NanoImpact: Special Issue of ProSafe

A condensed paper which serves as a reader's guide of the NanoImpact Special Issue. An overview of general findings for policy makers; for the policy conference on 'A future-proof approach to nanomaterials' (17-18 April 2018).

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Executive summary

Scientists all over Europe have cooperated to clarify how nanomaterials can be used safely. Now that the European NANoREG and ProSafe projects have ended, European policy makers are provided with information, new insights and proposals that will enable them to take action to ensure future safe use of nanomaterials.

ProSafe represented a collaborative effort by twelve organisations from nine EU Member States and associated nations, working together on more effective and cost-efficient regulatory human and environmental health assessment of nanomaterials. In this context, the results of several EU research projects on health, safety and the environment were assessed. The aim was to translate these results into building blocks and recommendations for regulatory actions, boosting the impact of each individual project by placing it into larger context.

Various papers arising from relevant projects in the field were published in NanoImpact, a scientific journal. Key recommendations ('no regret' measures) included harmonising test methods and exposure limits, selecting test methods for further development, and requiring that substance identification data must include particle size distribution, shape, porosity and reactivity. Issues were also addressed that are relevant to future-proof approaches, such as interim changes during lifecycle and risk assessment frameworks to secure material safety.

The published information and insights needed for policy makers are summarized below. The link between brackets directs to a more extended summary. The interested reader can also access the original publication in NanoImpact (see the link below the title of the paper). The aim is to support policymakers and regulators in reducing uncertainty in regulatory assessment of nanomaterials, supporting a climate in which nanotechnology will be able to reach its full innovative potential. These topics are of utmost importance for the safe production and safe use of nanomaterials.

Standardisation of nanomaterial identification and characterisation
In a regulatory context, nanomaterials are subject to the same risk assessment paradigm and associated endpoints as conventional chemicals. However, chemical composition is not the only relevant aspect here; other parameters are needed to identify specific manufactured nanomaterials for regulatory purposes. It is also necessary to develop new methods for material characterisation and for determining hazard, exposure, fate, and risk assessment. (1)

Standardised, validated characterisations are needed to measure the physicochemical properties of nanomaterials that are relevant in assessing health and safety risks. Many properties of manufactured nanomaterials and their behaviours are difficult to characterise using the available methods. New methods and modifications to conventional methods are being developed to address some of these shortcomings.

Overall, the nanomaterials industry has made good progress towards identifying the (unique) properties of nanomaterials that must be measured to predict human and environmental health risks. Additional investments in assessing the robustness of the measurements and developing standards for data reporting will greatly improve comparability of data. (2)

Exposure assessment and modelling

Assessment of nanomaterial exposure for workers, consumers and the general public via the environment is well developed; the biggest challenge here is harmonisation to create solid regulatory frameworks. Real-life studies are still needed so that models and assessment strategies can be evaluated in actual practice. (3)

Exposure modelling is a key part of the risk assessment process, because it can show predicted environmental concentrations. Modelling can help in assessing emerging technologies, like engineered nanomaterials, to predict environmental flows and concentrations of nanomaterials. Exposure models should include release during end-of-life processes (e.g. waste incineration or landfill disposal) as well as intended release (such as agricultural applications). A lifecycle-based analysis is needed to identify which release pathways are most relevant for each engineered nanomaterial. (4)

Inhalation is an important means of (accidental) exposure to manufactured nanomaterials, followed by oral and dermal exposure. Predictive testing strategies can be used to compare risks against confirmed benchmarks. After that, risks can be quantified and characterised. A framework has been developed which combines different strategies that cover all classes of nanomaterials and exposure routes, in order to optimise data collection methods for regulatory use. This framework could lead to more efficient and cost-effective regulatory risk assessment. (5)

Designing safe, functional nanomaterials requires a comprehensive understanding of how the physiochemical properties of a nanomaterial relate to its behaviour in biological systems. Computational models can help establish those relationships, and are becoming an increasingly important tool in helping us fill our knowledge gaps for untested chemicals. The (Q)SAR methodology is well known and extensively applied to drug discovery and chemical toxicity modelling. (Q)SAR models are currently being developed for regulatory assessment of nanomaterial risks. These models are not yet ready to be used as standalone regulatory testing methods. However, they can be used to support the findings of other, more robust *in vivo* and *in vitro* tests. (6)

