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A pan-European infrastructure for quality in nanomaterials safety testing

Final Report

Month 1 – Month 54

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4.1 Final publishable summary report

4.1.1 Executive Summary

Nanoscience constitutes a new scientific frontier in which specialists can, for the first time, engineer materials on the length scale of some millionths of a millimetre. This capacity enables faster computers, better mobile phones, harvesting of solar energy more efficiently, and more reliable batteries. It could change the face of human health for the better, both in diagnostics and in therapeutics.

The earliest reports or concerns about the safety of nanotechnology are almost as old as the technology. However, by 2009 the European Parliament had backed a report urging the European Commission to revise its stance on nanomaterials, and classifying all of them as new substances for which existing legislation did not fully take into account the risks, and asserting the principle of 'no data no market'.

The problem was that obtaining data was not a simple task. The concerns were legitimate, and science was not prepared. A bewildering array of conflicting data suggested that the same nanoparticles could be toxic, and safe, depending on which report from which group of scientists. It was rare for such a complete split to occur amongst scientists on the facts of an experiment, rather than its interpretation. Clearly, these issues could not be simply the consequences of a few bad experiments, but a systemic problem of how science was being organized and executed in this new interdisciplinary arena.

Into this complex situation entered QualityNano, as well as several other major initiatives driven by National agencies in US, Asia, Latin America, as well as international organizations such as OECD, ISO. The priority was to find some consensus on the actual laboratory results of short term toxicity tests. If one could not agree on those, then the feeling of uncertainty, confusion, and doubt would be uncontainable.

QualityNano applied all the tools of the Infrastructure to address these questions. Using blinded inter-laboratory comparisons the sources of these divergences and confusions were steadily identified, and eliminated, and consensus grown within a controversial and complex landscape.

QualityNano actively networked by canvassing opinions, engaging, and championing the concepts of methodological excellence, controls, and good practice by its own conferences, and dissemination at many others. It engaged with scientists, regulators, industry, and policymakers to keep all updated on the emerging understanding.

QualityNano educated and trained people, including hands-on development of several hundred young scientists who made transnational access visits, and themselves disseminated the new practices. It looked to the foundations of the field by building research methodologies and devices that could more easily automatically control the quality of the results.

QuaityNano was never designed to definitively and finally answer the question of whether nanomaterials as a class implied new hazards, and it has not done so. QualityNano did definitively establish the causes for the laboratory level disagreements on short term toxicity, and for exemplary systems showed how convergence could be achieved. Many reports of short term acute toxicity were found not to be correct, though some specific materials are found to be so. The broader community is now able to generate more robust and reliable results with some confidence. QualityNano therefore brought the field to a point where the key and more enduring questions for long term safety can now be addressed in a favourable context and balanced environment where focus on the right questions becomes possible. These should now be addressed.

4.1.2 Summary Description of project context and main objectives

Context within which QualityNano project was conceived.

Nanoscience constitutes a new scientific frontier in which specialists can, for the first time, engineer materials on the length scale of some millionths of a millimetre. Current applications of nanotechnology for the benefit of mankind range from information technology, energy storage and harvesting to radically new medical technologies.

The projected market for nanotechnology incorporated in manufactured goods was enthusiastically predicted[1], though in practice it was difficult to separate entirely the contribution from nanomaterials, and how much from the value added in the product. Meanwhile, many nanomaterials were finding their way into the general market and increasing efforts were made to set up reporting systems to track these issues. Those efforts are still under way.

Uncertainty: Despite significant R&D investment in the 10 years prior to QualityNano[2] real (and perceived) unknown hazards and risks of nanomaterials, the reliability of testing approaches were highlighted in all dimensions from science, media, parliaments, and government.[3] This was partially caused by the fact that current methods and assessment were not fully appropriate to test nanomaterial hazard and new protocols and approaches. Added to this was the even more unusual situation where different scientists, even from certified laboratories, came up with entirely different results in toxicity testing using those methods. Thus, for some a given nanomaterial was plainly toxic in new or complicated ways, while for others the same material showed no such toxicity. It is important to stress that all these remarks refer to the majority of material (some certainly have short term toxicity, now agreed by all) and to short term effects. The question of longer term effects remain largely unknown, even now, despite improvements in the arena.

Lack of standards in any arena: Additional complicating issues arose because manufacturing standards, and workplace practices for nanomaterials, are not uniform across market sectors, and this has lead in strong differences across different laboratories in different parts of the world. Serious issues were apparent, for example, from issues of impurities, unconventionally sequestered in nanomaterials, to basic characterization however others required a detailed understanding of the material and its properties to fully understand and predict its behaviour (eg agglomeration, aging, etc.).

Communication resolving aspects of confused and confrontational dialogue: The atmosphere prior to QualityNano being set up was therefore somewhat confused, and discussions between stakeholders had become polarised, based on opinions, rather than on science.[4] The role of communication was partly undertaken by QualityNano, illustrated for example by the constructive European mission on nanosafety in Brazil where a dialogue was needed between scientists and stakeholders in Brazil and Europe leading to sharing of results and ideas[5].

Scientific inputs lead to more confusion, not less: However, not everything could be resolved by communication. For some issues of laboratory science, people simply found different results, and

[1] Nanotechnology Market Forecast to 2013, RNCOS, May 1, 2009, Pub ID: CICQ2230088.
[2] The EU 6th Research Framework Programme (2002-2006) devoted over €1.3 billion to nanotechnology and new materials, and has allocated € 3.5 billion to the NMP theme for the period 2009-2013 (FP7).
[3] Foss Hansen S, Maynard A, Baun A, Tickner JA. Late lessons from early warnings for nanotechnology. Nat Nanotechnol. 2008 Aug;3(8):444-7.
[4] http://www.nature.com/news/2009/090818/full/460937a.html
[5] https://chemicalwatch.com/21383/eu-nanosafety-mission-to-brazil

this had to be addressed directly. It was at first very difficult for most people to accept that even some of the results at laboratory level could not be agreed. This variability of reported biological and toxicity outcomes at laboratory level on nominally identical materials began to cause controversy in science, and the media, with an impending feeling that there could be a loss of confidence in science: that single force capable of unifying societal views on this topic.

This was entirely distinct from the more subtle question as to how results on conventional toxicity tests would be interpreted in the context of hazard and risk. This was a substantive issue that required the consolidation of the community, and ongoing discussion, rather than simply agreement on laboratory results.

The need for Infrastructure: It was then recognized that there was a need for (1) community based infrastructure to transform and drive the community transition to more scientifically based opinions, (for academic research, regulatory or industrial) (2) to develop a cohesive framework of blind round robin (RR) to eliminate controversy on the basic laboratory outcomes. It was also recognized that part of the problem was that large group of scientists were being attracted into the field as a result of the important issues, exciting and new questions, and funding available from many sources, and that many of those would be entering for the first time relatively lacking in experience in the field. Finally, looking to the future, it was also noted that beyond the intra-community dynamic, other stakeholders interests had to be addressed, especially the priority of assessing specific material in a particular environmental scenario, if there was a real hazard involved. These factors suggested the need for networking (addressing the capacity to engage, check results and such like) training (addressing the needs of a growing interest, without the knowledge to draw robust conclusions), and research, addressing the longer term, 'real' needs of the European consumer, and others. These factors suggested the value of an Infrastructure, within the European Union framework.

The emergence of the Infrastructure: The vision of QualityNano was the creation of a 'neutral' scientific and technical space in which scientists from all stakeholder groups could engage, develop, and share the scientific best practices in the field. It was understood that such an organization could not resolve all challenges, nor even address all the important areas of the science; however, its aspiration was limited to the creation of an ethos, development of processes, and harnessing of the resources, to allow evidence-based dialogue in critical areas to flower. The infrastructure aimed to display ethical standards, processes and assessed protocols with a view to yielding clarity and unity of purpose from the uncertain atmosphere pertaining at the time. By processes (for example, blind round robins) it planned to determine (and provide the support to determine) facts, and report them to the scientific community, and stakeholders. By those methods it aimed to intervene and change the course of the evolution of the arena. .

Given the emergent nature of the field, the QualityNano Research Infrastructure was intended to remain open and flexible, responding to events on the ground, remaining responsive to the emerging concerns, providing the potential for new key communities, and key players to enter, and find a scientific base from which to operate responsibly. No one could predict quite what such an Infrastructure would face. It was clearly far from those built to serve more established communities.

The Concept: Within this context QualityNano was devised to be an accessible integrated European resource for research, regulatory, and industry (both small and large) developers in nanoscience and nanotechnology. It became that, and much more. It materially affected the outcome of the Nanosafety debate, stabilized it, settled many of the questions that were then considered key.

It sought to harness and integrate existing research expertise and facilities from across the EU member states into a cohesive interdisciplinary entity strongly focussed on scientific excellence and quality of execution in all aspects of nanomaterials processing and characterisation for assessment of their biological and environmental impacts. It consulted with, and remained in close alliance with Organisation for Economic Cooperation and Development (OECD), ISO, NanoImpactNet (NIN), the

International Alliance for NanoEHS Harmonisation (IANH), and numerous other national platforms. Thus, it did not achieve all these aims alone, but as part of a tapestry of activity that was seeking to address the issues. This must be considered as a durable achievement of all these institutions that it was possible in such a fast-changing environment to adapt to changing needs, and make space for each other to pick up challenges they were most suited to address.

The Annual and Other Periodical Meetings: Following on from NanoImpactNet, a previous community project aimed to ensure the continuity a forum in which all these issues could be openly discussed. In this regards, the key events were probably the annual meetings at which a plenary international discussion took place. Those meetings were well attended, often with hundreds of people in the audience from all over the world, and at them, the results being deduced from the different regions were exchanged. In addition, various sectional meetings were important, in which subgroups ranging from regulators, industry and other topical discussions.

Round Robins were considered Essential: Looking back, as we summarize this activity it becomes difficult to explain the exceptional levels of controversy associated even with the simplest tests of cell death toxicity (apoptosis) with simple materials such as silica and titania nanoparticles. Nevertheless, scientists persistently reported that this was a serious issue, and a few headlines in newspapers even suggested the possibility of serious diseases. While the results were confidently understood by some to contrary to this, in practice, unless a substantial number of scientists could simultaneously get the same results it was clear that the controversy could not be resolved. The favoured instrument for this was the blind round robin, with controlled central distribution of all materials. There were many other networking activities, but this was a strategic choice. Those studies connected many stakeholders, and were deeply challenging at every level, from logistics to execution, to communication and resolution. It was there ultimately where the axis began to turn, and many of the laboratory issues be resolved.

Training was envisaged to be Central: QualityNano sought to offer a distributed set of transnationally accessible facilities as well as a range of added-value services to users and stakeholders. These were to include high quality (‘approved’) Nanomaterials, training in advanced characterisation methodologies and round robin validated protocols for biological assays, as well as industry-oriented support, using flexibly configured distributed ‘hubs’ via which different constituencies could interact. It was always recognized that training of incoming scientists, especially of the young ones, would be central, and that likely hands-on visits to established laboratories would be important. In the event this element become even more central than envisaged. The numbers seeking training were large, and unlike more established communities the need for training was more diverse and complex. Thus, as a concept transnational access (TA) was ideally suited to the need, but its manifestation was not entirely expected, nor planned for.

Other kinds of training events, workshops, and related approached were also attempted. They were partially successful, and had positive outcomes. However, again, the unique circumstances, complexities, and lack of maturity within this field often required hands-on, specific training almost person-to-person. However, success was achieved. At the final plenary meeting of the infrastructure in Crete in July 2015, a retrospective on the whole program was framed in which the most successful TA visitors came together to present their developed ideas and outcomes, post training. A competition was held to identify the most successful outcomes, and this was judged by an international panel. Additionally, the poster sessions have been highly populated not only by students, but also by senior scientists, experts in the field from industry and academia, regulators and policymaker and they were all engaging in an horizontal and bilateral discussion. It was considered that this illustrated the power of training of several hundred young scientists to materially influence the whole dynamic and level of the field. Several of those went on to achieve recognition, and several were awarded ERC started grants, progressing on from their work in QualityNano.

4.1.3 Main S & T results/foregrounds

Overview of main S&T results

In this presentation we will try to summarize major directions and achievements of the QualityNano Infrastructure, focusing on the key impacts. In the first part we seek to integrate those achievements across the various pillars from which they were derived, and then we summarize similar information from the work-package perspective. The extent and variety of the outcomes within the program is however sufficiently large that it is recommended to consult the website, and the original deliverables reports. It is intended to progressively make those available on the web.

A major objective from the outset of QualityNano was to support the elevation of the overall research standards in the community. At the time of conception, this was considered to be of importance, with underlying current of confusion and uncertainty across all the domains from science, regulation, industry and policy. While a single roadmap would not suffice to address all of these dimensions, there were clearly immediate priorities to stabilise the role and reputation of science as a useful contributor of reproducible and agreed data for the community. In the early period of the QualityNano program the actual number of people in Europe, and internationally, that began to join the discussion grew rapidly, scientific controversy at an increasing rate, and it ultimately required an intra-community larger effort than was originally planned for. [1]

It should be stressed that in the presentation below no conclusions are being drawn about the ultimate and long term issues of nanomaterial hazard and those questions are commented on towards the end of this report. Rather we are discussing the outcomes of the then known and reported short term toxicity tests, which can now be carried out with some confidence.

1. **Highlights of Networking and Research**

Outcomes Addressing and Stabilizing Community Uncertainty:

The issues soon became relatively well defined at laboratory level. Large numbers of scientists (including those traditionally working in toxicology of chemicals, and therefore noteworthy) found that nanomaterials induced many different types of toxicity at cell level, also in specific toxicity tests that had long been considered reliable, and reproducible for chemicals. Similarly a number of other scientists found there to be no such toxicity. Other found a confusing array of irreproducibility.

To deal with this scientific controversy, before coming to any conclusions, the most urgent need was to decide on the origin of the differences in laboratory results being obtained all across Europe. Therefore what was required was essentially a process of deconstructing the origins of the uncertainty that was scientifically robust, drive agreement by process rather than argument and conviction, and that was publically transparent and convincing.

Interlaboratory Comparisons: The major tool to achieve this was chosen to be the blind round robin style in which large inter-laboratory comparison (ILC) studies involving multiple (>10) labs using agreed protocols and nanomaterials. The question to be addressed was how to construct such comparisons. A range of approaches were taken in order to assess the community's needs in terms of training in the use of the protocols. Modest groups of laboratories (around 10) were chosen from the more expert groups, and partners, and varied also to provide some form of sampling.

Physiochemical: To begin with the initial physico-chemical ILC had no protocol provided, and simply asked all participants to use their in-house protocol to assess the level of variability in existing practice. It should be appreciated that such measurements would be the basic pre-requisite of every published work in the field, and, therefore, should not require a centrally driven protocol, material by material. The results were striking, and revealing.[2] Across Europe, it was found that

many laboratories obtained different results for their physiochemical characterization regardless of the method used. It became evident that for the characterization alone some of the problems were due to poorly understood concepts of dispersion, including in biological media in which the systems had to be studied. With hindsight this is no longer considered controversial, nor indeed surprising. The study of chemicals was not necessarily a good pre-requisite for people unfamiliar with nanoparticles and their dispersion. The magnitude of the challenge was becoming clear however.

Toxicity ILC: Early steps were also taken to initiate ILC for toxicity tests, including some of the simplest (for example, classical 'cell death' evaluation). In all of this, a very small group of laboratories worked closely with the Coordinator to pre-test the viability of the comparison, and ensure that a uniform results was in fact to be expected. This became a highly intensive process, but ensured that the large overheads of time and effort for larger ILC were not wasted.

One of the key results involved the use of amine-modified polystyrene (NH2-PS) nanomaterials with 'cell death' toxicity tests, that have been largely used in QualityNano. [3-9] These were also then considered candidate positive controls for several endpoints, including cytotoxicity, apoptosis, cell cycle disruption, and reactive oxygen production. It should be stressed that in choosing this example for a large ILC it was possible to eliminate many of the complications mentioned above in characterization. These materials were of high quality, easily dispersed, and shown to be so, and therefore many of the outcomes were considered to be a result of the toxicity testing itself. It should also be noted that these were specifically chosen to be cytotoxic. The purpose of that was to establish the community capacity to determine the level of toxicity.

The identity of individual laboratories was concealed in presenting and compiling results, and this had the benefit that the discussion was neutral, and technical, rather than pointing to weaknesses of laboratories. This was considered important since, whatever the underlying problems, they were so widespread they had to reflect intrinsic problems in the field, and not individual weaknesses. Nevertheless, the outcome was quite striking, and an illustration of the type of result that became widely discussed across the whole scientific and policy community is illustrated below. Across all the laboratories, the range of outcomes was sufficiently varied that some found high levels of toxicity, others little. Other follow-up ILC with widely differing sub-groups of partners, and also with additional laboratories that entered via the expert groups in the networking elements were sometimes found to have even more severe variations. It was clear these difficulties were not a consequence of any particular sub-group, but a community wide problem.

These variations were indeed consistent with the confusion in the scientific debate, and huge variations, whatever their source, were clearly present. While in its conception QualityNano had been intended to address such variations, there was initial surprise among the partners that such wide variations could be present even with that grouping. It also became more widely appreciated across the scientific community, and beyond, that the then reports of widespread short-term toxicity observed in many laboratories would require more careful consideration and could not be taken simply at face value.

At this point, it cannot be determined the degree to which QualityNano alone thereby began the process of building a more systematic and balanced understanding of nanomaterial toxicity. Certainly, dating from these large round robins, and the illustration of the need for science to build its methodology, there was the beginnings of a more balanced and cautious view. There were other actors and stake-holders engaged right across the whole scientific, community, including OECD, ISO, CEN, FDA, NIST, and numerous others attempting to also find a firm footing in the arena. There was much overlap and communication between the primary actors in those and QualityNano, and consensus was progressively built between them. One of the enduring successes of QualityNano,

and the other projects and programs from such agencies, was the degree of cohesion and clarity that was built mutually.

Final Outcomes of ILC; The actual long term outcomes of the inter-laboratory exercises is discussed more below, but in summary progressive discussion, training, and refinement of the concepts (rather than protocols alone) were found to improve the situation.

By the end of the QualityNano projects all partners could attain acceptable agreement, and the most expert in the relevant methods could obtain a remarkable level of quantitative agreement. The whole round robin exercise was subsequently initiated within the United States, with much the same initial, and final outcomes, of a final high level of agreement between most laboratories on specified tests. Indeed, that quantitative agreement became an expectation within a number of laboratories, that several young researchers that helped build it within QualityNano subsequently won ERC starter awards, with the growing expectation of a level of excellence in the emerging science. This was a long way from the initial status and degree of controversy and surprise.

