News and opinions

The 3Rs as a framework to support a 21st century approach for nanosafety assessment

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A B S T R A C T

Due to the plethora of nanomaterials being manufactured, it is crucial that their effects on human health are understood. It is not feasible to assess the safety of all nanomaterials using animal-based toxicity tests. There are also scientific, business, legislative and ethical drivers to reconsider the use of such toxicity tests. Utilising non-traditional methods has the potential to improve the human relevance of nanosafety assessment, reduce the numbers of animals that are used, and shift the paradigm to a ‘21st century’ approach that exploits recent scientific and technological advances. This article considers how application of the 3Rs principles can be used as a framework to support and guide this paradigm shift in the short, medium and long-term. Bringing the community together to facilitate the transition is necessary to ensure that tangible impacts are made on the efficiency and robustness of the nanosafety assessment process.

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There are numerous advantages of utilising manufactured nanomaterials over their bulk chemical counterparts, due to their unique physiochemical properties. Over the last decade there has been a notable rise in nanomaterial development and production within a vast array of different applications. Nevertheless, concerns remain regarding their potential impact on human health. Science-led efforts are needed to understand the genuine effects of nanomaterial exposure and ensure that health protection goals are met.

Whole organisms, particularly rodents, continue to be the preferred test system for assessing the hazards of nanomaterials to humans. This is mainly because they capture the site of administration, systemic distribution and the target tissues. Due to the potentially vast number of nanomaterials being marketed, regulatory toxicity testing could use large numbers of animals. The studies can be technically demanding and relatively long in duration. Generating in vivo data for each nanomaterial is therefore not practical or sustainable. There has been a great deal of discussion within the field in recent years around whether traditional methods are the best way to assess nanosafety and the need to ensure that the data generated bears relevance to humans. There are also business, ethical and legislative motivations to re-evaluate the use of animal toxicity tests. For example, there have been recent geographical bans on the testing of cosmetic ingredients in animals and the marketing of products containing ingredients that have been tested on animals.

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Now is the ideal time to embrace a move towards a more scientifically-driven paradigm for nanomaterial safety assessment. The benefits of this will be (i) prioritisation of efforts to overcome the challenges associated with moving away from traditional toxicity testing, and subsequently decreasing the numbers of animals used, and (ii) capitalisation on all relevant scientific and technological developments, resulting in a ‘21st century’ approach to nanosafety assessment [1], which is as predictive of the human situation as possible. Application of the 3Rs principles (replacement, refinement and reduction of animals) offers a framework in the short (0–5 years), medium (5–10 years) and long-term (10+ years) to support and guide this transition. Further detail can be found in Table 1.

In the short-term, the numbers of nanomaterials requiring testing in regulatory animal studies could be reduced, and the necessary animal studies refined (i.e., levels of suffering minimised).

The first question that should be asked before embarking on any testing is whether the nanomaterial is likely to be exposed to humans. If exposure is not anticipated, for example if it is embedded within a matrix, the nanomaterial would not pose a risk, and thus regulatory studies could be waived. If exposure is likely, a nanomaterial’s toxicity could first be assessed in shorter, ‘preliminary’ animal studies (which can be completed within 28 days, as opposed to 90–day long regulatory studies). This preliminary data could give an early indication of potential effects. A decision could be taken at this point to halt further development of the nanomaterial, thereby avoiding the longer, resource-intensive studies. If the nanomaterial continues into regulatory testing, the preliminary data could be used to inform the design of these studies. This includes the selection of dose levels, to ensure that excessively high doses resulting in non-specific toxic effects are avoided. In both preliminary and regulatory studies the nanomaterial should be administered through the route most relevant to the human exposure scenario. Traditionally this would be via inhalation, to reflect occupational exposures. As the potential breadth of nanomaterial applications increases other routes may also become relevant, such as ingestion or dermal. As much relevant information as possible should be obtained from any animal study performed. This may include incorporation of additional measurements or analyses, providing they do not cause additional stress to the animals. This could include determining effects at both the site of exposure and secondary target sites. Results of short-term studies could also be used to inform read-across (see below).