Nanomaterials in the environment

Nanomaterials are being used more widely, making it even more important to describe and predict their environmental fate and behaviour. It is necessary to identify the data, testing protocols and assessment methods that are reliable and have regulatory relevance. How nanomaterials dissolve or are dispersed through water is relevant, as is biodegradability. In addition to gaps in testing methods, there are also simply knowledge gaps in what we know and understand about the environmental fate and behaviour of nanomaterials. (7)

Ecotoxicity and health hazards

Reliable, relevant aquatic ecotoxicity testing may be able to facilitate regulatory decision-making on manufactured nanomaterials. In general, reliable ecotoxicity testing faces two central issues: a) creating and maintaining stable suspensions, and b) appropriately characterising suspensions. Comprehensive protocol standardisation of environmental matrix components is not yet feasible; there simply is not enough information available. Tests should be developed to assess chronic effects of long-term exposure to low concentrations of nanomaterials. (8)

Engineered nanomaterials are developing at lightning speed, creating a strong need for fast, reliable assessment of their health hazard potential. There are a range of different testing methods that have been developed for toxicology of chemicals. However, since nanomaterials are solids, conventional *in vitro* assessments have to be adapted. More standardisation is needed here in order to use the resulting data in risk assessment. (9)

Ongoing development of risk assessment frameworks

Nanomaterial-specific risk assessment frameworks are being developed that prioritise or substitute nanomaterials of very high concern and bring safety considerations into the innovation chain. These risk assessment frameworks are designed to efficiently prioritise, rank or assess the safety of a nanomaterial by targeting critical information. Where nanomaterials are involved, certain issues deserve special attention, including exposure, bioaccumulation, *in vitro*/*in vivo* comparisons and long-term effects. More work also needs to be done to standardise and develop protocols and guidance documents, as well as suitable controls and reference materials. (10)

As nanomaterials continue to evolve, regulatory guidelines must keep pace with advancements in technology. While existing regulatory frameworks designed for conventional chemicals can be applied to nanomaterial manufacturing, the unique characteristics of nanomaterials may demand a more tailored regulatory approach. Scientific research has identified the test protocols, data sets, models, guidances, and assessment methods that are most relevant to regulatory needs. Reliable methods are already available – and are ready to be integrated into regulatory policy, or will be soon. (11)
In parallel to regulatory frameworks, other concepts, like Safe by Design (SbD), might be the way to keep pace with innovation.

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Standardisation of nanomaterial identification and characterisation

1. Methods and data for regulatory risk assessment of nanomaterials: Questions for an expert consultation

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Nanomaterials are subject to the same risk assessment paradigm and associated endpoints as conventional chemicals. However, chemical composition is not the only relevant aspect here; other parameters are needed to identify specific manufactured nanomaterials for regulatory purposes. It is also necessary to develop new methods for material characterisation and for determining hazard, exposure, fate, and risk assessment.

This paper covers the regulatory questions for each nanomaterial risk assessment endpoint. They were derived in part from the EU FP7 research programme NANoREG, and augmented by issues raised by regulatory authorities. Relevant topics were grouped into themes, marked in bold below.

A nanomaterial's **physicochemical properties** determine whether it falls under regulatory action and make it possible to predict its potential hazard and fate outcomes. Two groups of properties were identified (see also Table 1): intrinsic or part of the nanomaterial itself; and extrinsic, or influenced by external factors such as the media in which they are tested for their toxic potential. On that basis, the endpoints were evaluated to see whether intrinsic or extrinsic properties would work best to identify nanomaterials and to determine their fate and effects. The conclusion was that intrinsic particle properties would be most relevant to regulatory definitions of a nanomaterial.

Regulatory authorities evaluate **exposure** throughout the lifecycle of a nanomaterial to assess its potential to harm the environment and human health. Important considerations here include identifying the best ways to measure and simulate exposure, defining models to estimate exposure and determining dose metrics for reporting exposure.

Nanomaterials behave differently than conventional chemicals, so it is difficult to use conventional models to verify environmental exposure and **fate**. In particular, methods should be devised that can help identify major sinks of nanomaterials. Also, OECD test guidelines for conventional chemicals should be reviewed to see which parts may need revisions to make these applicable for nanomaterials testing. New guidelines or guidance will be needed for specific nanomaterial aspects such as solubility and size distributions.

New **models** need to be developed or current ones need to be adapted to support a regulatory risk assessment framework for nanomaterials. These models should be included that address the entire lifecycle, from production to long-term disposal.