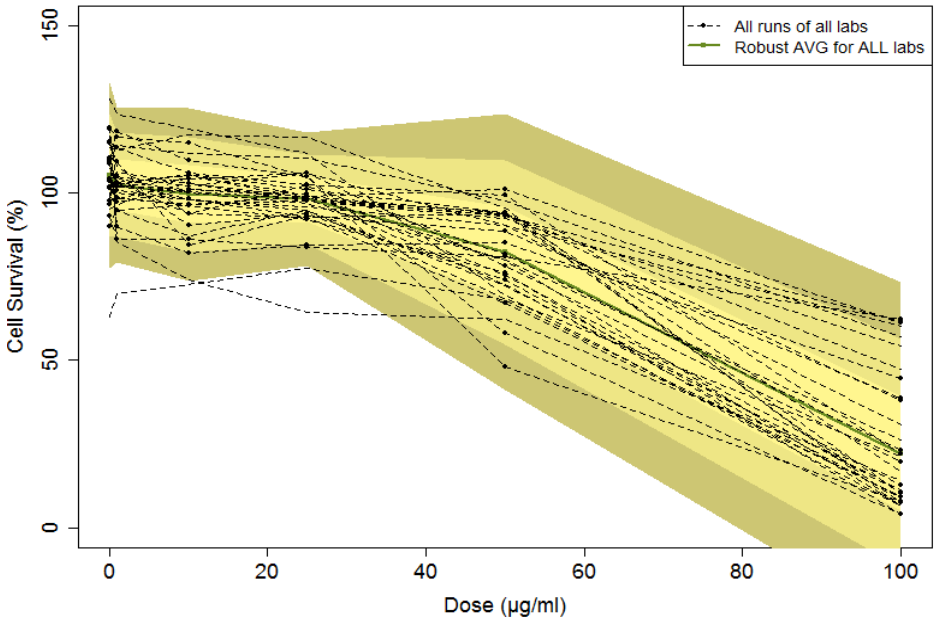


Figure 1: Protocols and Understanding of Irreproducibility; Early ILC results, without highly evolved protocols showed large variations in results between partner laboratories, far beyond anything that could be seen for equivalent tests. Such results were iconic, and surprising. Protocols helped, but dissection of the origins of the errors were also important.

There was for some time an internal discussion within QualityNano about the real long term value of such specific protocols, because each test and each materials required a significant degree of refinement of the protocol. Some felt that if one acted alone via protocols to obtain agreement then the longevity of the impact of QualityNano would be doubtful. Thus, considerable focus should be given to uncovering and publishing the general range of difficulties associated such tests, and explaining the origins of the irreproducibility and differences that had arises, rather than simply protocol development. By doing so, it was felt, those differences that had beset the field would be eliminated by scientists themselves, and not only by project with a finite life-span, but by knowledge widely disseminated. In the event, protocols were also developed as well as shared with other projects (many other grouping also worked on protocols), while a number of publications clarified the potential for incorrect result from toxicity testing, and the likely origins of the problems.

It was clear to all that there was need for a core of trained young scientists in Europe who could take forward the conclusions, and this was addressed via training and transnational access initiatives.

In the event the issues became less complex as time went on. A mixture of all of the tools of QualityNano progressively converged opinion about the nature of much of the reported short term toxicity. And that opinion progressively also grew to be shared widely across the community world-wide. In the event, there are fewer and fewer reports with widely different outcomes, and a broader level of understanding has progressively eliminated the need for such interventions, either for protocols, and the community is now much more expert and prepared to undertake such studies.

The Importance of Materials Quality and Growth of Concept of Positive Controls: The early ILCs also highlighted several other weaknesses of the then common practices. These included may detailed issues in relation to quality of materials. [10, 11] It is not possible in this short summary to detail the range of these, but only to cite a few that were confronted and resolved. They ranged from issues of contamination of nanomaterials and leeching of those impurities causing apparent toxicity, to the use of labile markers on nanomaterials that separated during use and give confusing results about nanoparticle distributions. The range of such practices was then so large, and so varied as to not easily be summarized, and most were addressed, and progressively eliminated.

Materials Repository: A central repository of nanomaterials was synthesized and maintained, and samples distributed from there to users for the purposes of careful experiments. These materials were examined in very great detail to ensure that they were free from impurities, and had sufficiently well-characterized properties that any toxicity they exhibit would be a consequence of their nanomaterial property, and not of more trivial aspects of their nature. This was a highly time and resource intensive aspect of QualityNano, for samples were not always found to maintain their quality over time, degrading, or otherwise altering slowly over time. This became a subject of such concern for some materials, that a significant effort was made in research to understand and eliminate it. A great deal of knowledge was accrued about this topic and discussed below where we discuss the assessment of approaches for curation and long-term stability of nanomaterials

Another key gap identified at the project mid-term review, was the lack of data and understanding regarding the ageing of NMs during storage, whether as powders or as dispersions. Thus, a new task was introduced to address this gap and assess the long-term stability of NMs in powder and dispersion form as a function of the storage conditions. From the broader point of view it became more clear that the challenges of maintaining a single sample over extended periods of time was a challenging and time-intensive exercise, requiring an in-depth knowledge of specific materials, and ongoing monitoring. It was agreed at the mid term of QualityNano to progressively withdraw from that activity of seeking to maintain a general materials repository, and key elements of this were brought forward by JRC in a materials repository that provides samples in a transparent and easy accessible manner.

Henceforth the materials that were supplied within QualityNano were based on the idea of being able to reproduce the syntheses in useful ways. There was also a key development of positive controls.

Concept of Positive Control Materials: Henceforth, the concept of materials hub focused on more specific outcomes that were closer to QualityNano objectives of community integration and convergence around harmonization of results. Within the field there not yet been time to develop and disseminate the concept of positive (and negative) controls, and this was considered a limitation. First and foremost, in its absence, there was no real way of calibrating an inter-laboratory comparison, especially during a period of controversy. One usual way of ensuring that a specific test is being carried out correctly locally is the use of a positive control. While such positive controls were in existence for chemical toxicity testing, and were sometimes used in conjunction with

nanomaterials they were invariably found to have different mechanistic action from that of nanomaterials. Thus, while they provided some element of control for some of the materials being used, they did not control for the specific mechanism under investigation. Examples considered within QualityNano were cell death (apoptosis and others), cell cycle arrest, reactive oxygen species and others. Initially commercially available particles were used to test out the concept of distributing particles, but the mechanisms of their action were investigated, and more refined versions synthesized within QualityNano to ensure a range of more controlled outcomes. Besides the explicit examples of materials, the whole concept of positive control was validated within ILC, showing that indeed rather specific and precise measurements could be made on the biological impacts. The role of positive controls became much more cross-cutting across the whole program. Instead of an acceptable level of variation between partners and many Transnational Access visitors and other laboratories, the provision of a single well defined control for a given endpoint gave a new focus and impetus to the possibility, and ultimately the requirement for laboratory level agreement in toxicity testing. This device of the positive control, in combination with the wider use of ILC became form some period somewhat iconic internationally, and the need for, and possibility of reproducible science in nanosafety became increasingly accepted. This had implications far beyond the particular positive controls introduced. It materially changed the level and standard of expectations in the whole field.

These positive control materials are still supplied (by Partner 1 beyond the lifetime of QualityNano) to scientists from around the world on an informal basis, most especially providing a useful tool when new arenas of nanosafety are opened up where there is little previous experience. An interesting example has been the recent growth of interest in oceanic polymer pollution in which particulate degraded polymers have been of increased concern in the last few years, public attention having been focused on the high level of contamination, and the role of ocean currents.

The degree to which such materials should be transformed into a formal standards of control materials is under consideration, and in particular the degree to which the community still requires such materials, supplied centrally now that the concept is widely disseminated and practices so much improved. Likely the most valuable role such standard controls will have is for new communities such as those mentioned above, involved with the ocean, and others involved in life cycle investigations. Whatever the outcome, one should not underestimate the impact this initiative had at the time it was instituted. It contributed to what is now a fully accepted standard of agreement between laboratories.

Storage, Reproducibility and Synthesis: The recognition of creating and maintaining highly reproducible batches of nanomaterials was recognized from the beginning, and its implications for nanosafety was appreciated. There were difficulties in making nanoparticles in the same way every time, and in making a large batch, and storing it. These challenges were significant for nanosafety research, but also throughout the whole industrial chain. In depth research efforts in JRA1, and in different parts of the whole project workflow were made to understand the issue. Hundreds of batches were examined in an effort to understand the limits of reproducibility. A number of nanomaterials were stored and maintained in different conditions by a small sub-group of the partners, and periodically examined up to a period of two years to determine the nature of any changes, and the role of the storage conditions. Significant progress was made in understanding the controlling factors in these topics, some of which have been implemented in broader practices within the community.

Traceability and Detectability: It was widely recognized from the beginning that a strategic problem was the lack of methodology to detect, and trace nanoparticles in the many environments where they would be applied and found, from cells, to organs in animals,[12, 13] to food products. [13]This was an overarching problem that required not just a single solution, but the evolution of a wide range of

approaching both in labelling the particles themselves (for instance with fluorescence, isotopes, radioactivity and others), to detection methods, particle-induced X-ray Emission (PIXE),[13] fluorescence correlation spectroscopy (FCS),[14] to single particle -inductive coupled mass spectrometry (sp-ICP-MS).[15] The challenges to apply these methods in the context of nanoparticles, and in the relevant context in which they were applied were taken up in JRA2 & 3 as well as within various of the networking initiatives. As an example, single-particle inductive coupled mass spectrometry applied to complex matrices such as in food products was advanced, and validated in one of the QualityNano round robin studies.[15] Similarly, the radiolabelling of several nanoparticle materials was achieved, labels shown to be fixed to the particles, and the outcomes used in early biodistribution studies. [13, 16, 17]Small details had sometimes large implications. A problem in application of fluorescent labelling was noticed within the activities of the NanoMaterial hub, and the challenge of internalizing and preventing facile leakage of dye resolved for many nanomaterials within these research activities.[10, 18] Though focused in nature, and unnoticed by many, this changed important outcomes in the literature. Instead of widespread reports of large amounts of nanoparticles being detected in the nuclei of cells (as was then common) such reports came to be almost unknown. In turn this alerted those involved in genotoxicity testing that the nature of the established tests for chemicals for example, those focused on physical contact of materials with nuclear components may be incomplete. Many such modifications of understanding took place, driven from advances of this type, which, instead of residing only within individual laboratories, disseminated through the whole community and became widely accepted, almost without note.

Modes of Presentation of Nanoparticles to living organisms, and alternative testing:

While inter-laboratory comparisons and studies of all kinds were found to eliminate many sources of irreproducibility, and conflicting outcomes, simply by clarifying the nature of the control parameters and ensuring they were more tightly controlled via protocols, some aspects of the arena could not be further improved without either conceptual or infrastructural developments. This point, though now clear, and relatively well accepted was far from so at the beginning or indeed throughout QualityNano. That it is now relatively clear is in part a tribute once more to the whole complex of QualityNano activity, not just a specific form of networking or research.

Working *in vitro* toxicology is in practice very different with nanoparticles, than with cells. Chemicals dissolve (usually) and are presented to cells in usual tissue culture, and the presence of serum at low concentrations merely has the role of feeding the cells. Nanoparticles constitute a completely different scenario. They may be considered mostly surface, and their interaction with cells in the first stance is the interactions of that surface. Anything adsorbed to that surface become the primary mode of contact between nanomaterial and target. Initial reactions of this understanding were confused. One approach was to avoid any ‘prejudice’ in the presentational mode by carrying out studies in serum free media, with the outcome that the high energy surface of the nanoparticles in contact with cells caused damage of different kinds, depending on the particle. [19, 20] The apparent outcome was a wide range of apparent ‘toxicities’ all of which would never been seen in exposures to animals (for instance). There every route of contact between animal and nanoparticles first leads to a coating (named the biomolecular corona) that protect against such direct contacts. Indeed, the absence of such mechanisms would make all forms of dust in the environment immediately hazardous. Progressively it was appreciated that more realistic forms of presentation should be arranged if one was to avid entirely spurious biological outcomes. Ultimately many of the most acute and dramatic reports in the literature citing toxicity were derived from such issues, and these were conclusively clarified. This was a good example where understanding, rather than a protocol, was the solution to the problem, both in science, and far beyond. Nowadays this understanding has progressed further, and it is believed that the nature of the biointerface is of such critical importance

that the nature of the organism's cells should be matched to the exposure medium, to ensure correct recognition by receptors. Thus, for example, human cells should be exposed to appropriate human biofluids. This more advanced understanding is still in the process of being widely integrated.

The role of medium does not alone define all important aspects of presentation. Indeed, the implications of allowing gravity and convection to allow particles to come into contact was investigated, and standard presentational formats devised.[21-25] Indeed there was development of new exposure devices, and subsequently small commercial grouping are seeking to promote the use of such devices in biological and toxicologicial assessments. All of these questions were relevant to, and of substantial support to new and alternative testing strategies. The avoidance where feasible of animal testing is a strategic effort throughout all of research in the Europe, and the capacity to present cells, and nanoparticles to each other by approaches that are sufficiently realistic is the means to advance that agenda here also. For instance, investigations of the blood-brain and other biological barriers required the development of supports allowing the presentation of cells to nanoparticles and passage through the support. [24]These and other such advances for alternative testing are increasingly being brought into routine use, rather than exceptional investigations.

2. Highlights of Training and Conferences:

There were a number of key achievements in the sector of training and education. Once more, as the program QualityNano evolved, other projects and activities arose with more specific tasks and focus. For example, the NanoToes (Marie Curie Initial Training Network) was focused entirely in structured education, and progressively, as it evolved, QualityNano become the supporting infrastructure and backdrop for its activities. For example, with NanoTOES a survey of the needs of current students and of the courses available in 2011 was conducted via the QualityNano website and reported as Deliverable report D3.1 (publicly available). Building on this, an outline training pathway was built which aims to establish a "gold standard" for the training of young experts in nanosafety. Full details are included in QualityNano Deliverable report D3.5. Some structured courses were constructed by QualityNano, and European Training schools, some including hands-on training sessions, were developed.

Each training course had a number of expert speakers from QualityNano and beyond, and each involved a significant hands-on element, on computers for the modelling school, via analysing experimental protocols for the Good Laboratory Practice school, or the practical details of mixing nanoparticles and soil (and subsequently trying to get them back out for characterisation) in the Ecotox training schools. Some training materials are available via the QualityNano website (repository of training materials; http://www.qualitynano.eu/the-qnano-knowledge-hub/repository-of-training-materials/qnano-funded.html).

QualityNano International conferences

QualityNano hosted three major integrating conferences during its 4.5 years: the first was co-organised with its predecessor project NanoImpactNet in Dublin from 27-29th February 2012, the second was held in Prague from 27th February - 1st March 2013. While one purpose of this was to increase the participation of nanosafety scientists from new member states, the main thrust of the meeting was to settle major remaining controversies.

The final meeting was held jointly with FP7 project

NanoReg in order to maximise transfer of knowledge between the projects and its main agenda was to hand over many of the ongoing skills, connections and effort to others in the community. The conference was attended by many of the key partners of QualityNano throughout its lifetime, from OECD, regulators and others. The final conference, from 15-17th July 2015 in Crete, was attended by over 150 delegates. One of the high-points in this meeting was the poster session, part of which involved the nominees from all of the TA visitors accumulated over the program. Each Partner that had hosted TA visitors was invited to nominate such visitors who then took part in an overall poster competition, with a prize-winning celebration, and award of certificates. Overall, this conference was widely considered to represent an impressive end to a complex and important EU infrastructure project.

3. Highlights of Training via Transnational Access:

In the conception of the QualityNano Infrastructure the role of transnational access (TA) was envisaged to play an important role, but the true relevance and importance of it was not fully appreciated until after the first set of visits. TA access was simplified by a portal-based peer reviewed proposal system wherein any researcher working in Europe could apply. Over 8 calls candidates from 30 countries have applied and 200 visitors made visits. The program is considered one of the most outstanding success stories in any Infrastructure, both in the suitability and quality of its delivery, including by those that had extensive experience of other Infrastructures. Full statistics are available in the extended report of the program, and summarized below.

Early visits began to reveal that many of the main issues and difficulties that had been experienced by the researchers was not mainly the absence of facilities or equipment, but in reality the expertise that resided in the host institutes. In many cases it was found that comparable collections of equipment were available in other locations, but that the TA visitor was seeking highly focused training and support in the design of the experiments and application of the methodologies in this new context of nanosafety. This had not been expected by all Partner institutions, but was generally consistent with all of the other dynamics in the community, and in the Infrastructure. This outcome certainly placed exceptional pressures on some of the young researchers residing at the host institutions, who had expected visitors to require less hands-on support. Furthermore, the range of techniques and skills that were transferred were much greater than envisaged. Above all dispersion and characterization methodologies were of exceptional interest throughout the program, though that often involved small scale equipment scattering and centrifugation. While there were some discussions about the potential to limit applications to those who has already shown success in such skills, thereby focusing more resource, broader realization (and consultation) pointed out that, since some of the sources of irreproducibility and uncertainly derived from precisely those issues. It was considered appropriate to continue to give intensive support to the TA visitors in preparing the systems. Some of those visitors continued their associations with the laboratories and returned via other sources of funding. There as emerged as a consequence, a cadre of competent and well prepared young researchers from all around Europe that were sufficiently prepared to avoid some of the difficulties in Nanosafety research. Though there is no specific quantitative evidence to support this, the opinion has grown that those young scientists and the ideas they carried back with them played a major role in resolving much of the confusion and irreproducibility that had arisen in the Nanosafety research community. In any case, the TA visit program has been universally appreciated.

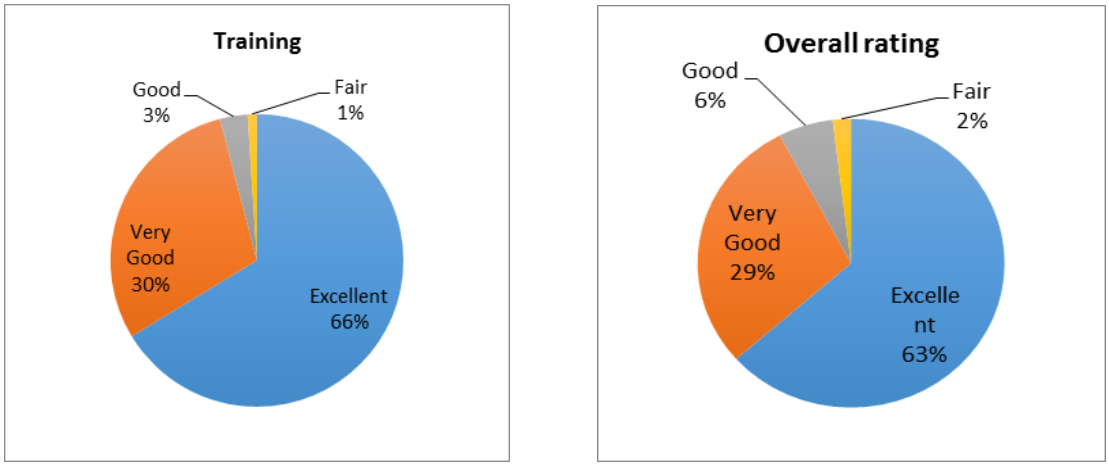


Figure 2 *The overall TA and training associated was strongly positively evaluated, and most importantly the users remarked that the TA had a dramatic role in the feasibility of the project, as without this platform, it would not have been performed.*

Overarching Impacts: It might be argued that no other single project in the nanosafety arena, and few in any arena, delivered so many diverse advances, large and small in such a short time, with so many implications and impact as did QualityNano Many of these outcomes are now so intrinsic to current thinking that they are barely recorded and understood as achievements of QualityNano, and those critical years in which those issues were resolved. In our summary below we will also carefully note what was not achieved. That is just as important, for it defines what is left to be done.

The outcomes that have had the broadest and deepest impact on the community and indeed society at large, are those that were framed in the narrower technical terms, but expressed via networking, dissemination and training outcomes. Very great progress was made in removing from consideration the then broadly held belief that most or at least many nanoparticles possessed acute toxicity. There is now broad agreement that they do not. No single technical achievement could have ensured that clarification of science. The inter-laboratory comparisons were necessary to develop consensus that tests in laboratories were not reliable, but could be made so. It was not sufficient that some laboratories claimed to have understood those issues, the topic was simply too controversial. In themselves the ILCs could not have had this impact without the key role of positive controls, and the realization that not just broad but quantitative agreement would be possible. This changed the conception of the field. It was not satisfying and entirely convincing for overall conclusions that in individual examples agreement could be won by well-prepared groups. It was necessary to understand what had gone wrong, and what continued to go wrong, with the early toxicity tests, and for that the appreciation of just how different the chemical paradigm of exposure and presentation conditions had to be clarified. Above all it was not sufficient that only a few laboratories could have these realizations. It was necessary, month by month and year by year that all of the lines of communications, from networking conferences, to transnational access researchers, to expert groups reaching into the many different domains from industry, regulations and many others should hear of those developments, often, and in different contexts.

