunt, Luigi Cazolai, Darren Hart and Juan Riego-Sintes, 09.09.2011, Deliverable 4.1d).

3.1 What has NANOIMPACTNET achieved?

NANOIMPACTNET has achieved its aim of generating strategies for involving very diverse stakeholders in the nanosafety debate, keeping them involved, and engaging in a two-way and transparent learning process. It has succeeded in creating a set of consensual key considerations on health impacts of nanomaterials. Over the four years NANOIMPACTNET has achieved these aims through conferences, workshops, web links, newsletters, reports, surveys utilising Delphi methods, questionnaires, debates, electronic polls and other methods.

Numerous meetings of formal and informal sub-groups have convened and shared ideas as a result of networking contacts. These include an Anglo-French series of meetings, contact with FP7-NaPolyNet (Polymer nanomaterials), CEN/ISO contacts, the Prague meeting in 2010, NANOFutures meeting, EU-US dialogue at SOT 2011, Washington DC;Italian-Swedish meeting in Rome, 2010 (outcome: book on nanotox edited by Bengt Fadeel, Antonio P, Anna Shvedova) to be followed up by Swedish-Italian meeting in Stockholm 2012,?Nanotox Autumn School Venice – held annually since 2009,?Nanotox conferences in Edinburgh 2010 and Beijing 2012, NANEX/RIPON industrial stakeholders meeting at NANOIMPACTNET conference Lausanne - 9th March 2010, and the promotion and dissemination of the NANOIMPACTNET project has been undertaken through SAFENANO. Most importantly, NANOIMPACTNET formed the community out of which the NanoSafety Cluster has emerged.

NANOIMPACTNET was able to establish, by means of a survey of expert stakeholders (n=92), priorities for the development of nano-safety. Organised around a conference in Prague in late 2010, a modified Delphi Method was used to determine the opinions of a range of experts from academia, industry and government in the field of nanotechnology, mainly those working in the areas of safety, occupational and environmental health, and nanotoxicology. The focus was on uncertainty and complexity. The participants identified the following ten priorities in the development of nano-safety:

- the need for realistic exposure scenarios,

- better established dose-response relationships,

- improved extrapolation from in vitro to in vivo,

- identification of the most relevant assessment parameters,

- understanding the dynamic biological interfaces,

- long term studies,

- information about stability and reactivity,

- understanding the behaviour of the protein corona,

- having test guidelines adapted to manufactured nanomaterials, and

- development of more advanced statistical and computational methods.

The discussions also investigated the basic nature of the uncertainties and how to distinguish between mere lack of data and intrinsic uncertainties that are a consequence of the complexity

of living systems. The findings of the survey were published in "Nanotechnology Perceptions" (Hunt, G. & Riediker, M. 2011 Building Expert Consensus on Uncertainty and Complexity in Nanomaterial Safety, Nanotechnology Perceptions, Vol. 7 (July) 82-98.) and widely distributed as part of the ongoing stakeholder dialogue process.

As the project comes to a conclusion, the initial 24 consortium partners have been joined by hundreds of members, with over 3500 members reading its e-newsletter. By coordinating research between scientists from over 40 countries, NANOIMPACTNET is helping to harmonise methodologies and communicate results, leading to increased consensus on best practice and priority research areas. NANOIMPACTNET also communicates with over 1,000 individual companies involved with development or application of nanomaterials and over 200 contacts from industry-related organisation. NANOIMPACTNET throughout its lifetime was deepening the quality of these contacts. The contact addresses remain available to the Cluster and QNano via the coordinator of NANOIMPACTNET, even though the active update ended with the end of the project.

3.2 What use are the lessons?

Nanotechnologies represent a paradigm-shift encouraging technological convergence and innovation. Due to this fundamental nature of nanotechnology's impact on technology, engineering and the economy there is an attendant widespread concern about broad and uncertain impacts on biological systems at all levels, from human to bacterial, from sub-cellular to ecological.

To take advantage of the promise of nanotechnologies in facing the challenges of moving towards a sustainable, high-efficiency, low-waste, alternative-energy economy it is imperative that industrial policy and innovation develop hand-in-hand in an accountable and transparent manner with all stakeholders. Dislocations in understanding, trust and communication between the different stakeholders can only hinder the exploitation of the benefits of nanotechnologies.

There can be no doubt that NANOIMPACTNET stakeholder events and communication activities are having and will continue to have an influence on regulation and legislation, and such issues as standardisation, and the labelling of nano-products.

3.3 What work still needs to be done?

In the field of nanomaterials, health and the environment there is no monopoly of knowledge, priority-identification or problem-resolution; so its development depends on the nurturing and facilitation of communications between stakeholders at various levels with differing expertise and conceptual frameworks.

There are many opportunities arising at the end of the NANOIMPACTNET project. Work still needs to done to develop and continue stakeholder communication. Some of this work could be done in the context of NanoSafety Cluster (NSC) and some aspects in QNano. NSC provides an opportunity to bring together and consolidate the nanosafety data, concepts and

emerging problem areas in thematic hubs to be explored in more focused workshops, publications, policy recommendations and media reports.

To some extent the same could be done in QNano to involve some of the more significant second-tier parties - such as educationists, manufacturers with specific problems, production process innovators and instrument designers in particular access arrangements and exchanges.

3.4 Outlook for stakeholder dialogue

Based on the discussions and observations above, the WP4 Communication team recommends that:

- The work and achievements of NANOIMPACTNET should be fully utilised in the development of NSC.

- Lists of contacts developed by NANOIMPACTNET be maintained by FP7 project QNano and used to continue regular spreading of EHS knowledge about manufactured nanomaterials, developments in nanosafety best practice, the activities of the NanoSafety Cluster and the EC's Action Plan on Nanosciences and Nanotechnology;

- More attention be paid to the communication and consideration of the 10 priorities in nanosafety development identified by a large group of experts at a NANOIMPACTNET event in Prague in late 2010 (see What has NANOIMPACTNET achieved? above).

- Nanosafety regulation and EHS events should be attached to Europe's most important commercial nano-conferences to encourage industry participation and interaction with academic researchers.

- More attention should be given to generating dialogue on the issues of commercial confidentiality and possible mandatory reporting in relation to safety data held by companies in Europe.

- Within the ambit of NSC and QNano, to concentrate on qualitatively deepening and consolidating rather than simply widening the network of stakeholders.

- Regular Delphi-style expert opinion surveys to get feedback from stakeholders for analysis and consensus-building.

- Setting up thematic hubs of stakeholder dialogue to deepen well-informed dialogue between significant parties (e.g. liability and insurance).

List of Websites:

Public website address, as well as relevant contact details.

The NANOIMPACTNET public website is http://www.nanoimpactnet.eu

The coordinator can be reached by e-mail using coordinator@nanoimpactnet.eu

General information can be requested through info@nanoimpactnet.eu

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