The **ecological effects** of nanomaterials are challenging to measure. There are concerns on how well a nanomaterial is distributed in the environmental media and and substantial knowledge is lacking on interactions with test systems and organisms. In this context, it is important to find methods for keeping the nanomaterials dispersed in test solutions.

The **human health effects** of nanomaterials are obviously important. Yet there is limited specific guidance on testing. Regulators in multiple countries have been working on this problem at OECD level, and some test guidelines have already been adapted to accommodate testing of nanomaterials. Protocoled test in experimental animals are used to predict human health effects. These can be improved – for instance by adding tests that show whether nanoparticles can cross biological barriers and penetrate cells. The OECD standards for tests using cell cultures (*in vitro*) are hard to meet here; other, separate validation criteria defined specifically for nanomaterials could lead to more regulatory confidence and more reliable test results.

Computational methods (*in silico*) can also be a helpful supplement to *in vitro* and tiered testing. Regulators are already using methods like structure-activity relationships to assess conventional chemicals. Those kinds of analyses could also offer promising ways to make nanomaterial assessment faster and more resource-effective. It is an area that has only recently started attracting attention, but more work should be done to continue developing these SAR models for nanomaterials.

Ecological and health **risk assessment** components can also be applied to nanomaterial risk assessments. However, general and specific nanomaterial risk assessment and grouping frameworks should be evaluated. Case studies are a key factor in building useful risk assessment frameworks. Nanomaterials are difficult to track from production to disposal, so it will be a challenge to include the whole lifecycle in these models.

2. Progress towards standardised and validated characterisations for measuring physicochemical properties of manufactured nanomaterials relevant to nano health and safety risks

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To manage the risks of manufactured nanomaterials, it must be possible to accurately and reproducibly measure the physical and chemical properties relevant for risk assessment. However, many properties of manufactured nanomaterials and their behaviours are difficult to characterise using the available methods. New methods and modifications to conventional methods are being developed to address some of these shortcomings.

The properties of nanomaterials can be categorised into intrinsic and extrinsic (see below). This is relevant because characterisation of extrinsic properties is often less validated and harder to reproduce, since the medium in which the nanomaterial is found affects the results. Metadata that explains the other factors should be included in reporting the results, so extrinsic properties can be compared.

Table 1: Intrinsic and extrinsic nanomaterial properties	
Intrinsic (system-independent)	*Extrinsic (system-dependent)*
Particle size distribution Specific surface area Particle shape Hydrophobicity Chemical composition Redox potential Band gap Density	Effective density in media, Dustiness, Zeta potential, Agglomeration rate Surface affinity Dissolution rate & solubility Reactive oxygen species generation.

Easy, reproducible, reliable methods that can be used as routine tests to characterise the physicochemical properties of nanomaterials are a top priority for the nano industry. **Most of the methods that we now use to characterise nanomaterials are not new at all.** Some of them have been well-developed through research done in other materials like colloids. After some additional testing and validation, these methods can be directly applied in nano-related characterisations. However, in many cases, these measurements still **need to be standardised** and more nanomaterials need to be tested to achieve both reproducibility and reliability (validation).

Many nanomaterial properties are **significantly affected by the passing of time**. Time-dependent differences can be controlled by developing standard operating procedures. Even then, **test results may vary more widely** for particle properties that change quickly in time. The challenge is to limit variability amongst laboratories. It may be worth reporting the time of measurement down to the level of seconds.

Overall, the nanomaterials industry has made good progress towards identifying the (unique) properties of nanomaterials that must be measured to predict human and environmental health risks. Some methods are becoming standardised and automated. The usability of reported data is at present limited when it comes to predicting the behaviour of nanomaterials. Additional investments in **assessing the robustness of the measurements** and **developing standards for data reporting** will greatly improve comparability of data.

Exposure assessment and modelling

3. Nanomaterial exposures for worker, consumer and the general public

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Nanomaterial exposure assessment is the most fully developed branch of nanomaterial assessment; a variety of measurement devices, methods and significant data are already available. The biggest challenge here is harmonisation to create solid regulatory frameworks. This summary of the current state of the art and challenges for nanomaterial exposure assessment for workers, consumers and the general public via the environment is intended to promote advancements in different exposure assessment fields by facilitating cross-fertilisation.

Exposure to nanomaterials encompasses any exposure to nano-objects, nanostructured materials or nanocomposite. That includes nanomaterials in a relatively pristine state during production, or in a more aged condition at some later stage.