Flexibility and Responsiveness of Infrastructures: The future may again raise the issue of how controversial, complex and novel scientific arenas can be clarified and strengthened. Acknowledging that the Infrastructure modality more usually focuses on larger scale, and more established communities, with quite different dynamics, there is a natural question as to whether this could all have been achieved by a different approach. The answer is, probably not. Certainly there were incompatibilities of some elements with the rules of the infrastructure with this kind of challenge. The changing nature of the target or source of controversy as understanding developed rendered a few of the Partners methodologies less central than others with less requests for access,

while others found themselves under pressure to deliver sufficient high-grade effort. The nature of the TA visits were sometimes different to what had been expected, and perhaps usual for an infrastructure and enormous effort in preparation, and clarification of the nature of the sample was required prior to submission to the intended tests.

The rapid growth of interest from young researchers from Eastern Europe was not envisaged, and eventually lead one of the major conferences to be located there, and a new partner adopted to manage the communication and support of those young people, including also career planning. Still, all the necessary changes were accomplished, and the program was enabled and supported to remain flexible and achieve much.

It may also be worth considering the positive benefits of the infrastructure modality, and the means by which the positive outcomes were achieved, and those features that enabled, such a high impact. The multi-tier, and multi-tool aspect of the pillars of an Infrastructure was of central importance in achieving these outcomes. The internal flexibility implied by the Infrastructure concept allowed re-configuration of the balance of effort between Networking, Research, and Training to address the evolving situation on the ground, rather than only what had been envisaged. Had the challenge been undertaken by a single-purpose research project, with fixed aims and approaches the progress that was possible might not have been made. The whole workflow was informed by a capacity to adapt and modify elements to fit the evolving situation, at every level. The adoption of positive controls, and their validation in ILC was more fruitful at some point than seeking to maintain the a broader range of materials. New projects, and new activities also grew up, making specific actions or intentions of QualityNano less important, a good example being the nanomaterial repository. On the other hand, the difficulty and limitations of storing materials in a repository could then have more directed attention. The disentangling of the causes of confusion and controversy also required flexibility, and adaptation as the shape of the controversy and uncertainty evolved.

At an early stage, with the measure of the origins of the irreproducibility and uncertainty more clear it was considered that the best approach was to separate the question of ‘real’ toxicity from ‘perceived’ toxicity (resulting from methodological limitations). This issue of perceived toxicity was then dealt with using Networking tools, as well as Transnational Access to strengthen community practices. It also grew to become a priority and a prerequisite in building up excellence within the community, for until those issues were settled, no real progress could be considered, at least across a wide variety of laboratories. The means and tools to ensure the discipline then became an overarching concern. The degree to which there could be real toxicity progressively (but not exclusively) became focused via the Research pillar, and the two strands were then re-unified within the ‘Integrating Conferences’.

What remains to be done, and reflections:

In such a large activity, with so many dimensions, and actors, not everything is accomplished. It is too early to tell, as the final status of the activity will only be seen in future years, as to its final impact. However, there are some considerations that may be of interest in future, including also to those who confront such challenges in future emerging areas of science. Looking back it is difficult to imagine the extraordinary passions and controversy raised by some questions now considered clear. Above all is the message that despite temporary confusions, controversy, often deemed difficult and intractable at the moment, science works, when we have confidence in it, and pursue it to its end. It is not people alone who resolve controversies, but the scientific process, as long as the process remains flexible, open, and persistent, and insistent.

In our current understanding, the Infrastructure played perhaps even a central (but not sole) role in disentangling and restructuring the highly complex mixture of uncertain and irreproducible science

that was driving such uncertainty at every level from research to policy, and stabilizing the science. It has progressively changed the focus from those issues that were dead-ends, and spurious, to those that are truly substantive. It did not, nor could it, decide the higher level issue as to whether nanoparticles exhibit a new form of toxicity not previously understood, likely (if such exist) at longer time periods than current acute toxicity tests.

Future actors in new fields where similar cocktails of issues arise may also find themselves asking if the balance between addressing and resolving uncertainty in the community, and addressing the most deep questions of long-term hazards, for which a much more narrow focus would have been required, was the right one. There is little doubt that, when QualityNano was conceived and throughout much of its existence, its focus and role was so vital in addressing the complexities and confusions of short term toxicity that no such focus was possible.

We have therefore made very great progress, but we are far from finished. We have little understanding of the nature and consequences of long term accumulation within organs, and few tools have developed to allow for a depth of understanding there to grow.

We would not wish the very success and conclusiveness of the message and outcome in short term toxicity won within QualityNano and beyond to endanger and obscure the need and commitment required to address remaining uncertainties. Those uncertainties are very real, as are the challenges. The success though of QualityNano should however send a clear message to all. Science when properly and fully executed clarifies, and resolves, and consensus grows by the process of excellent disciplined reproducible science, widely communicated. If we have confidence in that, we can finish the process.

Appendices by Work Package

WP2 addressed the overall QualityNano objective of "Establishment of the Nanomaterials Hub which involves the development of positive control nanomaterials for selected biological end-points (e.g. apoptosis) and their testing via RR, lead by Beneficiary 1 (NUID UCD).

As a WP in the networking pillar, the objectives of WPs were primarily to support and integrate the European nanosafety community, which was achieved in large part via:

(i) round robin (RR) and Interlaboratory Comparison (ILC) studies to develop and implement Standard Operating Procedures (SOPs) and best practice in nanomaterial safety characterization;

(ii) development of guidelines and protocols for storage and curation of nanomaterials and assessment of the impact of storage conditions on NMs ageing as indicated by changes in their physic-chemical characteristics; and,

(iii) the development and documentation of representative positive and negative control nanomaterials for a series of endpoints including apoptosis and cell cycle arrest.

Activity

A major objective from the outset of QualityNano developed within WP2 was also to support the elevation of the overall research standards in the community via the implementation of large interlaboratory comparison (ILC) studies involving multiple (>10) labs using agreed protocols and nanomaterials, and the provision of training in implementing these protocols. Thus, a significant effort has been dedicated to the design, implemention and data interpretation from a number of ILC and Round Robin (RR) tests, assessing both physico-chemical characterisation of NMs and impacts of NMs on cellular end-points. A range of approaches were taken in order to assess the community's needs in terms of training in the use of the protocols. Several interesting long term results emerged.

One of those was an analysis of the different mathematical models underpinning the software in different instruments, and some clear recommendations regarding the optimal algorithms for calculating NM size and size distribution. This certainly was found to be relevant to problems in measurement, though perhaps not the core of the issue that was causing widespread controversy.

A summary of the physico-chemical ILCs/RRs is presented in Table 1 below.

Table 1: Summary of the physico-chemical characterisation ILCs/RRs undertaken in QualityNano

Physico-chemical endpoint	**Method utilised**	**RR / ILC or both**	**NMs utilised***
Particle size (in water)	DLS	ILC before/after training	Silica NPs (20 and 100nm) 50nm NH_2-PS and COOH-PS (positive / negative control) NPs
Particle size (in water, in cell media ± BSA)	NTA	ILC – 4 rounds of discussion & refinement of protocol	NIST PS NPs (100 & 200 nm) Gold NPs (60 and 80 nm)
Particle size (in water)	DCS	RR (4 expert labs) + further SOP refinement	Silica nanoparticles (100nm)
Particle size (in water)	DCS	ILC (7 labs)	Silica nanoparticles (100nm) 50nm COOH-PS NPs

* Results to be published in special issue of outcomes from QualityNano.

In parallel, optimization of existing standard operating procedures (SOPs) for test methods evaluating selected biological end-points (cell viability, apoptosis, NM uptake, cell cycle disruption, and oxidative stress) via focused round robin (RR) studies within small groups (3-4) of expert QualityNano laboratories and ILCs with the community more broadly was also progressed. The most extensive ILC was that of cell viability using the MTS assay, for which 2 complete cycles were performed, before and after training in terms of the protocol and all aspects of good laboratory practice, such as why the order of mixing reagents might be important, and what is considered acceptable in terms of variability etc. The results of this show an increase in the number of partners whose datasets meet the method's compliance requirements, and an improvement in the comparability of the data across laboratories following the training. Another important outcome from this is the detailed comments from ECVAM on the protocol for the assessment of NM-induced *apoptosis (caspase activity)* which provided additional insight into the level of detail and thought involved in standardisation of SOPs.

Table 2: Summary of the biological ILCs/RRs undertaken in QualityNano

Biological endpoint	**Assays utilised**	**RR / ILC or both***	**NMs utilised**
Cell Viability	MTS assay	ILC before/after training + further SOP refinement	50 nm NH_2-PS and 40 nm COOH-PS (positive / negative control) NPs
Nanoparticle uptake	Flow cytometry	RR (3 expert labs) + further SOP	40nm and 100 nm Fluorescent COOH-PS NPs

		refinement	
Apoptosis	Caspase activity	RR (3 expert labs) + further SOP refinement & standardization	50 nm NH_2-PS and 40 nm COOH-PS (positive / negative control) NPs
Cell proliferation	Edu incorporation	RR (3 expert labs) + further SOP refinement	50 nm NH_2-PS and 50 nm COOH-PS (positive / negative control) NPs
ROS generation	DCF assay	RR (4 expert labs) + further SOP refinement	50 nm NH_2-PS and 50 nm COOH-PS (positive / negative control) NPs

Assessment of approaches for curation and long-term stability of nanomaterials

Another key gap, identified at the project mid-term review, was the lack of data and understanding regarding the ageing of NMs during storage, whether as powders or as dispersions. Thus, a new task was introduced to address this gap and assess the long-term stability of NMs in powder and dispersion form as a function of the storage conditions. This was achieved by three independent laboratories undertaking detailed physico-chemical characterisation of stock samples of four solid NMs (ZnO, CeO_2, TiO_2, SiO_2) and their dispersions in MilliQ water monthly over a period of 6-9 months. Similar studies were performed on the -COOH -NH_2 functionalised PS NM dispersions to assess their long-term stability.

WP3 NA3 Knowledge Hub for Nanosafety

The vision of QualityNano's Knowledge Hub was to develop a centralised resource to address the training and outreach needs in the area of processing, analysis and characterisation of nanomaterials for use in biological applications, focussing initially on the priority needs of regulators and industry for a consensus approach to nanosafety assessment.

At the outset of QualityNano, there was a critical need to provide training in good practice (as it existed then and the advances that emerged from with the QualityNano Joint Research and Networking Activities WPs, as well as from FP6 and FP7 projects, and national efforts) for young researchers and for analysis and characterisation facilities who are only beginning to apply their tools to the field of nanobiology. While re-training of existing scientists in this arena was seen to be an immediate priority, strong emphasis was also placed on the framing of a new generation of experimental scientists who were comfortable working at this interface between materials and biology, thereby strengthening not just nanosafety, but many aspects of nanomedicine, and other field where these skills were relevant. This was seen to be a very considerable challenge, and QualityNano sought to initiate, promote and otherwise push for that development. Even within the group of the people that helped run QualityNano, a number of young people emerged to academic positions, and ERC fellowships, having seen and understood first-hand the needs going forwards. They will be a durable asset in the coming years.

Activity

Assessment of training needs and the draft nanosafety training pathway (model curriculum)

Jointly with NanoTOES (a Marie Curie Initial Training Network) a survey of the needs of current students and of the courses available in 2011 was conducted via the QualityNano website and reported as Deliverable report D3.1 (publicly available). Building on this, an outline training pathway was built according to experience mostly derived in the ongoing ITN NanoTOES which aims to establish a "gold standard" for the training of young experts in nanosafety.

The training pathway integrates topics specific for nanosafety (e.g. regulation of nanomaterials) with topics that are standard content for a modern PhD program (e.g. scientific writing). However, even training modules which are general in topic can be matched specifically to the need of nanosafety training. For example, a scientific writing course can include information on what should be reported for studies in this field, like number, mass and surface area of all particles used, controls for contamination of batches, etc. It is structured into an introductory stage, an intermediate stage and an advanced stage. While the assignment of some topics is flexible, it is clear that some issues should be covered at specific times, like good laboratory practice in the beginning and job seeking skills towards the end.

European Training schools, including hands-on training sessions

A number of highly successful training schools, covering topics ranging from *modelling of nanoparticle toxicity* to *good laboratory practice for nanosafety assessment* through *embedding environmental realism in nanosafety assessment* were organised and delivered via the QualityNano platform. Each training course had a number of expert speakers from QualityNano and beyond, and each involved a significant hands-on element, be that on computers for the modelling school, via analysing experimental protocols for the Good Laboratory Practice school, or getting stuck-into mixing nanoparticles and soil (and subsequently trying to get them back out for characterisation) in the Ecotox training schools (see photo above). Deliverable reports from each of the training schools are available on request, and slides, recordings and other materials are available via the QualityNano website (repository of training materials; http://www.qualitynano.eu/the-qnano-knowledge-hub/repository-of-training-materials/qnano-funded.html).

WP4 NA4 – Support for NanoSafety Cluster and community activities

Support for the NanoSafety Cluster in terms of developing a roadmap:

- Contributions to 11 of the 14 original chapters of the EU NanoSafety Cluster Research Roadmap *Nanosafety in Europe 2015 – 2025: Towards Safe and Sustainable Nanomaterials and Nanotechnology Innovations,* including Chairing of 3 of the chapters.
- Developed cross-cutting chapter on Infrastructure needs for nanosafety assessment for that publication.
- Supporting efforts in Europe and US-EU collaborations to harmonise research database requirements and ontology for nanosafety assessment
- Comparison of range of occupational exposure methodologies developed in FP7 project NanoDevice underway as basis for best practice in occupational exposure monitoring and risk management

QualityNano International conferences

QualityNano hosted three major integrating conferences during its 4.5 years: the first was co-organised with its predecessor project NanoImpactNet in Dublin from 27-29th February 2012, the second was held in Prague from 27th February - 1st March 2013, in order to increase the participation of nanosafety scientists from new member states; and the final one was held jointly with FP7 project NanoReg in order to maximise transfer of knowledge between the projects. Indeed, NanoReg is one of the main targets for the special issue from QualityNano (see below) in terms of the hand-over of protocols, data regarding the

positive and negative control nanomaterials, outcomes from Inter-laboratory and Round Robin tests, and more. The final conference, from 15-17th July 2015 in Crete, was attended by over 150 delegates, and represented an impressive end to a complex and important EU infrastructure project.

Facilitation of the Expert Resource Groups

QualityNano interacted on various different levels with different governmental and public bodies. The project implemented four expert panels to ensure an efficient dialogue between different international stakeholders and to provide input and advice to the project.

In the **regulatory resource group** QualityNano interacted regularly with different European agencies including EMA (European Medicines Agency), ECHA (European Chemicals Agency), EFSA (European Food Safety Authority), national regulatory authorities, the European Commission, in particular DG (Directorate General) ENTR (Enterprise and Industry), DG ENV (Environment) and DG SANCO (Health & Consumers) and international agencies such as US EPA (Environmental Protection Agency) or CPSC (Consumer Product Safety Commission) or Australian APVMA (Australian Pesticides and Veterinary Medicines Authority). Via these interactors QualityNano also contributed to ongoing efforts on the OECD level.

In the **standardization resource group**, QualityNano interacted regularly with members of ISO (International Organization for Standardization), CEN (European Committee for Standardization) or NIST (US National Institute for Standards and Technology), which linked it to ongoing activities in ISO/TC 69 (Application of statistical methods), ISO/TC 229 (Nanotechnologies) and to CEN/TC 352 (Nanotechnologies).

Via the activities within the standardization resource group, QualityNano was invited to a standardization workshop with different EC project officers on 27th March 2015 in Brussels.

In particular in the field of standardization QualityNano was able to develop best practice methods to measure, characterize, quantify and analyse biological/ toxicological effects of nanomaterials. As described above, QualityNano was already able to engage with different governmental bodies and policy makers. The expertise of QualityNano has been handed over the EU FP7 project NanoREG, which is interacting even more strongly and directly on different levels with policy makers. NanoREG will therefore also use expertise from QualityNano and will give scientific advice to policy makers.

Documentation on the positive and negative control Nanomaterials

At the time of writing the QualityNano proposal, and again at the revision as part of the mid-term review process, provision of positive control nanomaterials that exert specific and reproducible biological impacts and negative control nanomaterials to eliminate a particle-specific effect, were identified as amongst the most pressing needs for the EU nanosafety research community. Ideally, a positive control should behave mechanistically as close to the study material of interest as possible, and certainly apoptosis is a broad endpoint of interest. A successfully applied positive control nanomaterial has the general presumption that the particles were dispersed appropriately, and that the organism is responding normally. This task has required intense work by many people, and the technical challenges were significant in implementing.

During the QualityNano conference in Prague in 2013, the first in a series of positive and negative control nanomaterials indicated in the Description of Work was launched:

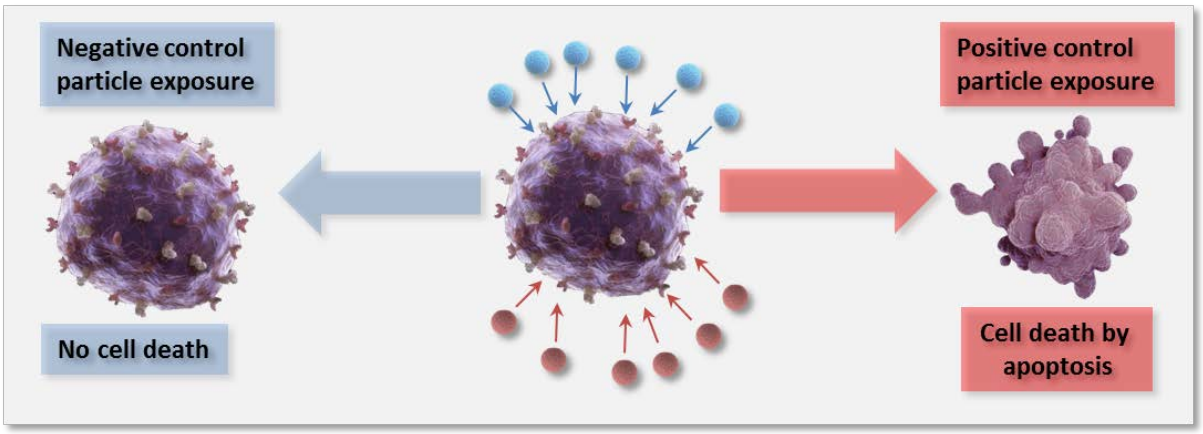


Figure 3: Schematic illustration of the positive and negative control NMs for apoptosis.

Progress in the round robin assessment of the cytotoxicity of positive and negative control nanomaterials

Following the completion of the first round of the benchmarking studies of partner laboratories for performance of biological tests, which involved A549 cell culture and mycoplasma testing (phase 1), and determination of A549 cell growth rate (cell doubling time; phase 2), the first RR assessment for cytotoxicity, both chemical- and nanomaterial (NM)-induced, was undertaken using the MTS assay (phase 3). In total 13 participants sent their test results to VITO for review and assessment of compliance with the test acceptance criteria stated in the SOP. Results have either been provided in excel templates via e-mail or reported through a web-based form created by UCD. VITO has performed statistical analysis of the data.

The cytotoxicity data have been evaluated in R using methods for proficiency testing by interlaboratory comparisons, as laid out in ISO 13258:2005. The statistical approach here is based on a consensus value from the participants that is calculated as the robust average of the results reported by all participants in a round of the proficiency testing scheme. Laboratory biases are interpreted on the basis of the calculated robust standard deviations. Within laboratory results of laboratory L12 are shown as an example in the left hand column of Figure 4, and the between laboratory comparison is shown in the right hand column of the same Figure. When a laboratory reports a result that gives rise to a laboratory bias greater than 3 SD or less than 3 SD, the result shall be considered to give an ‘action signal’. A laboratory bias above 2 SD or below 2 SD shall be considered to give a ‘warning signal’. A single ‘action signal’ in one round, or two ‘warning signals’ in successive rounds shall be taken as evidence that an anomaly has occurred that requires investigation. Corrective actions, such as refinement of the SOP based on a detailed investigation of the laboratories’ filled out forms, and repeat of the tests are planned in this case to achieve reproducibility within and across labs.

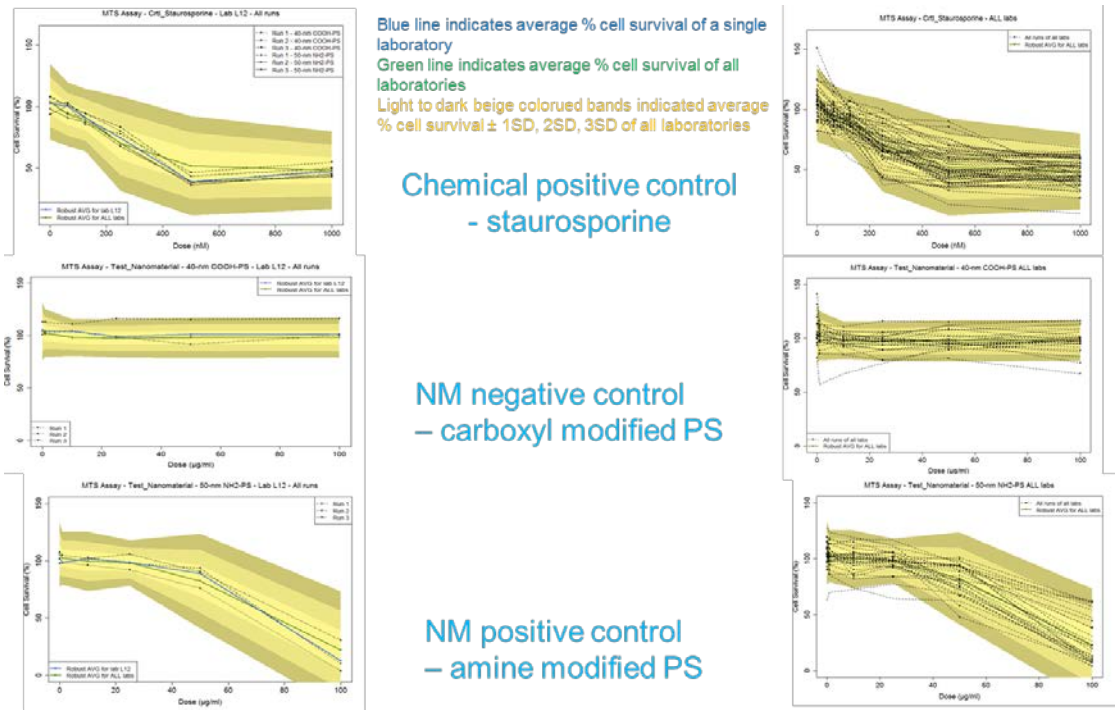


Figure 4. Left hand side: **Within laboratory comparison of nanomaterial-induced cytotoxicity using 50 nm amine-modified polystyrene (NH_2-PS) as positive control nanomaterial.** NH_2-PS nanomaterial (50 nm) was tested as a positive control nanomaterial in 3 runs concurrent with a positive control chemical. Cell survival (%) as compared to untreated cells is plotted as response to a concentration series of 50 nm NH_2-PS (0, 1, 10, 25, 50, 100 µg/ml). For each run a mean of 3 replicate measurements per concentration is shown. A robust average (AVG) for laboratory L12, calculated on the basis of the mean % cell survival of all runs, is indicated with a blue line. For interpretation of intra-laboratory bias, the robust average and standard deviation (SD) calculated from the mean % cell survival of all runs from all laboratories are shown as a green line and boundary areas (yellow: AVG ± 1 SD, light brown: AVG ± 2 SD and dark brown: AVG ± 3 SD), respectively. Right hand side: **Interlaboratory comparison of nanomaterial-induced cytotoxicity using 50 nm amine-modified polystyrene (NH_2-PS) as positive control nanomaterial.** NH_2-PS nanomaterial (50 nm) was tested as a positive control nanomaterial in 3 runs concurrent with a positive control chemical by all laboratories. Cell survival (%) as compared to untreated cells is plotted as response to a concentration series of 50-nm NH2-PS (0, 1, 10, 25, 50, 100 µg/ml). For each run a mean of 3 replicate measurements per concentration is shown. For interpretation of laboratory bias, the robust average and standard deviation (SD) calculated from the mean % cell survival of all runs from all laboratories are shown as a green line and boundary areas (yellow: AVG ± 1 SD, light brown: AVG ± 2 SD and dark brown: AVG ± 3 SD), respectively.

High Content Analysis assessment of cellular response to the positive/negative control NPs:

High Content Analysis (HCA), an automated epifluorescence microscopy approach with proprietary acquisition/analysis software was used to assess cellular cytotoxicity in a multiparameter approach. HCA was performed.[5] Briefly, 5×10^3 cells were seeded in a clear flat bottom 96 well plate (Cell Star) in 100 µl of cell culture medium containing 10% FBS. After 24 hours nanoparticle dispersions were prepared as 3× the final concentration required in cell culture medium containing 10% FBS, then 50 µl of particle suspensions were added to the cell-containing wells to reach a 1× concentration. Equivalent volume of water to the highest volume of nanoparticles was applied as vehicle in all experiments. The dispersants from the nanoparticle suspensions was tested at the same volume which did not induce any alteration of the parameters analysed (data not shown). Cells were incubated with nanoparticles at a final concentrations of 0.3 µg/ml, 0.7 µg/ml, 1.5 µg/ml, 3 µg/ml, 6 µg/ml, 12 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml, for 24 or 72 hours. After 1 hour incubation, cells were analysed by High Content Analysis using the Arrayscan VTI 740 (Thermo Scientific). Images were acquired using a 20× objective and fluorescence intensities were collected using the following combination of excitation/emission filters: Data was exported to Prism where the EC_{50} and IC_{50} were

calculated by fitting the data with a sigmoid curve. Data are shown as mean +/− SD of 45 fields acquired from three independent experiments performed in triplicate. The detailed properties of Lysotracker green positive lysosomes were analysed using the Spot Detection Bioapplication. Lysotracker positive objects were identified and separated using a 3 sigma algorithm. Cell details were acquired and they were shown as mean +/− SD of a representative experiment performed in triplicate.

WP5 JRA1: Strategies to eliminate / reduce nanoparticle batch-to-batch variability

Current industrial and laboratory processes for synthesis of nanoparticles introduce quite significant variability from batch-to-batch. Such variations are quite well known, and some efforts are being made to reduce them for high added value applications. However, while such variability may not impact on the industrial applications of nanoparticles (such as catalysis, material strengthening, energy conversion etc.), they may have very significant implications for the evaluation of biological impacts of the nanoparticles. Thus, different detailed biological outcomes from different batches (even if none implies any real hazard) introduce a lack of certainty in the science. To some degree the (immediate) need for standard materials (for say the OECD materials) can be addressed by purchase of a large single batch. However, it is clear that in the long term this will not work, because of the limited shelf life of nanoparticle dispersions, which may be as short as 3 months, and full *in vivo* investigations, which can take 2 years. There is also the question of how 'representative' a single batch is. For these reasons it is necessary to have more reproducible batches (or sufficiently 'representative' batches in sufficient quantities) in order to really progress knowledge and remove uncertainty in the field. Thus, research is needed in order to identify the source of these variations and to develop strategies to eliminate or reduce them, including the evaluation of currently available methods (for example continuous flow) to address the problem and assessment of band width in biological response. The creation of negative nanosafety controls will also be possible.

Variability sources: The identification of potential sources of variability of physico-chemical properties of nanoparticle batches and their reduction is therefore an important task. As a first step towards to reach this goal OECD proposed physico-chemical parameters for nanoparticle batches were measured. Fifty-two batches of SiO_2, TiO_2, CeO_2, PS and ZnO were prepared under carefully controlled conditions to relate the synthesis conditions with the measured results.

The initial observation was that the size distributions of the particle batches vary significantly. The size distributions of particles from liquid phase synthesis are narrow; however, partial agglomeration is observed. The batches produced by liquid phase synthesis are partly contaminated by low concentrations of metals (e.g. Cu). Additionally, It became apparent that post-synthetic treatments commonly applied in the purification of nanoparticle dispersions, such as centrifugation approaches, can cause shifts of particle size distributions in dispersions, as particles can cross-link. For liquid phase synthesis, dispersion reproducibility can be "designed into" the synthetic procedure by subsequent optimisation processes, which involve careful parameter variation coupled with extensive characterisation. Materials from flame synthesis are mostly aggregated and have broader size distributions. However, silica particles from flame synthesis have a higher chemical purity than the ones produced by Stöber synthesis.

The first strategy was to work with industry groups of suppliers and require exact manufacture procedures for specific processes and products. A questionnaire for ZnO synthesis routes, raw materials selection, handling and storage of raw materials and products, and possible contaminations has been sent to 16 companies. Characterisation of commercial ZnO NPs (16 products) in terms of

the morphology, dispersion characteristics, and solubility, have been performed by SEM, TEM, XRD, DLS and FTIR. TiO2 NPs synthesized at ICN have been compared with the commercial aeroxide P25 form Sigma Aldrich, which is one of the most used TiO2 NPs. Characterization in terms of morphology, crystallinity, and dispersion characteristics has been done by TEM, XRD and DLS. The results showed that current industrial routines for synthesis of NPs have quite significant variability from batch-to-batch.

The second strategy is to work with QualityNano partners, turn to lab synthesis routines, and assess the sources of batch-to-batch variability. The major variability factors that influence the synthesis of NPs have been identified as impurities, atmosphere, reaction time, temperature, mass gradients and synthesis conditions. The third strategy was to analyse experimental data to identify the effect parameters and address the challenge of process control of NP synthesis. Depending on the raw materials selection, synthesis condition control, and post cleaning methods, the size distribution of NPs was significantly different. We have demonstrated that higher levels of reproducibility are possible through using parallel reactor systems and strictly controlled reaction parameters. Absolute size distribution reproducibility may not be possible, but levels could be specified to suit specific applications. The fourth strategy was to develop SOPs on methods for synthesis of NPs that minimize variability and to minimize the sources of batch-to-batch variability in lab synthesis routines.

The studies performed included cleaning, extraction of undesirable catalysts, monomers/reactants, and undesirable biological contamination of the nanoparticles for use in studies of selected biological end-points (apoptosis, cell cycle disruption, oxidative stress and genotoxity). Batches of candidates for positive and negative control nanomaterials were evaluated for their suitability for biological safety assessment using mini round robin studies organized by endpoint or mechanistic effect.

This work was performed with the aim to identify sources of batch-to-batch variability of candidate positive/negative control nanoparticles for biological safety assessment, and to produce test batches with low variability and evaluate them using most sensitive, representative assays organized by endpoint or mechanistic effect.

WP6 JRA2: Optimisation of nanoparticle traceability and reliable labelling strategies

Three main labelling strategies were investigated within QualityNano: radiolabelling, stable isotope labelling (enrichment) and optical labelling. Work during the second part of the project looked also at dual labelling strategies such as labelled core and labelled shell as a means to independently trace the fate of the core and the shell, as the shell may be broken down and removed from the particle surface. Some preliminary investigation of the impact of chemical labelling (i.e. doping) of nanomaterials, and the effects of substitutions of one atom for another on resulting particle stability and interactions have also been undertaken. Highlights from WP6 are presented below.

Radiolabelling strategies for nanomaterials

A number of priority nanoparticles were identified at the outset for labelling with radio isotopes, including SiO_2, TiO_2, CeO_2, Polystyrene, ZnO (all on the OECD priority nanomaterials list for testing via their sponsorship programme), with the requirement that the labelling should not affect the particles' dispersability or surface characteristics. In reality a much wider variety of NMs were labelled than originally envisaged, and a wide range of approaches for radio-labelling of NMs was developed. Approaches utilised included ion-beam activation of existing particles, a novel recoil method that involves implantation of nanoparticles with Be-7 created in a Li-containing source material under proton irradiation, and a bottom-up approach that starts with a radioactive precursor material that is used for chemical synthesis of the nanoparticles. Each of the approaches investigated

has been published and a summary of the approaches and the particles that have been labelled in this manner is provided in Table 3 below.

Table 3: Strategies for radiolabelling of nanomaterials

Radio-labelling method	Nanomaterials labelled via this method
Direct ion-beam activation of particles	^{48}V labelled TiO_2 nanoparticles ^{56}Co labelled radioactive Fe_3O_4 nanoparticles ^{7}Be labelled carbon-based nanoparticles ^{139}Ce labelled ceria nanoparticles ^{141}Ce labelled ceria nanoparticles ^{65}Zn labelled ZnO
Recoil implantation method	^{7}Be labelled SiO_2 nanoparticles ^{7}Be labelled TiO_2 nanoparticles ^{7}Be labelled carbon-based nanoparticles
Radiochemical synthesis	^{56}Co labelled SiO_2 nanoparticles ^{105g}Ag labelled silver nanoparticles TiO_2 radiolabeling with $^{44,45}Ti$ radionuclides ^{195}Au labelled gold nanoparticles

Dual labelling strategies – radio-labelled core and labelled shell

Dual radiolabeling using two gamma emitters with different emission energies has been developed by CIC to independently label the core and the surface of NPs. Poly(lactide-co-glycolide) NPs (PLGA NPs) were selected as the demonstrator for this approach. Because of their size and the use of emulsion techniques for their preparation, a new approach for the radiolabelling could be implemented based on the encapsulation of smaller radiolabelled metal oxide nanoparticles inside the core. First, iron oxide NPs entailing ^{111}In and stabilized with oleic acid were prepared; iron oxide NPs were encapsulated into the core of PLGA NPs by mixing them with the PLGA phase during emulsification. Bovine Serum Album (BSA) was used as stabilizing agent for the emulsion droplets, facilitating the incorporation of ^{125}I, the second radioisotope, by electrophilic substitution on the tyrosine residues of the protein. These particles have been utilised in biodistribution studies very effectively, with results indicating that ^{125}I labelled BSA is removed progressively from the core and follows a different biodistribution pattern and clearance than the core.

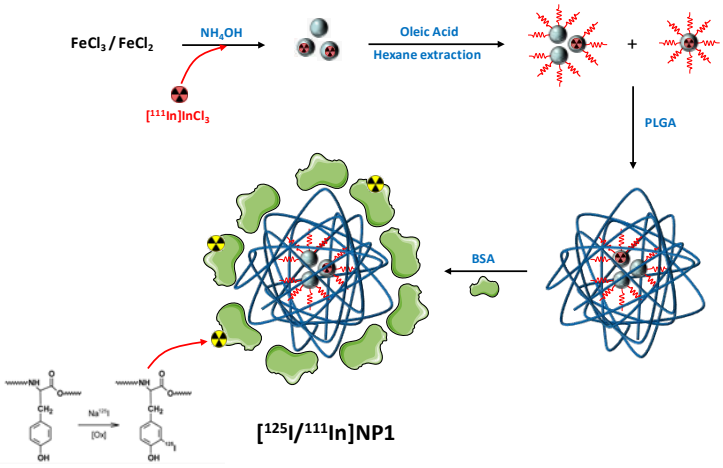


Figure 5. Schematic route followed for the preparation of dual radiolabelled NPs.

Stable isotope belling strategies for nanomaterials

Stable isotope labelling strategies involve the enrichment of rarer stable isotopes such that the labelled particles can be distinguished from background particles of similar elemental composition, and thus are especially useful for tracing nanoparticles in the environment. Similar particles were selected as priorities for stable isotope labelling as above, although the range of strategies available is lower, since the principle approach is to start from an rare isotope and use chemical synthesis approaches – thus labelling cannot be performed on pre-existing particles in this case.

Within QualityNano, procedures for stable-isotope labelling of a number of particles were refined, including AgNPs, CuO/CuO_2 NPs, CeO_2 NPs and ZnO NPs, and the processes for stable isotope labelling NPs have been taken forward towards standardisation via CEN TC352.

Optical labelling of nanoparticles (fluorescent and near infrared)

A wide range of optical labelling strategies were developed and optimised within QualityNano and their dispersion stability and properties compared to the unlabelled equivalents. In the case of optical labelling, core and surface labelling strategies are possible but surface labelling has generally been assumed to alter the surface properties of the nanoparticles, unless a subsequent shell is added, either of the unlabelled core material or using a polymer coating for example, which is a common strategy for stabilising and capping nanoparticles.

Table 4: Strategies for optical labelling of nanoparticles.

Nanomaterial	**Labelling strategy**
SiO_2	FITC labelling -label added directly to Stöber synthesis (altered NP stability) - Core shell approach - additional silica shell around labelled core (Stober-ArgSil NPs)
CeO_2	Surface modification with a fluorescent dye followed by formation of a polymer shell such that the labelling didn't affect the surface - Electrostatic attachment - Covalent attachment
AuNPs	Glucose-coated Au NPs labelled with a HiLyte Fluor 647 label

WP7 focus has been the development of new methods that were capable of detecting and provide quantitative information on nanoparticles in complex media.

A large variety of nanoparticles with different characteristics and properties are on the market and applied in consumer products where these particles show high interaction with their surroundings, which makes them difficult to measure and quantify.

Furthermore, all particles, and in particular nanoparticles in a physiological or biological milieu, are rapidly coated with a range of biomolecules (forming the 'corona') and it is this (if sufficiently long lived) that is presented to the organism. Thereby it is these biomolecules that confer a biological identity onto the nanoparticles, while the pristine nanomaterial surface remains buried and not available for binding.

Indeed, the situation is additionally complicated by the fact that aggregation may occur over the time period of the experiment (*in vitro* or *in vivo* during digestion and in the various body and cellular compartments), and inattention to detail of which biological fluid (for example, bovine or human serum are quite different) may be an additional factor in interpretation of observed biological impacts. Thus, characterising nanoparticles in aqueous solutions or simple buffers is of value in standardising the basic understanding of materials, but not sufficient to truly understand the nature of

the entity in situ in a biological system. A full, time resolved understanding of the nanomaterial *in situ* is obligatory, even to make meaningful reproducible studies.

Within WP7 it was the development and implementation of new approaches for time-resolved characterisation of nanomaterials (*in situ*) in complex biological milieu. Strength and limitations of methods such as Dynamic Light Scattering (DLS) and Differential Centrifugal Sedimentation (DCS), Particle-Induced X-ray Emission (PIXE), Confocal Raman Microscopy (CRM), Fluorescence Correlation Spectroscopy (FCS), and Coherent anti-Stokes Raman spectroscopy (CARS) have been studied.

The conclusion of these studies is that classic physico-chemical characterisation techniques such as DLS, which is well suited for monodisperse and homogeneous samples, need to be combined with other methods which are more reliable in the case of polydisperse samples and in the presence of complex fluids, such as the biological media in which NPs are dispersed prior to these tests.

Overall DCS has shown to be a really powerful technique, and during QualityNano its use has been extensively evaluated for different kinds of nanomaterials and in different complex media. A significant advantage of DCS measurement is that the instrument can successfully resolve multiple populations over wide size ranges from a few nanometers to microns within the same sample. Moreover, it allows measurement of NPs incubated with fluids of any kind (in situ) as the biomolecule background will not significantly impact the NP measurement due to their very different sedimentation time.

Moreover, the dispersions need to be characterised not only at the time of mixing, but also for the full length of the experiment, in order to monitor eventual changes during exposure to cells (or other organisms) and in the conditions applied.