Assessments of consumer exposure to nanomaterials mainly focus on combining release measurements and exposure scenario modelling. A tiered approach like the OECD-approved methods for measuring airborne emissions in the workplace could also be useful here.

Very little data is available on **exposure to the general public** via the environment. Measurement and analysis methods are limited and relatively expensive. Accordingly, most models rely on environmental nanomaterial concentrations. Many of the parameters used are just estimates because actual measurements are either not feasible (level of detection) or absent (too expensive), which means the level of uncertainty is often very high.

Overall, good progress has been made on exposure assessment, showing considerable advancements over the last ten years. Measurement devices and strategies are becoming more readily available and measured values are more robust, with comparability rates for airborne particle measurements at around 30% to 50%. Measurement devices are (or are becoming) easier to handle, portable and more affordable.

Workplace and regulatory risk assessments still rely heavily on mass concentrations, even though many workplace measurements have been done to determine particle number concentration, as this is easier to measure. Neither method can distinguish between the nanomaterial of interest and background particles. Nanofiber measurement should be based on the same procedure as for asbestos. However, the methods and strategies for measuring nanofibers are not as well developed as for nanoscale particles.

Reliable data, measurement methods and strategies that can be used to estimate exposure for consumers, the general public and the environment are still in their infancy. Consumer exposure is not measured on a regular basis, nor can it be. The major problem for environmental exposure measurement is the inability to routinely identify nanomaterials in an environmental matrix.

One option could be improved guidance for assessing consumer exposure to nanomaterials within existing frameworks like REACH, cosmetics regulation and food legislation. State-of-the-art environmental exposure models also include nanomaterial transportation and transformation, but further evaluation is needed before they are used for regulatory purposes.

Nanomaterial release, potentially leading to exposure, takes place in response to mechanical, thermal or chemical processes. Ways in which nanomaterials are released should be identified and tested; this research should include fragment types as well as the original nanomaterial. A Framework of Release is being developed to identify relevant methods for assessing exposure, define output, and specify how the data should be used in exposure modelling and safer-by-design applications.

Real-life studies are still needed so that the models and tiered assessment strategies presented here can be evaluated in actual scenarios. Some will be useful in the regulatory framework, and some have already been adopted for harmonisation and standardisation, in guidance to regulation, and even in legally binding regulation in a few cases. That kind of bridging between scientific developments and regulation should be continued and intensified to facilitate adaptations in regulation where needed and appropriate.

4. Evaluation of environmental exposure models for engineered nanomaterials in a regulatory context

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Exposure modelling is a key part of the risk assessment process, because it can show predicted environmental concentrations even without analytical data. Such modelling can help in assessing emerging technologies, like engineered nanomaterials, for which limited data is available. Various models have been used recently to predict environmental flows and concentrations of nanomaterials.

Environmental exposure modelling can be grouped into two categories: **material flow models** and **environmental fate models**. Material flow models track the entire lifecycle of nanomaterials, from production and use to end-of-life processes, and then into the environment. Environmental fate models use the data on material flows to determine what happens to nanomaterials at each step, predicting the form the nanomaterial will take and how it will be distributed.

Material flow models are more common. One recent development is **dynamic material flow modelling**, which acknowledges that nanomaterials may be trapped in a product for a long time before they start being released into the environment. As a result, nanomaterial release may be delayed or occur very slowly. Because dynamic models take that into account, they are able to provide a more accurate, comprehensive impression of environmental flow.

It is important to quantify release at every stage of the lifecycle, and sufficient data for this is still lacking. Only 20% of the engineered nanomaterials used industrially and 36% of the product categories involved have been investigated in release studies, and only a few relevant release scenarios have been described – and that data is often incomplete.

Overall release of materials from consumer products has been well documented. Limited experimental release data is available on nanomaterials and there is a critical need for such data to be used in environmental exposure models.

Releases during end-of-life processes have so far received only passing attention. Flows of engineered nanomaterials into waste incineration plants and landfills have been quantified in existing models. The release from these waste-handling processes has garnered little attention. Landfills as final sink are an important compartment where most of the engineered nanomaterials end up, but there has been almost no research on possible

releases from landfills. There are landfills for hazardous waste, but they have never been considered in nano-modelling.

Intended release applications, soil or groundwater remediation, or agricultural applications, also have high exposure potential. However, current use in agriculture seems very limited and no exposure model includes it. EU pesticide regulations do not include nano-specific provisions, so there is no specific data available on the use of engineered nanomaterials as active ingredients, but it is thought to be low.