Another objective of WP7 was the development of new approaches to characterise the interaction of nanomaterials with their matrix in complex matrices as consumer products and food. To undertake meaningful toxicological and risk assessment studies, a proper understanding of the size, shape, composition and agglomeration state of nanoparticles and their interactions with the surrounding matrix is of the utmost importance. Experiments show that nanoparticles in consumer products, food, and biological fluids are rapidly coated. Often they are coated with proteins or other organic materials originating from the matrix. In addition, matrix constituents in food can stabilize as well as destabilize nanoparticles, and even break-up agglomerates, resulting in more coated nanoparticles. As a consequence it is likely that exposure of organisms and consumers to nanoparticles is often not to the bare, but to coated nanoparticles and agglomerates.

Successful methods were developed and published in scientific papers. Furthermore, their strength and limits were compared in the Round Robins (RR) and method comparison conducted in this work package.

WP8 JRA4: Optimal modes of presentation of nanoparticles to cells, tissues, organisms

Introduction

Reliable predictions of nanoparticle fate and impact based on toxicity studies suffer from insufficient standardization and methods for quantification of nanoparticle presentation. This summary highlights strategies to improve comparability and standardization of nanotoxicological studies. The objective of WP8 was to advance the exposure scenario for toxicity studies and to develop controls, including the evaluation of various types of cell culture dishes, measurement of dose, and dose rate, and critically the time-dependent concentration of the presumed paracrine signallers.

WP8 pursued a survey of the *optimal modes of presentation of nanoparticles to cells, tissues, organisms and animals.* It developed recommendations for surface-weighted particle dosing and nanoparticle number per cell as the biologically relevant dose parameters. Effects of the dispersion methods on the size and the surface composition of nanoparticles and their implications in toxicology assessment were studied by all partners in WP8. In addition the role of fluidic containers and culture dishes on accumulation and uneven particle distributions was studied.

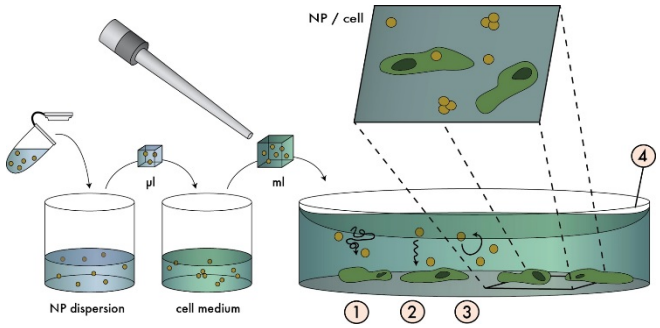


Figure 6: Schematic drawing optimal modes of presentation of nanoparticles to cells in an in-vitro assay

Label free assessment of nanoparticle dose is important for testing industry nanoparticles in a routine fashion. WP8 compared the quantitative capacity of previous radioactive aerosol inhalation and biokinetics studies at HMGU with new non-radioactive aerosol inhalation and biokinetics methods at RIVM. The RIVM received equipment from HMGU-Germany as part of the replacement of HMGU as a partner by RIVM. A Radioactive Isotope laboratory was set up and expose rodents in the facilities previously not meant to have animals. Technicians were trained with procedures for handling and exposing rats with an initial exposure to nanogold particles and to perform the intratracheal intubation of laboratory animals to nano-particles in the plethysmographs of the HMGU equipment. The amount of gold in the organs, lung, heart, liver, spleen, kidney, blood, skin, was analysed using Instrumental Neutron Activation Analysis (INAA). Optimisation and advancements of transport studies using isotopically labelled material was carried out by *Partner 7 (FUNDP).* FUNDP analysed and quantified nanoparticle uptake and crossing throught a multilayer tissue model, the epidermis barrier. FUNDP received characterized radiolabelled NPs from the JRC cyclotron facility and investigated nanoparticle uptake and transport in a fully stratified reconstructed epidermis at the air-liquid interface.

The role of *autocrine and paracrine signallin g*is potentially a missing link in understanding chronic effects from lower doses of nanoparticles. In recent years it became evident that we should shift the focus of toxicological studies from 'live–dead' assays to the assessment of cell function. Up-regulations of various inter-cellular signalling processes induced by nanoparticle entry into a specific cell have the capacity to lead to cell damage in neighbouring cells, whilst leaving the cell into which the nanoparticle actually entered, intact. UCD (Partner 1) has shown that nanoparticles can induce *indirect effects* to cells not directly in contact with the nanoparticles themselves by activating signalling pathways leading to release of cytokines and other signals to neighbour cells. UCD has investigated paracrine signalling activated by in vitro blood brain barrier models exposed to carboxylated polystyrene nanoparticles. They found that exposure of the BBB models to these nanoparticles in the presence of underlying astrocytes caused a significant increase in pro-survival signalling in comparison to what observed in their absence, confirming that paracrine signalling can occur across different cells upon exposure to nanoparticles.

The LMU group developed a single cell platform based on micropatterning techniques for standardized exposure of cells with NPs. The use of micro-patterned single-cell arrays allows for

real-time recording of nanoparticle-induced apoptosis in thousands of cells in parallel. The fluorescent signals of the apoptosis indicators are simultaneously monitored after adding cationic nanobeads. Experiments showed that the distribution of onset times shifts to later times and broadens as a function of decreasing NP dose. The systematic change in the time differences of the early apoptotic and late apoptotic markers indicate that the apoptotic process alters during the time course of the experiment. The work demonstrated the potential of array-based single-cell cytometry for higher-throughput screening and kinetic analysis of nanoparticle toxicity.

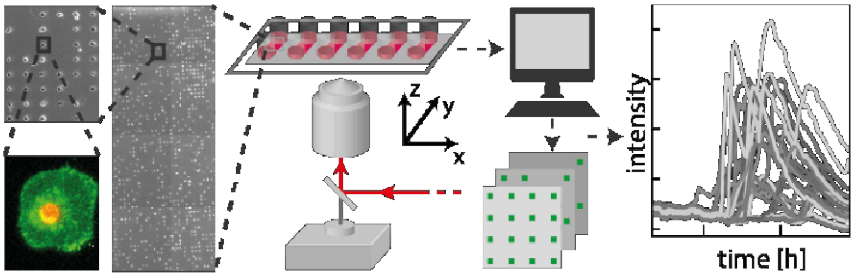


Figure 7: Single cell array for parallel recording of individual apoptosis events.

WP9 aimed at promoting the development of alternative methods for risk and benefit characterization of manufactured nanomaterials and consisted of four tasks.

Co-Culture and tissue / organ models as alternatives to animal testing

In vitro models were selected to represent the main relevant NPs uptake routes: skin, intestinal tract and respiratory tract. In addition, cells of the immune system were considered. We included different cell lines, primary cells, co-cultures and differentiated or 3D models. We considered cytotoxicity, oxidative stress, genotoxicity, and inflammation as relevant endpoints. For each, several possible methods and SOP's were selected.

For cytotoxicity, we compared the colorimetric MTS viability assay, the luminometric ATP viability assay, and the fluorimetric LDH assay. Fifty nm PS-NH2, but not fifty nm PS-COOH NPs, induced a dose-dependent toxic effect in all tested cell lines. However, differentiated or 3D models (i.e. reconstituted human epidermis, differentiated Caco-2 cell monolayers) were insensitive to PS-NH2, while primary keratinocytes and undifferentiated Caco-2 cells exhibited cytotoxicity, suggesting that NP toxicity depends on the differentiation status of the cell models. Primary cells (i.e. CD34-DC) displayed a higher variability. Results obtained with MTS, ATP and LDH assays were in general comparable. However, MTS assay appears to be most robust. Thus, this assay was transferred for a large interlaboratory comparison study in WP2. Furthermore, our results show that PS-NH2 NPs can induce oxidative stress and cell cycle arrest, while PS-COOH NPs do not. Again, the ROS production was dependent on the differentiation status as also observed for cytotoxicity.

We could show that PS-NH2 are good candidates for positive control NP in a variety of tested cell models for different endpoints, i.e. cytotoxicity, oxidative stress and cell cycle arrest. PS-COOH appear to be suitable negative control candidates.

Assessment of protein corona

WP9 compared different currently available techniques for isolation of NP-hard corona (HC) complexes, in particular centrifugation, size exclusion chromatography, and magnetic isolation using silica coated iron oxide and PS-COOH NPs. Overall we observed similar protein corona patterns and total protein intensities by all three methods with only minor differences. Thus, the hard protein

corona composition seems to be very stable. It also indicates that the yields of NP recovery are rather similar. Centrifugation is the most popular protocol, but may promote aggregation and it struggles when assessing NPs of low density and small size. Size exclusion chromatography certainly is not a high-throughput approach, but is certainly well chosen for NPs being prone to agglomeration or for low density NPs. Magnetic isolation is limited to superparamagnetic materials.

Furthermore, we have developed an SOP for isolation of NP-HC complexes by centrifugation and subsequent analysis by SDS-PAGE, which was used for RR exercise. Overall the results of three different laboratories looked very similar with comparable protein intensity and fingerprints.

WP10 – Transnational Access provision

Transnational Access (TA) has been an exciting pillar of QualityNano dedicated to provide users of the European nanosafety community access to state-of-the-art facilities to nanomaterial processing, characterization, and exposure assessment facilities. The instruments available for TA were quite unique and impressive, and has reflected high interest from the scientific community. The programme offered the users a full range of services from standard nanomaterials, tuition in best practice, laboratory support and training, and a suite of protocols for all aspects of nanomaterials processing and characterisation in a biological context.

16 laboratories located in 9 European countries have adhered to the TA programme,.

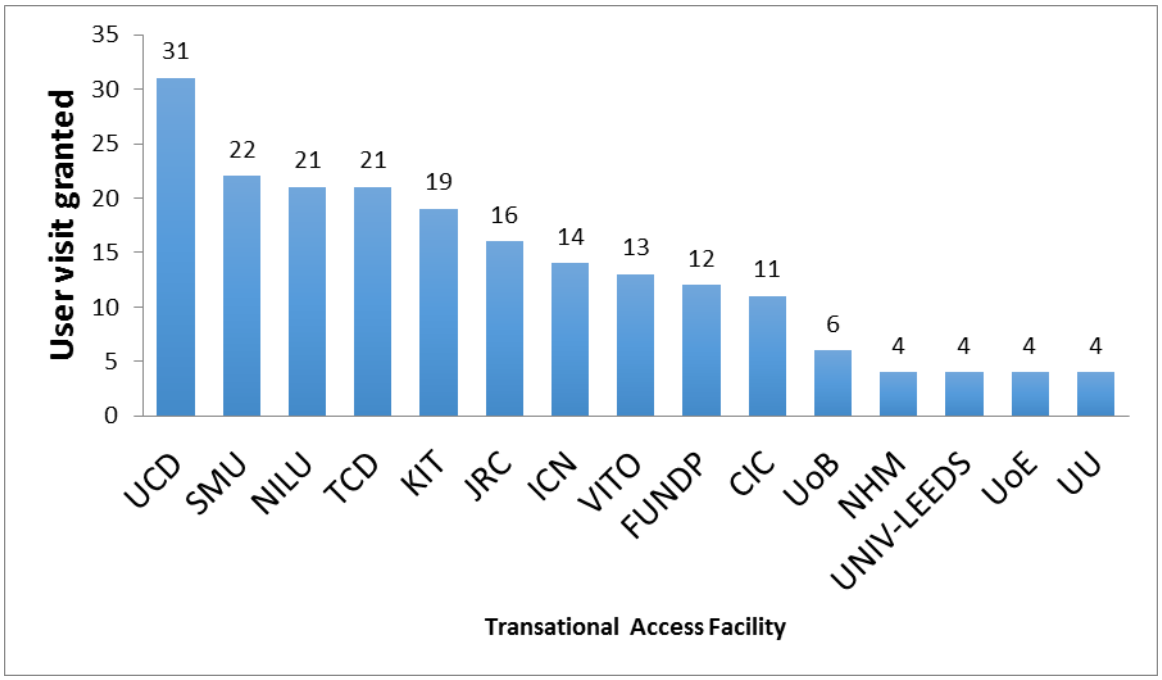
Figure 8. TA facilities located around Europe

TA access was simplified by a portal-based peer reviewed proposal wherein any researcher working in Europe (regardless of his/her origin) could apply. Overall the researchers could apply when a call was open, and overall 8 calls have been successfully launched during QualityNano. TA has been a very successful story as over 300 applicants from 30 countries have applied during 8 calls and nearly 200 visitors have benefited from this program. The interest in TA has been constant and persistent throughout, even towards the end of the study as the number of applications remains high. This high interest was assured by the dissemination activity at the project coordination level and by each TA

Leader of the single institutions that have jointly reached the nanosafety community by means of mailshots, dissemination at conferences, at their host institutions, and by personal contact. Gender balance was also taken into consideration, and the statistical analysis have shown that 57% of the users were female. Dissemination flyers have also been produced and distributed at crucial conferences to ensure reaching a vast audience. The TA applications were divided into four categories, according to the techniques and instrumentations required; category C (particle characterisation in situ & ex situ) and category D (Particle exposure assessment) were the most used. Examples of instruments used in category B are Analytical Centrifugation, Dynamic Light Scattering, Nanoparticle Tracking Analysis, Mass Spectrometry, SEM/TEM/X-Ray diffraction/Raman Spectroscopy, and Fluorescence Correlation Spectroscopy, while the most popular instruments of categories D are *in vitro / in vivo* exposure system, *in vivo* exposure system, high content platform, flow cytometry, and transcriptomics.

The dissemination of the TA visits has been also highly successful as the results gathered during the visits have been published in 41 peer reviewed papers, 34 non peer reviewed papers, and 41 theses, and the full output is likely yet to come as more manuscripts are likely to be published. Additionally, over 40 TA users attended the QualityNano conference and workshop in Crete, and presented their work in a dedicated poster session.

Table 5 user visit per TA facility



4.1.4 Socio-economic impact, main dissemination, exploitation

Exceptional Societal Impact: QualityNano was neither a usual project, nor an easy one in execution. But one thing is clear. Few research projects can ever claim to have had such a material impact on wider societal implications. QualityNano entered into an arena where almost nothing was agreed at laboratory level, all around the world, and the level of public and policy controversy was exceptional. When it exited, there was broad agreement on the outcome of all at laboratory level, and the public and policy debate is placid. That consensus in science at least, is born of transparent, clear and well-judged measurements.

It will be for the readers of this report in future to follow the final outcome, and to understand also that this accomplishment was not solely due to QualityNano. Many organizations, agencies and actors also helped create this more balanced understanding, however QualityNano was often the first to clarify key results, was the first to create the roadmap, and was often amongst the earliest in highlighting the issues. It also worked well with those other projects, and institutions, and handed over responsibility to them as they became better positioned to deal with those issues. It was therefore a key part of a tapestry that brought clarity at the technical level to many questions.

Project Dissemination

QualityNano was, during the key periods, visible at all key locations. Besides many invited lectures of the researchers in it, and its own key integrating conference, it sponsored an exhibit booth at a number of various conferences. QualityNano took the opportunity to disseminate information in an effective way, including recent advances, difficulties and breakthroughs in high quality research and nanosafety, besides expanding the network along the field of nanotechnology and nanomedicine.

Table 6:

Dates	***Conference***	***Link***	***Type of activity***
18th – 21st June 2012	Industrial Technologies Arhus, DK	http://www.industrialtechnologies2012.eu/	Joint booth with other EU projects
24th -27th June 2012	COMS 2012 Tönsberg, Norway	http://www.norfab.no/news/coms-2012-in-tonsberg-24th-27th-of-june/	Special Session Nanosafety *(UCD, NILU)*, Joint Booth *(KIT)*
4th – 7 th Sept 2012	Nanotoxicology 2012 Beijing, China	http://english.nanoctr.cas.cn/nanotoxicology2012/	Booth with display + flyers (UCD)
17th – 21st Sept 2012	E-MRS Fall Meeting Warsaw, Poland	http://www.emrs-strasbourg.com/index.php?option=com_content&task=view&id=500&Itemid=172	Joint Booth with KNMF (KIT)
27th Feb – 1st March 2013	2nd QualityNano Conference Prague		Display + Booth, TA Poster Show, TA Special Session, TA-"Clinic"
18-20 June, 2013	EuroNanoForum 2013 - Dublin	http://www.enterprise-ireland.com/en/Events/OurEvents/EuroNanoForum2013/	Booth
16th Sept – 20th Sept 2013	E-MRS Fall Meeting Warsaw	http://www.emrs-strasbourg.com/index.php?option=com_content&task=view&id=572&Itemid=1584	Display + Booth, Special Symposium on RI, Satellite Workshop on Nanoparticles for Industry
23rd-26th April 2014	NANOTOX 2014, 7th International	http://nanotox2014.org/sci	Booth

	Nanotoxicology Congress, Antalya	entific-program.html	
18th – 21st Nov 2014	NanoBio & Med 2014, Barcelona, Spain	http://www.nanobiomedconf.com/NBM/index.php	Booth
15th -17th July 2015	*QualityNano Conference, Crete*	http://www.qualitynano.eu/conference/welcome.html	Display and booth TA poster Show TA Poster Session

Co-operation with other projects/programmes

NanoSafety Cluster & Vision Document

As highlighted in various WP reports, QualityNano has been an active participant in the Nanosafety Cluster, and in the preparation of the NanoSafety Cluster *Nanosafety in Europe 2015 – 2025: Towards Safe and Sustainable Nanomaterials and Nanotechnology Innovations*, publication, and in supporting with science, positive controls and other assets various projects. NanoMILE and NanoSOLUTIONS, eNanoMapper/FutureNanoNeeds

MODENA COST action; The QualityNano Modelling Expert Group was handed over to MODENA COST during this period.

NanoEIS ; QualityNano plan on making a contribution to the final NanoEIS Meeting on nanospecific aspects of training, for industry & society.

QualityNano has achieved the above in a global perspective through its global collaboration and partnerships (e.g. US, JN, CA, AU, KR, RU etc). It has enabled Europe to become a global champion for quality systems for both research and downstream implementation. In so doing it has put nano EHS regulatory testing fully onto the long journey for global consensus so necessary for industry and society.

In September 2014, the Brazilian Ministry of Science, Technology and Innovation organised the EU-Brazil mission on nanosafety to promote the interactions between Brazil and the European Union on the field of Environmental, Health and Safety (EHS) aspects of nanomaterials. The project and participated in a 5-day mission to explore and elaborate the possibilities for (further) transatlantic cooperation and also highlighted in chemical watch and other websites.

The impact of QualityNano as it relates to the regulatory field.

QualityNano interacted on various different levels with different governmental and public bodies.

The project implemented four expert panels to ensure an efficient dialogue between different international stakeholders and to provide input and advice to the project.

In the regulatory resource group QualityNano interacted regularly with different European agencies including EMA (European Medicines Agency), ECHA (European Chemicals Agency), EFSA (European Food Safety Authority), national regulatory authorities, the European Commission, in particular DG (Directorate General) ENTR (Enterprise and Industry), DG ENV (Environment) and DG SANCO (Health & Consumers) and international agencies such as US EPA (Environmental Protection Agency) or CPSC (Consumer Product Safety Commission) or Australian APVMA (Australian Pesticides and Veterinary Medicines Authority). Via these interactors QualityNano also contributed to ongoing efforts on the OECD level.

In the standardization resource group, QualityNano interacted regularly with members of ISO (International Organization for Standardization), CEN (European Committee for Standardization) or

NIST (US National Institute for Standards and Technology), which linked us to ongoing activities in ISO/TC 69 (Application of statistical methods), ISO/TC 229 (Nanotechnologies) and to CEN/TC 352 (Nanotechnologies).

Via the activities within the standardization resource group, QualityNano was invited to a standardization workshop with different EC project officers on 27 March 2015 in Brussels.

In particular in the field of standardization QualityNano was able to develop best practice methods to measure, characterize, quantify and analyse biological/ toxicological effects of nanomaterials. As described above, QualityNano was already able to engage with different governmental bodies and policy makers. The expertise of QualityNano has been handed over the EU FP7 project NanoREG, which is interacting even more strongly and directly on different levels with policy makers. NanoREG will therefore also use expertise from QualityNano and will give scientific advice to policy makers.

QualityNano has impacted on regulation in the following ways:

- QualityNano organised a training school for professionals, which was dedicated specifically to experts doing risk assessment at regulatory bodies or panels as well as industry. Within this training school, QualityNano approached the experts in risk assessment and discussed specific needs for nanomaterials with them. This training school was well received by the experts and will impact regulation as we have been training the regulators here on which specific needs have to be taken into account for nanomaterials from a basic science perspective. Further, questionnaire responses from participants attending the training school identified that there is a need for similar activities to be continued over time.

- All relevant outputs of QualityNano have been directly discussed with various regulatory bodies (e.g. ECHA, EMA) in various teleconferences. In particular there is strong need for method validation. QualityNano has addressed this in a first step by performing round robins and interlaboratory comparisons, which might be continued later on in formal validation studies. Furthermore there is a need for positive and negative benchmark nanoparticles, which also has been addressed by QualityNano. None of the methods has been formally validated (which was out of the scope of this project) but the structure of QualityNano expert groups regulatory bodies ensured that all relevant results have been communicated such that they can be used for regulatory purposes by them. There are now several methods of analysis that are well prepared for elevation to standards.

Exploitation :

As has been outlined in this presentation, the dimensions of exploitations are still being understood and worked on in many different dimensions. It was envisaged that the projects NanoReg and ProSafe would take up the concept (if not always able to take over all practices) of executional excellence that had been built up within QualityNano. However, in practice, the whole concept of methodological excellence seems increasingly embedded in the practices of the broader community, and at the point of writing it is not clear if a formal structure to do so is still required. This issue could be monitored in the light also of new people joining the community from many countries, and the need for ongoing education.

Some of the methodologies are also moved into common use, and individual partners are promoting them for use in all sectors of the community.

The impact of QualityNano as it relates to industry

QualityNano was established to provide a pan-European research infrastructure to provide science platform to explore critical health, safety and environment properties of engineered nanomaterials. Its core aim has been the creation of a 'neutral' scientific & technical space in which all stakeholder groups can engage, develop, and share scientific best practice in the field. Initially, it harnessed the talents of researchers and resources from across Europe and developed efficient, transparent and effective processes. Progressively, it formed important alliances with leading practitioners and organisations across the globe. Its foundation occurred at a critical time in the evolution of nano-science and –technology. From 2003 onwards, anticipating the socio-economic potential of this new technology very large public investments in nanotechnology research and innovation (R&I) were made in the US (National Nanotechnology Initiative NNI), Europe (Frameworks 6, 7 and Horizon 2020), Japan and Korea [6]. Latest estimates of the world market for nanotechnology products are ~$1billion per year [7]. By 2005, an analysis of UK Engineering & Physical Science Research Council grants indicated nanoscience was now in the mainstream of these specialisations. This conclusion was further supported in 2009 by an analysis of FP7 ERC Advanced and Starting investigator grants in these same disciplines, where 75% of all grants involved nanotechnology [8]. By 2015, it was clear that the Nanotechnology commercial revolution was following a classic Gartner "hype to reality" cycle.

The peak of the "hype" phase occurred ca 2010-11. From then to 2015, and despite a world market of $1billion sales/year, the US appears to be in the "trough of disillusion". Factors affecting progress of the technology up the "slope of enlightenment" to the "zone productivity" include: a) further investment in R&I by industry and b) resolution of perceived uncertainties associated with EHS risks (hazard and exposure).

An interesting new dimension has arisen during the period of the QualityNano project that could have a positive influence on the *hype* to *reality* tipping point and thus enhance the longer term impact of its results. This is illustrated in Fig.9 below:

[6] Wilkins TA: Mid-term assessment FP6-TP3 : Nanotechnology and Nanosciences, Knowledge-based Multifunctional Materials, New Production Processes and Devices Expert Advisory Group Sixth Framework Programme (2002-2006),Thematic Priority 3, European Commission 31 January 2005. http://ec.europa.eu/research/evaluations/pdf/archive/fp6-evidence-base/evaluation_studies_and_reports/evaluation_studies_and_reports_2005/mid_term_assessment__fp6_thematic_priority_3.pdf

[7] Harper T: 2015: "The Year of the Trillion Dollar Nanotechnology Market?": http://www.azonano.com/article.aspx? ArticleID=3946; Jan 2015

[8] Wilkins TA (Chair, Kiperassides C (Editor) et al. Position Paper on Future RTD Activities of NMP for the Period 2010 – 2015: Publications Office of the European Union, Luxemburg, 2010 Nov 2009; ISBN 978-92-79-14065-5 and doi 10.2777/77895

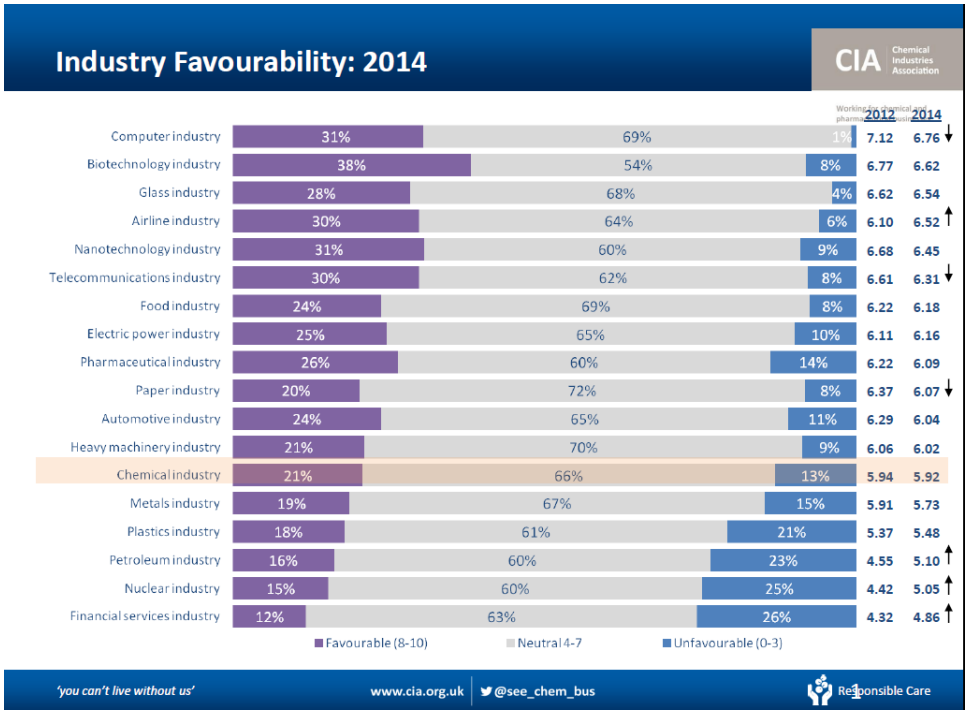
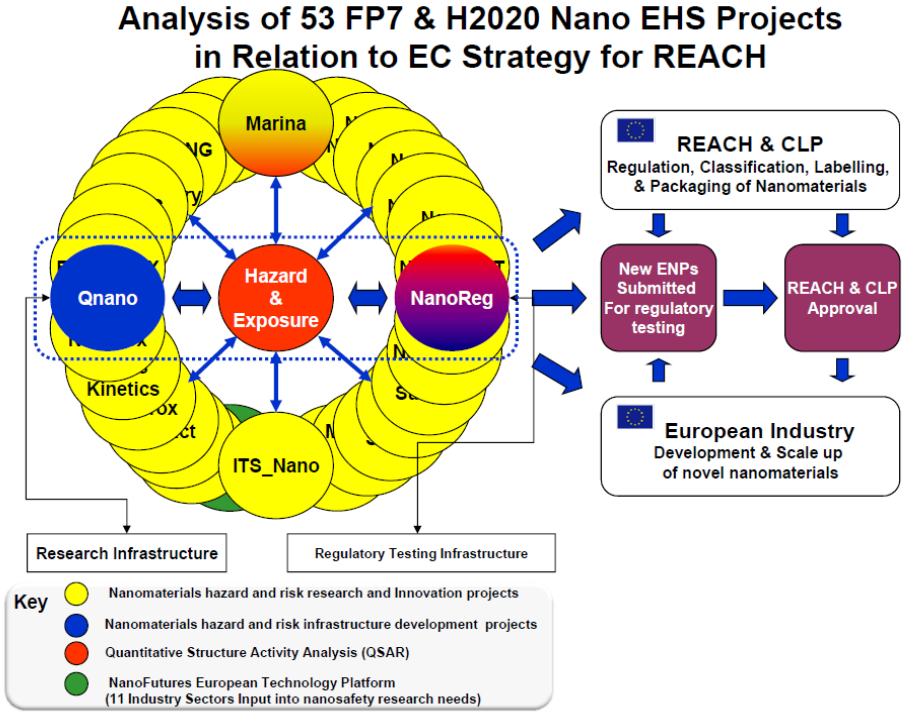


Fig. 9 European Public Attitudes to Industries Inc. Nanotechnology[i]

The survey covered both EU and non-EU countries. There was much agreement between countries and little change since the 2012 survey. In essence, nanotechnology is amongst the most favourably viewed sectors by the public. This observation follows a similar study carried out in the US by the NNI. The timing of QualityNano in relation to the 'tipping point' in the journey from new science to translational research and then to innovation has been pivotal in respect of a) support to building a coherent and united research community and the 53 projects. The work of the Quality Nano has laid the foundations for translation to industrial and societal application.



During its 4 years, the FP7 H2020 project portfolio's science has migrated towards "*safe by design*' manufacturing as exemplified in FP7 SUN, GUIDENANO, Nanoreg projects. In turn, this transition has underpinned the work of the Nanoreg, ProSafe projects and the OECD in the preparation of the white paper for accelerating Europe's ambitions for impact as expressed in Figure 10. These two achievements plus the very many young researchers trained in this new science and responsible innovation processes may prove to be QualityNano's greatest societal and economic impact.

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4.2 Use and dissemination of foreground

Section A (public)

This section includes two templates

- Template A1: List of all scientific (peer reviewed) publications relating to the foreground of the project.
- Template A2: List of all dissemination activities (publications, conferences, workshops, web sites/applications, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters).

These tables are cumulative, which means that they should always show all publications and activities from the beginning until after the end of the project. Updates are possible at any time.

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES

Peer reviewed papers

N°	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers	Is/will open access provided to publication
1	Nanoparticle Adhesion to the Cell Membrane and Its Effect on Nanoparticle Uptake Efficiency	Anna Lesniak	Journal of the American Chemical Society	Vol. 135/Issue 4	American Chemical Society	United States	30/01/2013	1438-1444	10.1021/ja309812z	
2	The biomolecular corona is retained during nanoparticle uptake and protects the cells from the damage induced by cationic nanoparticles until degraded in the lysosomes	Fengjuan Wang	Nanomedicine: Nanotechnology, Biology, and Medicine	Vol. 9/Issue 8	Elsevier Inc.	United States	01/11/2013	1159-1168	10.1016/j.nano.2013.04.010	
3	Low Dose of Amino-Modified Nanoparticles Induces Cell Cycle Arrest	Jong Ah Kim	ACS Nano	Vol. 7/Issue 9	American Chemical Society	United States	24/09/2013	7483-7494	10.1021/nn403126e	
4	Tracing Bioavailability of ZnO Nanoparticles Using Stable Isotope Labeling	Fiona Larner	Environmental Science and Technology	Vol. 46/Issue 21	American Chemical Society	United States	06/11/2012	12137-12145	10.1021/es302602j	
5	Mechanisms of Silver Nanoparticle Release, Transformation and Toxicity: A Critical Review of Current Knowledge and Recommendations for Future Studies and Applications	BogumiBa Reidy	Materials	Vol. 6/Issue 6	MDPI AG	Switzerland	01/06/2013	2295-2350	10.3390/ma6062295	
6	Fate of SiC and TiC nanoparticle	Jorge Mejia	Internationa	Vol. 4/Issue	Inderscience	United	01/01/2012	243	10.1504/IJ	

N°	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers	Is/will open access provided to publication
	dispersions in human reconstituted gastric fluid		l Journal of Nano and Biomaterials	3/4	Enterprises Ltd	Kingdom			NBM.2012.051706	
7	A novel method for synthesis of 56Co-radiolabelled silica nanoparticles	I. Cydzik	Journal of Nanoparticle Research	Vol. 14/Issue 10	Springer Netherlands	Netherlands	01/10/2012	p, 1185	10.1007/s11051-012-1185-x	
8	Radiolabelling of nanoparticles by proton irradiation: temperature control in nanoparticulate powder targets	Uwe Holzwarth	Journal of Nanoparticle Research	Vol. 14/Issue 6	Springer Netherlands	Netherlands	01/06/2012	2-15	10.1007/s11051-012-0880-y	
9	Feasibility study of production of radioactive carbon black or carbon nanotubes in cyclotron facilities for nanobioscience applications	K. Abbas	Applied Radiation and Isotopes	Vol. 73	Elsevier Limited	United Kingdom	01/03/2013	44-48	10.1016/j.apradiso.2012.11.012	
10	Strategies for radiolabeling of commercial TiO2 nanopowder as a tool for sensitive nanoparticle detection in complex matrices	Heike Hildebrand,	Journal of Nanoparticle Research	Vol. 17/Issue 6	Springer Netherlands	Netherlands	01/06/2015	278	10.1007/s11051-015-3080-8	
11	Iron oxide nanoparticle toxicity testing using high-throughput analysis and high-content imaging	Georgina Harris	Nanotoxicology	Vol. 9/Issue S1	Informa Healthcare	United Kingdom	01/05/2015	87-94	10.3109/17435390.2013.816797	
12	High Content Analysis Provides Mechanistic Insights on the Pathways of Toxicity Induced by Amine-Modified Polystyrene Nanoparticles	Sergio Anguissola	PLoS One	Vol. 9/Issue 9	Public Library of Science	United States	19/09/2014	e108025	10.1371/journal.pone.0108025	
13	Suppression of nanoparticle	Jong Ah Kim	Nanoscale	Vol. 6/Issue	RSC	United	01/01/2014	14180-14184	10.1039/C4	

N°	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers	Is/will open access provided to publication
	cytotoxicity approaching in vivo serum concentrations: limitations of in vitro testing for nanosafety			23	Publishing	Kingdom			NR04970E	
14	Controlling aqueous silica nanoparticle synthesis in the 10–100 nm range	Delyan R. Hristov	Chemical Communications	Vol. 51/Issue 98	Royal Society of Chemistry	United Kingdom	01/01/2015	17420-17423	10.1039/c5cc06598d	
15	A TEM protocol for quality assurance of in vitro cellular barrier models and its application to the assessment of nanoparticle transport mechanisms across barriers	Dong Ye	Analyst, The	Vol. 140/Issue 1	Royal Society of Chemistry	United Kingdom	01/01/2015	83-97	10.1039/c4an01276c	
16	Time resolved study of cell death mechanisms induced by amine-modified polystyrene nanoparticles	Fengjuan Wang	Nanoscale	Vol. 5/Issue 22	RSC Publishing	United Kingdom	01/01/2013	10868	10.1039/c3nr03249c	
17	The need for in situ characterisation in nanosafety assessment: funded transnational access via the QNano research infrastructure	Dawson KA	Nanotoxicology	8 per year	Informa Healthcare	London	26/01/2012	E-published ahead of print		Yes
18	Quantification of nanoparticles in aqueous food matrices using Particle-Induced X-ray Emission	Omar Lozano ,	Analytical and Bioanalytical Chemistry	Vol. 403/Issue 10	Springer Verlag	Germany	01/07/2012	2835-2841		No
19	Cytotoxicity of surface-functionalized silicon and germanium nanoparticles: the dominant role of surface charges	Sourav Bhattacharjee	Nanoscale	Vol. 5/Issue 11	RSC Publishing	United Kingdom	01/01/2013	4870	10.1039/C3NR34266B	

Nº	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers	Is/will open access provided to publication
20	The complexity of nanoparticle dissolution and its importance in nanotoxicological studies	Superb K. Misra	Science of the Total Environment	Vol. 438	Elsevier	Netherlands	01/11/2012	225-232	10.1016/j.scitotenv.2012.08.066	
21	Stabilising fluorescent silica nanoparticles against dissolution effects for biological studies	Eugene Mahon	Chemical Communications	Vol. 48/Issue 64	Royal Society of Chemistry	United Kingdom	01/01/2012	7970	10.1039/C2CC34023B	
22	Reversible	Silvia Milani	ACS Nano	Vol. 6/Issue 3	American Chemical Society	United States	27/03/2012	2532-2541	10.1021/nn204951s	
23	Are stirring and sonication pre-dispersion methods equivalent for in vitro toxicology evaluation of SiC and TiC?	Jorge Mejia	Journal of Nanoparticle Research	Vol. 14/Issue 4	Springer Netherlands	Netherlands	01/03/2012	815-833	10.1007/s11051-012-0815-7	
24	Effects of the dispersion methods in Pluronic F108 on the size and the surface composition of MWCNTs and their implications in toxicology assessment	Jorge Mejia	Journal of Nanoparticle Research	Vol. 13/Issue 2	Springer Netherlands	Netherlands	01/02/2011	655-667	10.1007/s11051-010-0063-7	
25	Characterizing Nanoparticles Reactivity: Structure-Photocatalytic Activity Relationship	J Piella	Journal of Physics: Conference Series	Vol. 429	Institute of Physics Publishing	United Kingdom	10/04/2013	012040	10.1088/1742-6596/429/1/012040	
26	Stability of polymer encapsulated quantum dots in cell culture media	Ojea-Jiménez	Journal of Physics: Conference Series	429	Institute of Physics Publishing		04/10/2013	1-5	10.1088/1742-6596/429/1/012009	
27	Radiochemical synthesis of	C. Ichedef.	Journal of	Vol. 15/Issue	Springer	Netherla	01/11/2013	NA	10.1007/s1	

N°	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers	Is/will open access provided to publication
	105gAg-labelled silver nanoparticles		Nanoparticle Research	11	Netherlands	nds			1051-013-2073-8	
28	Cryo-electron Microscopy Specimen Preparation By Means Of a Focused Ion Beam	Stefano Rubino	Journal of Visualized Experiments	Issue 89	MYJoVE Corporation	United States	01/01/2014	NA	10.3791/51463	
29	Highly repeatable synthesis of nHA with high aspect ratio	Song Chen	Materials Letters	Vol. 159	Elsevier	Netherlands	01/11/2015	163-167	10.1016/j.matlet.2015.06.086	
30	Quantification of nanoparticles in aqueous food matrices using Particle-Induced X-ray Emission	Omar Lozano	Analytical and Bioanalytical Chemistry	Vol. 403/Issue 10	Springer Verlag	Germany	01/07/2012	2835-2841	10.1007/s00216-012-5895-9	
31	Effects of SiC nanoparticles orally administered in a rat model: Biodistribution, toxicity and elemental composition changes in feces and organs	Omar Lozano	Toxicology and Applied Pharmacology	Vol. 264/Issue 2	Academic Press Inc.	United States	01/10/2012	232-245	10.1016/j.taap.2012.08.004	
32	Copper(ii) oxide nanoparticles penetrate into HepG2 cells, exert cytotoxicity via oxidative stress and induce pro-inflammatory response	Jean-Pascal Piret	Nanoscale	Vol. 4/Issue 22	RSC Publishing	United Kingdom	01/01/2012	7168	10.1039/C2NR31785K	
33	Tracing engineered nanomaterials in biological tissues using coherent anti-Stokes Raman scattering (CARS) microscopy – A critical review	Rhys M. Goodhead	Nanotoxicology	May 2015	Informa Healthcare	United Kingdom	11/05/2015	1-12	10.3109/17435390.2014.991773	
34	A comparative analysis on the in vivo toxicity of copper	Lan Song	Chemosphere	Vol. 139	Elsevier Limited	United Kingdom	01/11/2015	181-189	10.1016/j.chemospher	