General conclusions on the most important sources of releases to the environment cannot be made. The release is determined by the uses of the engineered nanomaterial, and only an analysis using a lifecycle perspective can identify which release pathways are most relevant for each engineered nanomaterial.

5. *In vivo* effects: methodologies and biokinetics of inhaled nanomaterials

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Inhalation is an important means of (accidental) exposure to manufactured nanomaterials, followed by oral and dermal exposure. Predictive testing strategies can be used to compare risks against confirmed benchmarks. After that, risks can be quantified and characterised. Various strategies are needed to cover all classes of nanomaterials and exposure routes. The framework presented here combines strategies in order to optimise data collection methods for regulatory use.

The three common exposure routes for nanomaterials are inhalation, oral exposure and dermal exposure. Inhalation is often considered the biggest risk, because nanomaterials may cause direct interactions in the lungs, and are then transported from there into the rest of the body in significant quantities.

Inhalation studies can be used to identify hazards and assess risks from inhaling nanomaterials. The best design would provide exposure-response data, since it can be used to characterise risks. Combining results from different types of studies for the same group of nanomaterials offers the most effective predictive method.

Comparing short-term versus longer-term inhalation exposure may be useful for toxicity ranking and risk assessment. Short-term exposure studies have limitations in identifying long-term effects. Sub-chronic and chronic inhalation studies could help, but the use of animals and high costs present ethical and economic restrictions.

In order to group nanomaterials based on physiochemical composition and exposure routes, it is necessary to use multiple strategies. Many different variables need to be considered in regulatory decision-making. The framework suggested in this paper could make it easier to use data on sub-chronic inhalation in combination with data from alternative test methods, leading to more efficient and cost-effective regulatory risk assessment.

6. Review of (Q)SAR models for regulatory assessment of nanomaterials risks

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Designing safe, functional nanomaterials requires a comprehensive understanding of how the physiochemical properties of a nanomaterial relate to its behaviour in biological systems. Computational models can help establish those relationships, and are becoming an increasingly important tool in helping us fill our knowledge gaps for untested chemicals. The (Q)SAR methodology is well known and extensively applied to drug discovery and chemical toxicity modelling, but nanomaterial applications still require major advancements.

A number of (Q)SAR models are currently available for regulatory assessment of nanomaterial risks. OECD member countries have agreed on five principles that can be used to validate these computational models for regulatory use: (1) a defined endpoint, (2) an unambiguous algorithm, (3) a defined domain of applicability, (4) appropriate measures for goodness-of-fit, robustness and predictivity, and (5) mechanistic interpretation. The available nanomaterial (Q)SAR models have been evaluated to see whether they meet the standards for each principle and to assess how useful they might be for nanomaterial regulation in the future.

Building this type of computational model requires various levels of detail about the nanomaterials. Since extremely detailed information is often not available and would take a lot of time to obtain, software is used to calculate descriptors for different parts of a nanomaterial's structure. These figures can then be used in model equations or classification methods to predict toxicity. However, effective risk assessment of a nanomaterial requires identifying its extrinsic and intrinsic physicochemical properties. Better computational models are needed to find descriptors that can represent both kinds of properties.

The (Q)SAR methodologies developed so far will certainly help in grouping nanomaterials and performing read-across for regulatory purposes. However, the nanomaterials tested so far have not been a diverse group, so this kind of testing is only really useful for a few materials at this point.

Overall, the majority of the (Q)SAR models evaluated by this review do not fully comply with all the five OECD validation principles and/or do not take relevant endpoints into consideration. Consequently, these models are not yet ready to be used as standalone regulatory testing methods. However, they can be used to support the findings of other, more robust *in vivo* and *in vitro* tests.

Nanomaterials in the environment

7. Regulatory relevant and reliable methods and data for determining the environmental fate of manufactured nanomaterials

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The widespread use of manufactured nanomaterials increases the need to describe and predict their environmental fate and behaviour. A review was conducted of data, testing protocols and guidance papers that describe the environmental fate and behaviour of nanomaterials, focusing primarily on their regulatory reliability and relevance.

Though the specific test considerations will differ between conventional chemicals and nanomaterials, the ultimate endpoints are similar. **Water** is one of the main points of entry, allowing nanomaterials to be dispersed into the environment and forging a link to other environmental compartments like soil, sediment, air, and biota. Once released into water, various processes have a huge impact on the fate of nanomaterials. Relevant processes include dissolution, agglomeration, sedimentation, interaction with natural organic matter, transformation and uptake by biota.

Once a nanomaterial reaches the environment, its fate and behaviour depend on its own physicochemical properties, the environmental transformation processes, and specific environmental conditions. When released into any environmental compartment, nanomaterials undergo a series of **dynamic transformation processes**. The complexity involved in understanding and describing those processes makes it difficult to define simple test methods that can meet the standards of regulatory relevance and reliability.

Although transformation processes are a major factor in a nanomaterial's environmental fate, few studies have provided data on transformative processes like biodegradability that are appropriate for a regulatory context. Bio-modification almost certainly takes place under environmental conditions, yet very little is known about the processes themselves or how they influence the fate and behaviour of nanomaterials in the environment, or the resulting effects.

Surface affinity and agglomeration are relevant parameters for predicting the environmental fate and behaviour of nanomaterials. **Surface affinity** is crucial for determining nanomaterial attachment in environmental matrices and their tendency to heteroaggregate, so it would be advisable to **incorporate test guidance for surface affinity** determination into future research. **Agglomeration** is relevant for every type of nanomaterial, **so OECD test guidelines** for **agglomeration in aquatic media** would be a helpful step towards data that meets high regulatory standards.

In addition to gaps in testing methods, there are also simply knowledge gaps in what we know and understand about the environmental fate and behaviour of nanomaterials. Test guidelines are in development, but a tiered approach could be a good solution to address this need. **More research could be incorporated along the way, similar to the current OECD testing scheme for biodegradability**.

Ecotoxicity and health hazards

8. Regulatory adequacy of aquatic ecotoxicity testing of nanomaterials

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It is time to question whether ecotoxicity testing can facilitate regulatory decision-making on manufactured nanomaterials. This paper looks at aquatic ecotoxicity testing for manufactured nanomaterials and identifies issues that challenge test reliability and relevance.

Several recent reviews addressing aquatic ecotoxicology emphasise how hard it is to test nanomaterials. There is an increased focus on characterising rather than controlling exposure, in order to facilitate meaningful comparison between studies. Without effective comparison, the regulatory reliability of the data is called into question.

Work is being done in multiple organisations to identify or revise endpoints for testing nanomaterials. While this work is important, there is debate about whether every nanomaterial needs to be subjected to every test. In general, reliable ecotoxicity testing faces two central issues: a) creating and maintaining stable suspensions, and b) appropriately characterising suspensions (see section 2 above).

There are no harmonised (OECD) guidelines for dispersion protocols in ecotoxicity testing. Comprehensive protocol standardisation of environmental matrix components is not yet feasible; there simply is not enough information available.

Test interference is also an issue, since test guidelines were designed with conventional chemicals in mind. Nanomaterials act and react differently, and they have a different effect on the testing environment. As a result, interference can sometimes muddy the waters when it comes to determining what is actual toxicity and what is not.

Long-term exposure to low concentrations of nanomaterials could be highly relevant. **Far fewer tests for chronic effects** have been reported compared to studies reporting on acute effects. This is because long-term chronic effect testing is expensive and labour-intensive, and it is difficult to maintain exposure conditions that are stable enough to produce good results.

Different tests are needed to fulfil different regulatory needs. Regulation usually focuses on two distinct areas: classification and protection. Those two areas have different testing needs. Classification requires tests under controlled conditions that give reliable, reproducible results, so benchmarks can be set. Protection is about assessing hazards to test concentrations under more realistic conditions in order to identify no-effect concentrations.

A lack of appropriate data for regulatory decision-making is still a pressing issue. It is advisable to consciously push developments towards increased regulatory reliability. When the implicit limitations in testing are acknowledged for hazard identification and hazard assessment, data can be generated that meets regulatory needs.

9. *In vitro* approaches to assess the hazard of nanomaterials

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Engineered nanomaterials are developing at lightning speed, creating a strong need for fast, reliable assessment of their health hazard potential. There are a range of different testing methods that have been developed for toxicology of chemicals. However, since nanomaterials are solids, conventional in vitro *assessments have to be adapted.*

The importance of the nanomaterial properties differs depending on the exposure scenarios. For inhalation exposure, it is important to study characteristics that deal with material deposition – how much actually will come in contact with the cells – and how the nanomaterials can be cleared. Every exposure scenario will have its own unique set of characteristics that will, in the end, help arrive at the best assessment of its health hazard potential. More standardization is essential for *in vitro* systems in order for the resulting data to be used and accepted in risk assessment.