N°	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers	Is/will open access provided to publication
	nanoparticles in three species of freshwater fish								e.2015.06.021	
35	56Co-labelled radioactive Fe3O4 nanoparticles for in vitro uptake studies on Balb/3T3 and Caco-2 cell lines	P. Marmorato	Journal of Nanoparticle Research	Vol. 13/Issue 12	Springer Netherlands	Netherlands	01/12/2011	6707-6716	10.1007/s11051-011-0577-7	
36	Radiolabelling of engineered nanoparticles for in vitro and in vivo tracing applications using cyclotron accelerators	N. Gibson	Archives of Toxicology	Vol. 85/Issue 7	Springer Verlag	Germany	01/07/2011	751-773	10.1007/s00204-011-0701-6	
37	Radiolabelling of nanoparticles by proton irradiation: temperature control in nanoparticulate powder targets	Uwe Holzwarth	Journal of Nanoparticle Research	Vol. 14/Issue 6	Springer Netherlands	Netherlands	01/06/2012	2-15	10.1007/s11051-012-0880-y	
38	Feasibility study of production of radioactive carbon black or carbon nanotubes in cyclotron facilities for nanobioscience applications	K. Abbas	Applied Radiation and Isotopes	Vol. 73	Elsevier Limited	United Kingdom	01/03/2013	44-48	10.1016/j.apradiso.2012.11.012	
39	Gold nanoparticle aerosols for rodent inhalation and translocation studies	Winfried Möller	Journal of Nanoparticle Research	Vol. 15/Issue 4	Springer Netherlands	Netherlands	01/04/2013	2-13	10.1007/s11051-013-1574-9	
40	7Be-recoil radiolabelling of industrially manufactured silica nanoparticles	Uwe Holzwarth	Journal of Nanoparticle Research	Vol. 16/Issue 9	Springer Netherlands	Netherlands	01/09/2014	2574	10.1007/s11051-014-2574-0	
41	Strategies for radiolabeling of commercial TiO2 nanopowder as a tool for sensitive nanoparticle detection in complex matrices	Heike Hildebrand'	Journal of Nanoparticle Research	Vol. 17/Issue 6	Springer Netherlands	Netherlands	01/06/2015	278	10.1007/s11051-015-3080-8	

N°	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers	Is/will open access provided to publication
42	Genotoxicity testing of PLGA–PEO nanoparticles in TK6 cells by the comet assay and the cytokinesis-block micronucleus assay	Alena Kazimirova	Mutation Research - Genetic Toxicology and Environmental Mutagenesis	Vol. 748/Issue 1-2	Elsevier	Netherlands	01/10/2012	42-47	10.1016/j.mrgentox.2012.06.012	
43	Mechanisms of genotoxicity. A review of in vitro and in vivo studies with engineered nanoparticles	Zuzana Magdolenova	Nanotoxicology	Vol. 8/Issue 3	Informa Healthcare	United Kingdom	01/05/2014	233-278	10.3109/17435390.2013.773464	
44	Interlaboratory comparison of size measurements on nanoparticles using nanoparticle tracking analysis (NTA)	Patrick Hole	Journal of Nanoparticle Research	Vol. 15/Issue 12	Springer Netherlands	Netherlands	01/12/2013	1-12	10.1007/s11051-013-2101-8	Yes
45	Can the comet assay be used reliably to detect nanoparticle-induced genotoxicity?	Hanna L. Karlsson	Environmental and Molecular Mutagenesis	Vol. 56/Issue 2	Wiley-Liss Inc.	United States	01/03/2015	82-96	10.1002/em.21933	No
46	Is the toxic potential of nanosilver dependent on its size?	Anna Huk	Particle and Fibre Toxicology	Vol. 11/Issue 1	BioMed Central	United Kingdom	01/01/2014	65	10.1186/s12989-014-0065-1	No
47	Nanoparticles in food. Epigenetic changes induced by nanomaterials and possible impact on health	Bozena Smolkova	Food and Chemical Toxicology	Vol. 77	Elsevier Limited	United Kingdom	01/03/2015	64-73	10.1016/j.fct.2014.12.015	
48	Critical factors to be considered when testing nanomaterials for genotoxicity with the comet	A. Huk	Mutagenesis	Vol. 30/Issue 1	Oxford University Press	United Kingdom	01/01/2015	85-88	10.1093/mutage/geu077	

N°	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers	Is/will open access provided to publication
	assay									
49	Effect of silver nanoparticles on mitogen-activated protein kinases activation: role of reactive oxygen species and implication in DNA damage	A. Rinna	Mutagenesis	Vol. 30/Issue 1	Oxford University Press	United Kingdom	01/01/2015	59-66	10.1093/mutage/geu057	
50	Biological impact assessment of nanomaterial used in nanomedicine. Introduction to the NanoTEST project	Lucienne Juillerat-Jeanneret	Nanotoxicology	Vol. 9/Issue S1	Informa Healthcare	United Kingdom	01/05/2015	5-12	10.3109/17435390.2013.826743	
51	Toxicity screenings of nanomaterials: challenges due to interference with assay processes and components of classic in vitro tests	Rina Guadagnini	Nanotoxicology	Vol. 9/Issue S1	Informa Healthcare	United Kingdom	01/05/2015	13-24	10.3109/17435390.2013.829590	
52	Coating-dependent induction of cytotoxicity and genotoxicity of iron oxide nanoparticles	Zuzana Magdolenova	Nanotoxicology	Vol. 9/Issue S1	Informa Healthcare	United Kingdom	01/05/2015	44-56	10.3109/17435390.2013.847505	
53	Immunotoxicity and genotoxicity testing of PLGA-PEO nanoparticles in human blood cell model	Jana Tulinska	Nanotoxicology	Vol. 9/Issue S1	Informa Healthcare	United Kingdom	01/05/2015	33-43	10.3109/17435390.2013.816798	
54	Health effects of selected nanoparticles in vivo : liver function and hepatotoxicity following intravenous injection of titanium dioxide and Na-oleate-coated iron oxide nanoparticles in rodents	Katarina Volkovova	Nanotoxicology	Vol. 9/Issue S1	Informa Healthcare	United Kingdom	01/05/2015	95-105	10.3109/17435390.2013.815285	
55	Suitability of human and	Hilary Cowie	Nanotoxicol	Vol. 9/Issue	Informa	United	01/05/2015	57-65	10.3109/17	

N°	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers	Is/will open access provided to publication
	mammalian cells of different origin for the assessment of genotoxicity of metal and polymeric engineered nanoparticles		ogy	S1	Healthcare	Kingdom			435390.2014.940407	
56	Towards an alternative testing strategy for nanomaterials used in nanomedicine: Lessons from NanoTEST	M. Dusinska	Nanotoxicology	Vol. 9/Issue S1	Informa Healthcare	United Kingdom	01/05/2015	118-132	10.3109/17435390.2014.991431	
57	Impact of nanosilver on various DNA lesions and HPRT gene mutations – effects of charge and surface coating	Anna Huk	Particle and Fibre Toxicology	Vol. 12/Issue 1	BioMed Central	United Kingdom	01/12/2015	1-13	10.1186/s12989-015-0100-x	Yes
58	The use of the comet assay for the evaluation of the genotoxicity of nanomaterials	Amaya Azqueta , Maria Dusinska	Frontiers in Genetics	Vol. 6	frontiersin.org	Switzerland	10/07/2015	1-4	10.3389/fgene.2015.00239	
59	Role of membrane disturbance and oxidative stress in the mode of action underlying the toxicity of differently charged polystyrene nanoparticles	Sourav Bhattacharjee ,	RSC Advances	Vol. 4/Issue 37	RSC	United Kingdom	01/01/2014	19321-19330	10.1039/C3RA46869K	Yes
60	Cell transformation assays for prediction of carcinogenic potential: state of the science and future research needs	S. Creton	Mutagenesis	Vol. 27/Issue 1	Oxford University Press	United Kingdom	01/01/2012	93-101	10.1093/mutage/ger053	
61	Hydrophobic sodium fluoride-based nanocrystals doped with lanthanide ions: assessment of in vitro toxicity to human blood lymphocytes and phagocytes	Bartlomiej Sojka	Journal of Applied Toxicology	Vol. 34/Issue 11	John Wiley and Sons Ltd	United Kingdom	01/11/2014	1220-1225	10.1002/jat.3050	

N°	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Permanent identifiers	Is/will open access provided to publication
62	Is the toxic potential of nanosilver dependent on its size?	Anna Huk	Particle and Fibre Toxicology	Vol. 11/Issue 1	BioMed Central	United Kingdom	01/01/2014	65	10.1002/em.21930	
63	Lessons learned from research on air pollution and other particles in the toxicology of nanomaterials and vice versa	Peter Møller	Environmental and Molecular Mutagenesis	Vol. 56/Issue 2	Wiley-Liss Inc.	United States	01/03/2015	77-81	10.1002/em.21930	
64	Green synthesis of silver nanoparticles using Coffea arabica seed extract and its antibacterial activity	Vivek Dhand	Materials Science and Engineering : C	Vol. 58	Elsevier	International Technology Disclosure	01/01/2016	36-43	10.1016/j.msec.2015.08.018	
65	Nano-sized polystyrene affects feeding, behavior and physiology of brine shrimp Artemia franciscana larvae	Elisa Bergami	Ecotoxicology and Environmental Safety	Vol. 123	Academic Press Inc.	United States	01/01/2016	18-25	10.1016/j.ecoenv.2015.09.021	
66	Tuning of nanoparticle biological functionality through controlled surface chemistry and characterisation at the bioconjugated nanoparticle surface	Delyan R. Hristov	Scientific Reports	Vol. 5	Nature Publishing Group	United Kingdom	01/12/2015	17040	10.1038/srep17040	
67	Surfactant Titration of Nanoparticle–Protein Corona	Daniele Maiolo	Analytical Chemistry	Vol. 86/Issue 24	American Chemical Society	United States	16/12/2014	12055-12063	10.1021/ac5027176	
68	Diagnostic nanoparticle targeting of the EGF-receptor in complex biological conditions using single-domain antibodies	K. Zarschler	Nanoscale	Vol. 6/Issue 11	RSC Publishing	United Kingdom	01/01/2014	6046	10.1039/c4nr00595c	

Article in a book or book series

Nº	Title	Main Author	Title of the book (series)	Volume	Date of publication	Publisher	Place of publication	Relevant pages	Permanent identifiers	Is/will open access provided to publication
1	Critical Evaluation of Toxicity Tests	Maria Dusinska ,	Adverse Effects of Engineered Nanomaterials		01/01/2012	Elsevier		63	10.1016/B978-0-12-386940-1.00004-0	No
2	Toxicological Aspects for Nanomaterial in Humans	Maria Dusinska ,	Nanotechnology for Nucleic Acid Delivery		01/01/2013	Humana Press	Totowa, NJ	1	10.1007/978-1-62703-140-0_1	No
3	Bhas 42 Cell Transformation Assay for Genotoxic and Non-Genotoxic Carcinogens	Kiyoshi Sasaki ,	Genotoxicity and DNA Repair		01/01/2014	Springer New York	New York, NY	343	10.1007/978-1-4939-1068-7_20	No
4	Critical Evaluation of Toxicity Tests	Maria Dusinska ,	Adverse Effects of Engineered Nanomaterials		01/01/2012	Elsevier		63	10.1016/B978-0-12-386940-1.00004-0	Yes
5	Toxicological Aspects for Nanomaterial in Humans	Maria Dusinska ,	Nanotechnology for Nucleic Acid Delivery		01/01/2013	Humana Press	Totowa, NJ	1	10.1007/978-1-62703-140-0_1	Yes
6	Analysis of Nanoparticle-Induced DNA Damage by the Comet Assay	Julia Catalán	Genotoxicity and DNA Repair		01/01/2014	Springer New York	New York, NY	241	10.1007/978-1-4939-1068-7_14	No
7	Formation and Characterization of the Nanoparticle–Protein Corona	Marco P. Monopoli	Nanomaterial Interfaces in Biology	Vol. 1025	01/01/2013	Humana Press	Totowa, NJ	137	10.1007/978-1-62703-462-3_11	No

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES

Nº	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
1	Web sites/Applications	UNIVERSITY COLLEGE DUBLIN, NATIONAL UNIVERSITY OF IRELAND, DUBLIN	www.qnano-ri.eu	01/02/2011	hosted in Ireland	Scientific community (higher education, Research) - Industry - Policy makers - Medias	6000	All Europe & international
2	Flyers	UNIVERSITY COLLEGE DUBLIN, NATIONAL UNIVERSITY OF IRELAND, DUBLIN	QNano A pan-European Infrastructure for Quality in Nanomaterials Safety Testing	15/09/2011	Widely distributed at conferences by partners	Scientific community (higher education, Research) - Industry - Policy makers	6000	Europe
3	Oral presentation to a scientific event	WAGENINGEN UNIVERSITY	Synthesis, characterization and toxicity of functionalized silicon nanoparticles	11/02/2014	Playa del Carmen, Mexico	Scientific community (higher education, Research)	100	International
4	Oral presentation to a scientific event	INSTITUT UNIVERSITAIRE ROMAND DE SANTE AU TRAVAIL	Characterizing nanoparticles reactivity: Structure-Photocatalytic activity relationship	11/01/2013	Grenoble, Nanosafe 2012	Scientific community (higher education, Research)	200	Europe
5	Posters	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Size tailoring TiO2 nanoparticles via agglomeration state and its relation to photocatalytic properties	05/03/2015	Strasburg, E-MRS 2013 Spring Meeting	Scientific community (higher education, Research)	200	Europe

N°	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
6	Oral presentation to a scientific event	UNIVERSITE DE NAMUR ASBL	PIXE: a tool for nanoparticle quantification in food (and other media as well)	11/02/2011	Prague	Scientific community (higher education, Research)	200	Europe
7	Oral presentation to a scientific event	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	The use of PIXE for nanomaterial quantification: case studies	03/01/2012	4th NanoImpactNet Integrating Conference and the 1st QNano Integrating Conference / Dublin	Scientific community (higher education, Research)	500	International
8	Oral presentation to a scientific event	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Engineered nanomaterial quantification in complex matrices: PIXE case studies	11/02/2013	Nanosafe 2012, Grenoble	Scientific community (higher education, Research)	500	International
9	Posters	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Quantification of Engineered NanoMaterials in complex matrices: PIXE case studies	03/01/2013	2nd QNano conference, Prague	Scientific community (higher education, Research)	400	International
10	Posters	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Effects of SiC nanoparticles orally administered in a rat model	03/01/2013	2nd QNano conference, Prague	Scientific community (higher education, Research)	200	International
11	Oral presentation to a scientific event	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Nanopaints: physicochemical characterization through Transnational Access at the University of Namur	03/01/2013	2nd QNano conference, Prague	Scientific community (higher education, Research)	400	International
12	Oral presentation to a scientific event	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	The use of PIXE in the field of nanosafety	09/12/2013	ECAART 11, Namur	Scientific community (higher education, Research)	200	Europe

Nº	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
13	Oral presentation to a scientific event	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	The use of CLS and PIXE in the context of nanosafety	09/06/2014	NanoValid Training Workshop, Zaragoza	Scientific community (higher education, Research)	100	Europe
14	Oral presentation to a wider public	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Seguridad de los nanomateriales y su impacto en la sociedad	10/11/2014	A.B.I.A., Antwerp	Civil society	30	Europe
15	Posters	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Quantification of ENMs in complex matrices: development of PIXE analysis methodologies	04/12/2015	NanoTox 2014, Antalya	Scientific community (higher education, Research)	500	International
16	Posters	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Do carbide ENMs exert a toxicity effect when orally administered to rats?	04/12/2015	NanoTox 2014, Antalya	Scientific community (higher education, Research)	500	International
17	Oral presentation to a scientific event	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Physico-chemical characterization of ENMs in complex matrices and their role in nanosafety studies	05/04/2015	UANL, Facultad de Ciencias Químicas, México	Scientific community (higher education, Research)	20	México
18	Oral presentation to a scientific event	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Nanoseguridad: un enfoque multidisciplinario hacia el uso seguro de nanomateriales	05/02/2015	UANL, Facultad de Ciencias Físico-Matemáticas, México	Scientific community (higher education, Research)	20	México
19	Oral presentation to a scientific event	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Nanoparticle measurement in aqueous media: challenges and opportunities	05/06/2016	IMTA, México	Scientific community (higher education, Research)	30	México
20	Oral presentation to a scientific event	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Nanoseguridad: un enfoque multidisciplinario hacia el uso seguro de nanomateriales	06/02/2014	UNAM, México	Scientific community (higher education, Research)	50	México

Nº	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
21	Oral presentation to a scientific event	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Nanosafety: a multidisciplinary focus towards the safe use of nanomaterials	06/03/2014	IPN, México	Scientific community (higher education, Research)	50	México
22	Oral presentation to a scientific event	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Nanoseguridad: un enfoque multidisciplinario hacia el uso seguro de nanomateriales	06/04/2014	CIMAV, México	Scientific community (higher education, Research)	50	México
23	Oral presentation to a scientific event	INSTITUT UNIVERSITAIRE ROMAND DE SANTE AU TRAVAIL	Nanoseguridad: un enfoque multidisciplinario hacia el uso seguro de nanomateriales	06/05/2014	UANL, FIME, México	Scientific community (higher education, Research)	50	México
24	Oral presentation to a scientific event	INSTITUT UNIVERSITAIRE ROMAND DE SANTE AU TRAVAIL	La caracterización físico-química de nanomateriales en matrices complejas y su rol en estudios de nanoseguridad	08/08/2015	TecSalud, México	Scientific community (higher education, Research)	3	México
25	Oral presentation to a scientific event	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	The impact of SiC and TiC nanomaterials in a rat model	11/08/2015	Nanosafe 2014, Grenoble	Scientific community (higher education, Research)	400	International
26	TV clips	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Air Pollution and Hypertension co-morbidity: Role of Titanium Dioxide in Cardiac Arrhythmogenesis	07/03/2016	QualityNano final meeting, Heraklion	Scientific community (higher education, Research)	150	International
27	Oral presentation to a scientific event	UPPSALA UNIVERSITET	QNano Research Infrastructure	05/08/2012	Uppsala, Sweden	Scientific community (higher education, Research)	110	Scandinavia

N°	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
28	Oral presentation to a scientific event	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Toxic effects of multi-walled carbon nanotubes and copper oxide nanoparticles at cutaneous, intestinal and hepatic levels	10/04/2014	Belvaux, Luxemburg	Scientific community (higher education, Research)	50	International
29	Posters	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Transnational Access Facility - University of Namur	09/05/2013	Kaunas, Lithuania	Scientific community (higher education, Research)	200	Europe
30	Posters	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Transnational Access Facility - University of Namur	10/08/2013	Brussels, Belgium	Scientific community (higher education, Research)	80	Europe
31	Oral presentation to a scientific event	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	The use of PIXE for engineered nanomaterials quantification in complex matrices	11/01/2013	Nanosafe 2012, Grenoble, France	Scientific community (higher education, Research)	400	Europe
32	Oral presentation to a scientific event	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	How do oxide nanomaterial dispersions evolve in an in vitro assessment?	11/01/2013	Nanosafe 2012, Grenoble, France	Scientific community (higher education, Research)	400	Europe
33	Posters	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	How do carbide ENM dispersions evolve in an in vitro assessment?	11/01/2013	Nanosafe 2012, Grenoble, France	Scientific community (higher education, Research)	400	Europe
34	Oral presentation to a scientific event	INSTITUT UNIVERSITAIRE ROMAND DE SANTE AU TRAVAIL	Promotion of Transnational Access	12/04/2012	'Enlargement' Workshop on the applications of nanobiotechnologies. Ispra, Italy.	Scientific community (higher education, Research)	80	Europe