It is important for researchers to report the characteristics of the cell culture they are using to test the nanomaterial. The type of cells chosen for testing is also relevant, but not currently standardised at all. Accurate metadata reporting will help increase the relevance of the information derived from the tests.

Ongoing development of risk assessment frameworks

10. Risk assessment frameworks for nanomaterials: scope, link to regulations, applicability, and outline for future directions in view of needed increase in efficiency

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The rise in nanomaterial applications, paired with the realisation that their distinct features compared to larger sized counterparts should be considered in safety assessment, has led to the development of nanomaterial-specific risk assessment frameworks. These frameworks are designed to efficiently prioritise, rank or assess the safety of a nanomaterial by targeting critical information.

Each nanomaterial framework has its own scope, advantages and disadvantages. It should be noted that all but one lack certain vital details, such as decision criteria, that will be needed before they can be applied in practice. The most interesting frameworks are those that aim to gather information and make decisions on regulatory submissions at national and EU levels, as well as those aimed at informing decision-making in the innovation chain. Exposure and hazard assessment issues, such as lifecycle, bioaccumulation and delivered dose, should also be considered in risk assessment frameworks.

Elements for improving practical risk assessment feasibility include standardised testing, knowledge of *in vitro*/*in vivo* comparison, and functional assays. Grouping and read-across

approaches can offer some efficiency compared to a case-by-case approach. However, current science is not advanced enough to fully substantiate decision criteria and specific protocols that are needed to considerably increase efficiency. One way forward would be to develop a pragmatic, internationally accepted nanomaterial decision-making framework with decision criteria that are based only in part on scientific data. Such an approach would require cooperation from policy-makers, scientists and industry alike.

A key issue in accepting and applying risk assessment strategies in a regulatory setting is the need for adequate nanomaterial safety assessment information. Outside the arena of the regulatory legal framework, it is important to address human and environmental risk early on in the innovation chain. The aim of early risk management is to reduce the number of potentially unsafe nanomaterials, thus avoiding investment in product development that will never yield a marketable product due to its high risk for humans and/or the environment. This could be a win-win situation for innovators and regulators, as both could benefit from a reduced level of uncertainty.

For nanomaterials that do eventually reach the regulatory evaluation stage, there are two options for increasing efficiency in information gathering:

1. *Continue in line with current regulation.*
   Grouping and read-across approaches can offer some extra efficiency gains, and work is already being done to weave this approach into future guidance.
2. *Prioritise information needs based on highest potential risk.*
   Pragmatic choices for decision criteria based in part on scientific evidence could be applied to nanomaterial risk assessments.

Reprioritisation of information gathering based on potential risk would require cooperation from policy-makers, scientists and industry, as well as agreement at an international level. Aspects most likely to be influenced by nano-specific properties could be used as a starting point for dialogue here. Relevant aspects include exposure, solubility/dissolution rate, coating stability, accumulation, genotoxicity, inflammation, ecotoxicity and environmental fate. For nanomaterials that are already on the market, it would be practical to identify all the forms within a single substance, differentiating between key physicochemical properties.

Ongoing efforts are being made to improve existing risk assessment frameworks, and those efforts need to continue. Where nanomaterials are involved, certain issues deserve special attention, including exposure, bioaccumulation, *in vitro*/*in vivo* comparisons and long-term effects. More work also needs to be done to standardise and develop protocols and guidance documents, as well as suitable controls and reference materials.

Risk assessment frameworks that prioritise or substitute nanomaterials of very high concern and bring safety considerations into the innovation chain are gradually becoming more concrete. Case studies and success stories are needed to improve these frameworks and pave the way for mainstream application and a more robust regulatory environment. Consensus is vital to making sure nanomaterials (or nanoforms) are incorporated into risk assessment regulations in an efficient manner, i.e. beyond the case-by-case approach.

11. Reliability of methods and data for regulatory assessment of nanomaterial risks

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As nanomaterials continue to evolve, regulatory guidelines must keep pace with advancements in technology. While existing regulatory frameworks designed for conventional chemicals can be applied to nanomaterial manufacturing, the unique characteristics of nanomaterials may demand a more tailored regulatory approach. Scientific research has identified the test protocols, data sets, models, guidances, and assessment methods that are most relevant to regulatory needs. Reliable methods are already available – and are ready to be integrated into regulatory policy, or will be soon.

It is not possible to subject every new nanomaterial to every test; that would not be financially or logistically feasible. Therefore, it will be important to develop clear benchmarking methods so new nanomaterials can be assessed based on the most important criteria. Methods that characterise the nanomaterial while also predicting its fate and effects would be the most cost-effective option. For instance, functional assays do both, making them a viable option for first-tier testing.

The OECD is finalising new test guidelines for the environmental fate of nanomaterials, including determination of dissolution rate and sludge retention. These guidelines will be helpful in crafting regulatory policy; they form a central part of continuing advances in ecotoxicity testing.

The most costly and time-consuming endpoints are often the ones that address mammalian hazard. Sub-chronic *in vivo* inhalation tests are complex and costly. There are proposals outlining ways to reduce the frequency of these tests by supplementing them with other, less involved testing methods. These proposals prove that *in vitro* tests can be standardised to meet regulatory requirements and linked to *in vivo* results, which means more efficient testing that is easier to integrate into regulatory guidelines.

Workplace exposure assessments are another important tool that needs to be included in regulatory frameworks for nanomaterials. Several well-developed environmental exposure models are currently available that could easily be used for regulatory purposes.

The recommendations discussed here should be considered in developing scientifically sound, cost-effective nanomaterial guidelines. It is also important to focus on the gaps identified here, so research can be designed to hone in on acquiring the knowledge that is currently lacking. The nanomaterials field is developing at lightning speed, and good regulatory research needs to do the same to make sure regulatory guidelines can keep up with the pace of industry development.

Further reading

For further reading, please refer to the following resources:

- Open Access articles in NanoImpact published between July 2017 and April 2018.
- Introductory to the articles: https://doi.org/10.1016/j.impact.2017.11.005
  The detailed summary of papers consulted for this overview, which included:
  1. Methods and data for regulatory risk assessment of nanomaterials: Questions for an expert consultation – *Philip G. Sayre, Klaus Günter Steinhäuser, Tom van Teunenbroek*
  2. Progress towards standardised and validated characterizations for measuring physicochemical properties of manufactured nanomaterials relevant to nano health and safety risks – *Xiaoyu Gao and Gregory V. Lowry*
  3. Nanomaterial exposures for worker, consumer and the general public – *Thomas A.J. Kuhlbusch, Susan W.P. Wijnhoven and Andrea Haase*
  4. Evaluation of environmental exposure models for engineered nanomaterials in a regulatory context – *Bernd Nowack*
  5. Regulatory relevant and reliable methods and data for determining the environmental fate of manufactured nanomaterials – *Anders Baun, Phil Sayre, Klaus Günter Steinhäuser & Jerome Rose*
  6. Regulatory adequacy of aquatic ecotoxicity testing of nanomaterials – *Rune Hjorth, Lars M. Skjolding, Sara N. Sørensen, Anders Baun*
  7. *In vitro* approaches to assess the hazard of nanomaterials – *Barbara Drasler, Phil Sayre, Klaus Günter Steinhäuser, Alke Petri-Fink, Barbara Rothen-Rutishauser*
  8. In vivo effects: methodologies and biokinetics of inhaled nanomaterials – *Günther Oberdörster, Thomas A. J. Kuhlbusch*
  9. Review of (Q)SAR models for regulatory assessment of nanomaterials risks – *Enrico Burello*
  10. Risk assessment frameworks for nanomaterials: scope, link to regulations, applicability, and outline for future directions in view of needed increase in efficiency – *Agnes G. Oomen, Klaus Günter Steinhäuser, Eric A.J. Bleeker, Fleur van Broekhuizen, Adriënne Sips, Susan Dekkers, Susan W.P. Wijnhoven, Phil G. Sayre*
  11. Reliability of methods and data for regulatory assessment of nanomaterial risks – *Klaus Günter Steinhäuser and Philip G. Sayre*
- ProSafe White Paper, the main outcome of the ProSafe project, which integrates and analyses the results of the EU funded projects NANoREG, ProSafe and numerous other nanosafety projects, and translates the findings into 15 recommendations for policy makers and regulators.
- Deliverables developed under the umbrella of the ProSafe project, including the report on the OECD-ProSafe Joint Scientific Conference in Paris 2016, the Joint Document and the White Paper.
- The ProSafe Final Report presenting a complete overview of all ProSafe results including links to all deliverables and other related documents.
- General information on the project. This includes a summary of the outline of the project, the list of partners and the newsletters that have been published during the course of the project, and some other documents.
- The NANoREG Results Repository.