N°	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
35	Posters	INSTITUT UNIVERSITAIRE ROMAND DE SANTE AU TRAVAIL	Time resolved characterization of carbide nanoparticle dispersions for in vitro toxicological evaluation	02/01/2014	2nd QNano Integrating Conference. Prague, Czech Republic	Scientific community (higher education, Research)	400	Europe
36	Posters	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Quantification of Engineered NanoMaterials in complex matrices: PIXE case studies	02/01/2014	2nd QNano Integrating Conference. Prague, Czech Republic	Scientific community (higher education, Research)	400	Europe
37	Oral presentation to a scientific event	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Nanosafety: a multidisciplinary approach towards the use of engineered nanomaterials	01/01/2015	salery range	Scientific community (higher education, Research)	80	Belgium
38	Posters	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Promotion of Transnational Access	09/03/2013	NanoReg WP2 Workshop. Copenhagen, Denmark	Scientific community (higher education, Research)	40	Europe
39	Posters	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	The use of PIXE in the field of contemporary nanotechnology issues: from nanoparticles in water pumps to the biopersistence in rat organs	09/01/2014	11th European Conference on Accelerators in Applied Research and Technology. Namur, Belgium	Scientific community (higher education, Research)	150	Europe
40	Posters	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Quantitative analysis of nanomaterials in complex matrices transnational access facility from the namur nanosafety center	09/01/2014	11th European Conference on Accelerators in Applied Research and Technology. Namur, Belgium	Scientific community (higher education, Research)	150	Europe
41	Oral presentation to a scientific event	INSTITUT UNIVERSITAIRE ROMAND DE SANTE AU TRAVAIL	The use of CLS and PIXE in the context of nanosafety	09/04/2014	NanoValid Training Workshop Zaragoza, Spain	Scientific community (higher education, Research)	40	Europe

Nº	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
42	Oral presentation to a scientific event	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Quantitative analysis of nanomaterials in complex matrices transnational access facility from the namur nanosafety center	09/04/2014	NanoValid Training Workshop Zaragoza, Spain	Scientific community (higher education, Research)	40	Europe
43	Posters	FUNDACIO INSTITUT CATALA DE NANOCIENCIA I NANOTECNOLOGIA	Quantitative analysis of nanomaterials in complex matrices transnational access facility from the namur nanosafety center	10/11/2014	Conference cycle A.B.I.A. Antwerp, Belgium	Civil society	40	Belgium
44	Organisation of Workshops	INSTITUT UNIVERSITAIRE ROMAND DE SANTE AU TRAVAIL	QNano WP7 meeting at FUNDP	06/02/2014	Namur, Belgium	Scientific community (higher education, Research)	10	Europe
45	Posters	THE UNIVERSITY OF EXETER	TAF Poster: University of Exeter	07/04/2017	Crete	Scientific community (higher education, Research)	150	International
46	Posters	THE UNIVERSITY OF EXETER	Imagind nanomaterials with CARS	06/08/2014	The EuroNanoForum, Dublin	Scientific community (higher education, Research)	1000	International
47	Organisation of Workshops	USTAV EXPERIMENTALNI MEDICINY AKADEMIE VED CESKE REPUBLIKY VEREJNA VYZKUMNA INSTITUCE	Workshop for students and young scientists to promote QNano Research Infrastructure	04/02/2015	Prague	Scientific community (higher education, Research)	20	Czech Republic
48	Organisation of Conference	USTAV EXPERIMENTALNI MEDICINY AKADEMIE VED CESKE REPUBLIKY VEREJNA VYZKUMNA INSTITUCE	Mini Summer School ºNano Safety - Opportunity for Young Researchers	06/08/2015	Ostrava	Scientific community (higher education, Research)	14	Czech Republic

N°	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
49	Oral presentation to a scientific event	UNIVERSITY OF LEEDS	Strategies to eliminate/reduce nanoparticles batch to batch variability	05/09/2015	Beijing	Scientific community (higher education, Research)	40	China
50	Posters	UNIVERSITY OF LEEDS	Synthesis of high quality NPs and reduce NP variability	07/01/2016	Leeds	Scientific community (higher education, Research)	150	UK
51	Oral presentation to a scientific event	NORSK INSTITUTT FOR LUFTFORSKNING	Can the comet assay be used reliably to detect nanoparticle-induced genotoxicity?	04/12/2015	Helsinki, Finland	Scientific community (higher education, Research)	400	International
52	Oral presentation to a scientific event	NORSK INSTITUTT FOR LUFTFORSKNING	Genotoxicity of nanosilver. Impact of size and surface properties	07/12/2015	Plymouth, UK	Scientific community (higher education, Research)	400	International
53	Oral presentation to a scientific event	INSTITUT UNIVERSITAIRE ROMAND DE SANTE AU TRAVAIL	Do we have testing strategy?	04/12/2015	Nanotox, Antalya, Turkey	Scientific community (higher education, Research)	400	International
54	Oral presentation to a scientific event	NORSK INSTITUTT FOR LUFTFORSKNING	Role of DNA damage and repair in pathogenesis of civilization diseases.	02/03/2014	Bratislava, Slovakia	Scientific community (higher education, Research)	100	Europe
55	Oral presentation to a scientific event	NORSK INSTITUTT FOR LUFTFORSKNING	DNA damage and repair by the comet assay in human biomonitoring.	05/08/2016	Kaposvar, Hungary	Scientific community (higher education, Research)	100	Europe
56	Oral presentation to a scientific event	NORSK INSTITUTT FOR LUFTFORSKNING	Genotoxicity testing of nanomaterial. New testing strategies.	10/09/2014	Kaposvar, Hungary	Scientific community (higher education, Research)	100	Europe
57	Oral presentation to a scientific event	NORSK INSTITUTT FOR LUFTFORSKNING	QualityNano: A pan-European infrastructure for Quality in NMs Safety Testing	10/12/2014	Prague, Czech Republic	Scientific community (higher education, Research)	400	International

Nº	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
58	Oral presentation to a scientific event	NORSK INSTITUTT FOR LUFTFORSKNING	Testing strategy for nanomaterials Workshop "in vitro choice"	11/04/2015	Barcelona, Spain	Scientific community (higher education, Research)	100	Europe
59	Oral presentation to a scientific event	NORSK INSTITUTT FOR LUFTFORSKNING	Genotoxicity of nanomaterials. Is the effect nano-specific?	08/02/2014	Warszaw, Poland	Scientific community (higher education, Research)	400	International
60	Posters	NORSK INSTITUTT FOR LUFTFORSKNING	QualityNano, Heraklion, Crete, Greece	07/03/2016	The effect of lead and cadmium nanoparticles on immune response of inhaled mice	Scientific community (higher education, Research)	150	International
61	Posters	NORSK INSTITUTT FOR LUFTFORSKNING	Toxicity of size-factionated airborne particulate matter in A549 cells.	07/03/2016	QualityNano final meeting, Heraklion	Scientific community (higher education, Research)	150	International
62	Posters	INSTITUT UNIVERSITAIRE ROMAND DE SANTE AU TRAVAIL	Multi-walled carbon nanotubes (NM401) induce ROS and HPRT mutations in Chinese hamster lung fibroblast cells	07/03/2016	QualityNano final meeting, Heraklion	Scientific community (higher education, Research)	150	International
63	Oral presentation to a scientific event	RIJKSUNIVERSITEIT GRONINGEN	Understanding how cells process nanoparticles for nanomedicine applications and quality in nanosafety testing	05/01/2015	Department of Pharmacy, University of Groningen	Scientific community (higher education, Research)	100	Netherlands
64	Oral presentation to a scientific event	RIJKSUNIVERSITEIT GRONINGEN	Characterising and quantifying nanoparticle interactions with cells	07/01/2016	QualityNano Conference, Heraklion, Crete	Scientific community (higher education, Research)	150	International

N°	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
65	Oral presentation to a scientific event	INSTITUT UNIVERSITAIRE ROMAND DE SANTE AU TRAVAIL	Understanding how cells process nanoparticles for nanomedicine applications and quality in nanosafety testing	06/12/2014	Bilthoven, The Netherlands, RIVM (National Institute for Public Health and the Environment)	Scientific community (higher education, Research)	20	Netherlands
66	Oral presentation to a scientific event	RIJKSUNIVERSITEIT GRONINGEN	Nanoparticle uptake and biointeractions for nanomedicine applications and nanosafety	07/01/2014	Conference of the CostAction AFMBio (European network on applications of Atomic Force Microscopy to	Scientific community (higher education, Research)	30	European
67	Oral presentation to a scientific event	NORSK INSTITUTT FOR LUFTFORSKNING	A common approach to the regulatory testing of nanomaterial.	10/05/2014	Bergen, Norway	Scientific community (higher education, Research)	70	Norway
68	Posters	NORSK INSTITUTT FOR LUFTFORSKNING	Is epithelial to mesechymal transition followed by global DNA metylkation changes?	12/10/2015	Olomouc, Czech Republic	Scientific community (higher education, Research)	100	Europe
69	Posters	NORSK INSTITUTT FOR LUFTFORSKNING	Salvia officinalis extract enhances the adhesion of surface-modified magnetite nanoparticles onto the cell membrane	07/03/2016	Crete, Greece	Scientific community (higher education, Research)	150	International
70	Posters	NORSK INSTITUTT FOR LUFTFORSKNING	Toxicity of the size-fractionated airborne particulate matter in A549 cells	11/05/2014	Brno, Czech Republic	Scientific community (higher education, Research)	400	International
71	Posters	NORSK INSTITUTT FOR LUFTFORSKNING	Application of the comet assay for nanotoxicity study on example of nanosilver	04/11/2015	Antalya, Turkey	Scientific community (higher education, Research)	400	International

Nº	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
72	Posters	NORSK INSTITUTT FOR LUFTFORSKNING	In vitro mutagenicity potential of different type of silver nanoparticles	04/11/2015	Antalya, Turkey	Scientific community (higher education, Research)	400	International
73	Posters	NORSK INSTITUTT FOR LUFTFORSKNING	Size, charge and stabilizer depended genotoxicity of nanosilver	09/06/2014	Porto, Portugal	Scientific community (higher education, Research)	400	International
74	Organisation of Workshops	JRC -JOINT RESEARCH CENTRE-EUROPEAN COMMISSION	CYCLEUR Cyclotron Research and Nanoparticle Radiolabelling Workshops	28/11/2011	Ispra, Italy	Scientific community (higher education, Research)	45	Europe
75	Organisation of Workshops	JRC -JOINT RESEARCH CENTRE-EUROPEAN COMMISSION	CYCLEUR Cyclotron Research and Nanoparticle Radiolabelling Workshops	29/11/2012	Ispra, Italy	Scientific community (higher education, Research)	45	Europe
76	Organisation of Workshops	JRC -JOINT RESEARCH CENTRE-EUROPEAN COMMISSION	CYCLEUR Cyclotron Research and Nanoparticle Radiolabelling Workshops	11/12/2013	Ispra, Italy	Scientific community (higher education, Research)	45	Europe
77	Organisation of Workshops	JRC -JOINT RESEARCH CENTRE-EUROPEAN COMMISSION	CYCLEUR Cyclotron Research and Nanoparticle Radiolabelling Workshops	13/11/2014	Ispra, Italy	Scientific community (higher education, Research)	45	Europe
78	Organisation of Conference	UNIVERSITY COLLEGE DUBLIN, NATIONAL UNIVERSITY OF IRELAND, DUBLIN	QNano Final Meeting	13/07/2015	Heraklion, Greece	Scientific community (higher education, Research)	150	International
79	Posters	UNIVERSITY COLLEGE DUBLIN, NATIONAL UNIVERSITY OF IRELAND, DUBLIN	Transnational Access Activity Across Europe	16/07/2015	QNano final meeting, Heraklion	Scientific community (higher education, Research)	150	International

N°	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
80	Press releases	UNIVERSITY COLLEGE DUBLIN, NATIONAL UNIVERSITY OF IRELAND, DUBLIN	Kenneth Dawson interview for local news coverage	17/07/2015	Heraklion, Greece	Medias	150	International

Section B (Confidential[9] or public: confidential information to be marked clearly)
Part B1

The applications for patents, trademarks, registered designs, etc. shall be listed according to the template B1 provided hereafter.

The list should, specify at least one unique identifier e.g. European Patent application reference. For patent applications, only if applicable, contributions to standards should be specified. This table is cumulative, which means that it should always show all applications from the beginning until after the end of the project.

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.					
Type of IP Rights[10]:	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant (s) (as on the application)

[9] Note to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

[10] A drop down list allows choosing the type of IP rights: Patents, Trademarks, Registered designs, Utility models, Others.

Part B2
Please complete the table hereafter:

Type of Exploitable Foreground[11]	**Description of exploitable foreground**	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	**Exploitable product(s) or measure(s)**	**Sector(s) of application**[12]	**Timetable, commercial or any other use**	**Patents or other IPR exploitation (licences)**	**Owner & Other Beneficiary(s) involved**
	Ex: New supercond uctive Nb-Ti alloy			*MRI equipment*	*1. Medical 2. Industrial inspection*	*2008 2010*	*A materials patent is planned for 2006*	*Beneficiary X (owner) Beneficiary Y, Beneficiary Z, Poss. licensing to equipment manuf. ABC*

In addition to the table, please provide a text to explain the exploitable foreground, in particular:

- Its purpose
- How the foreground might be exploited, when and by whom
- IPR exploitable measures taken or intended
- Further research necessary, if any
- Potential/expected impact (quantify where possible)

[19] A drop down list allows choosing the type of foreground: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, exploitation of results through EU policies, exploitation of results through (social) innovation.
[12] A drop down list allows choosing the type sector (NACE nomenclature) : http://ec.europa.eu/competition/mergers/cases/index/nace_all.html

4.3 Report on societal implications

Replies to the following questions will assist the Commission to obtain statistics and indicators on societal and socio-economic issues addressed by projects. The questions are arranged in a number of key themes. As well as producing certain statistics, the replies will also help identify those projects that have shown a real engagement with wider societal issues, and thereby identify interesting approaches to these issues and best practices. The replies for individual projects will not be made public.

A General Information *(completed automatically when **Grant Agreement number** is entered.*	
Grant Agreement Number:	262163
Title of Project:	A pan-European infrastructure for quality in nanomaterials safety testing
Name and Title of Coordinator:	Professor Kenneth Dawson

B Ethics	
1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	***No***
2. Please indicate whether your project involved any of the following issues (tick box) :	***YES***
RESEARCH ON HUMANS	
• Did the project involve children?	No
• Did the project involve patients?	No
• Did the project involve persons not able to give consent?	No
• Did the project involve adult healthy volunteers?	No
• Did the project involve Human genetic material?	No
• Did the project involve Human biological samples?	Yes
• Did the project involve Human data collection?	No
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	No
• Did the project involve Human Foetal Tissue / Cells?	No
• Did the project involve Human Embryonic Stem Cells (hESCs)?	No
• Did the project on human Embryonic Stem Cells involve cells in culture?	No
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	No
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	No
• Did the project involve tracking the location or observation of people?	No
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	Yes
• Were those animals transgenic small laboratory animals?	Yes

• Were those animals transgenic farm animals?	No
• Were those animals cloned farm animals?	No
• Were those animals non-human primates?	No
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	**No**
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	**No**
DUAL USE	
• Research having direct military use	No
• Research having the potential for terrorist abuse	No

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	1	5
Work package leaders	7	7
Experienced researchers (i.e. PhD holders)	49	74
PhD Students	17	11
Other	42	37

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	
Of which, indicate the number of men:	

D Gender Aspects

5. Did you carry out specific Gender Equality Actions under the project?	○ X	Yes **No**

6. Which of the following actions did you carry out and how effective were they?

		Not at all effective				Very effective
❑	Design and implement an equal opportunity policy	○	○	○	○	○
❑	Set targets to achieve a gender balance in the workforce	○	○	○	○	○
❑	Organise conferences and workshops on gender	○	○	○	○	○
❑	Actions to improve work-life balance	○	○	○	○	○
○	Other:					

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

○ Yes- please specify

X **No**

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

○ Yes- please specify

X **No**

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

X Yes- please specify: Developed materials for university/researchers via the Training Schools and protocols

○ No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

○ Main discipline[13]: 1.3 Chemical Sciences

○ Associated discipline[13]:1.5 Biological sciences

○ Associated discipline[13]: 1.2 Physical sciences

G Engaging with Civil society and policy makers

11a Did your project engage with societal actors beyond the research community? *(if 'No', go to Question 14)*	X ○	**Yes** No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

○ No

X Yes- in determining what research should be performed

○ Yes - in implementing the research

○ Yes, in communicating /disseminating / using the results of the project

[13] Insert number from list below (Frascati Manual).

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	○ X	Yes **No**

12. Did you engage with government / public bodies or policy makers (including international organisations)

○	No
X	Yes- in framing the research agenda
○	Yes - in implementing the research agenda
○	Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

X	Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
○	Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
○	No

13b If Yes, in which fields?

Agriculture x		**Energy x**		Human rights	
Audiovisual and Media		Enlargement		**Information Society x**	
Budget		**Enterprise x**		Institutional affairs	
Competition		**Environment x**		**Internal Market x**	
Consumers x		External Relations		Justice, freedom and security	
Culture		**External Trade x**		**Public Health x**	
Customs		Fisheries and Maritime Affairs		**Regional Policy x**	
Development Economic and Monetary Affairs		**Food Safety x**		**Research and Innovation x**	
		Foreign and Security Policy		Space	
Education, Training, Youth x		Fraud		Taxation	
Employment and Social Affairsx		Humanitarian aid		Transport	

13c If Yes, at which level?

- ○ Local / regional levels
- ○ National level
- ○ European level
- X International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?		**66**
To how many of these is open access[14] provided?		
How many of these are published in open access journals?		
How many of these are published in open repositories?		
To how many of these is open access not provided?		
Please check all applicable reasons for not providing open access:		
❑ publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available ❑ no suitable open access journal available ❑ no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[15]: ……		
15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		**0**
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	**0**
	Registered design	**0**
	Other	**0**
17. How many spin-off companies were created / are planned as a direct result of the project?		**0**
Indicate the approximate number of additional jobs in these companies:		

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

❑	Increase in employment, or	❑	In small & medium-sized enterprises
❑	Safeguard employment, or	❑	In large companies
❑	Decrease in employment,	X	None of the above / not relevant to the project
❑	Difficult to estimate / not possible to quantify		

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:	*Indicate figure:*

[14] Open Access is defined as free of charge access for anyone via Internet.
[15] For instance: classification for security project.

Difficult to estimate / not possible to quantify	❑

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

○ Yes x No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

○ Yes x No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

x	Press Release	x	Coverage in specialist press
x	Media briefing	❑	Coverage in general (non-specialist) press
x	TV coverage / report	❑	Coverage in national press
x	Radio coverage / report	x	Coverage in international press
x	Brochures /posters / flyers	x	Website for the general public / internet
❑	DVD /Film /Multimedia	x	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

❑	Language of the coordinator	x	English
❑	Other language(s)		

***Question F-10*:** Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]

1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)

1.3 Chemical sciences (chemistry, other allied subjects)

1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)

1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY

2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry,

etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]