Within the medium-term, further reductions in the number of regulatory studies needed could be achieved through the wider use of in vitro assays for screening and prioritisation. These could be assays already validated for the toxicity testing of traditional chemicals (for example, those to assess genotoxicity) with modifications where appropriate, or assays that have been specifically developed for testing nanomaterials. There is also the prospect of utilising unprotected species, such as invertebrates and early-lifestage fish embryos. Data from adapted in vitro assays, in combination with existing in vivo data and the use of computational models, will also help to identify links between structural, biological or physical

<table>
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<th>Table 1</th>
<th>Opportunities to align the 3Rs principles with improved approaches for nanosafety assessment in the short, medium and long-term.</th>
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<tr>
<td><strong>Timeframe</strong></td>
<td><strong>Opportunities to align with the 3Rs</strong></td>
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<td>Short-term (0–5 years)</td>
<td>- Using short-term studies (e.g. short-term inhalation studies, [2]) as early tier tests. This would determine whether further sub-chronic and chronic toxicity tests need to be carried out. Short-term studies could also be used as a tool to group substances for read-across purposes, and thus reduce the number of 90-day regulatory in vivo studies required. Toxicokinetic analyses could also be incorporated into short-term studies, to inform dose setting and refine subsequent chronic in vivo studies by ensuring high doses are only administered where necessary. - Combining several endpoints within studies. This could include determining toxicity at both the exposure site (e.g. lungs) and secondary target site (e.g. liver) as well as carrying out toxicokinetic assessments, to maximise the amount of information obtained from each study (e.g. see [3]). - Increasing consideration of exposure when designing in vitro and in vivo studies. For example, improving the relevance of toxicology studies to the likely routes of exposure within human risk scenarios. This will help when applying tiered approaches, which can be used to prioritise or waive testing. - Adaptation of existing in vitro test systems to facilitate nanosafety assessment and progress currently available nano-relevant in vitro assays towards formalised validation (e.g., see [4]). Efforts should be made to redress the problems associated with the relevance and reliability of existing in vitro assays for nanomaterial testing.</td>
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<tr>
<td>Medium-term (5–10 years)</td>
<td>- Leveraging existing information to prioritise the nanomaterials taken forward into animal studies. This could be achieved through grouping approaches. Effective grouping of nanomaterials would allow the utilisation of read-across approaches and could provide justification for waiving of tests. Although a complex area, particularly for nanomaterials, such approaches have started to be proposed e.g. [5]. Computational models would also be useful to establish correlations of toxicokinetics and adverse effects. At the present time these models can complement experimental data, but cannot replace them. - Expanding the use of in vitro approaches that are specifically targeted towards the fulfilment of regulatory data requirements. High throughput systems will also play a key role. They have the potential to provide information on nanomaterial physicochemical characteristics, hazard and internal exposure for use in risk assessment (as envisioned by the ITS-NANO framework [6]). However, these platforms may also be used as tools more widely in the early screening of candidate nanomaterials. This would help ensure that the toxicological potential of nanomaterials is detected and better understood prior to them being tested in animal studies [7]. - Investing further into innovative technologies for nano-specific use. These often offer the benefit of physiological relevance, e.g. microfluidic systems that can more accurately replicate conditions within a human organ (e.g. the lung [8]). - Refinement of necessary in vivo tests should continue within this timeframe. For example, developing short-term studies for routes other than inhalation e.g. oral, and improving the technical aspects of inhalation studies.</td>
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<td>Long-term (10 years+)</td>
<td>- Incorporation of exposure route and internal exposure considerations, by utilising in vitro systems that provide information on barrier penetration and translocation capabilities. Such systems may include 3D tissue models, which are more realistic and physiologically relevant systems than traditional 2D/monolayer methods. An emphasis on using human cells and tissues in these models where possible will further increase their relevance in human safety assessment. - Sufficient acquisition of further mechanistic knowledge on the key events that result in adverse effects at an organism level. This will support continued development of adverse outcome pathways (AOPs) specific for nanomaterials, which can begin now using currently available knowledge. The AOP framework has potential to identify data gaps and highlight the key aspects of toxicity pathways that require further investigation. These findings could then accelerate the development and implementation of non-animal methods for the prediction of apical endpoints [9]. - Progression of reliable and advanced in silico models, through the availability of more hazard, kinetic and physicochemical data.</td>
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The 3Rs are driven, studies to toxicity of a novel nanomaterial could then be inferred based on the presence or absence of specific properties or combinations of such properties, in a process known as read-across. Sufficient confidence in read-across techniques would obviate the need to assess whether the nanomaterial is toxic within animal studies. If such a judgement cannot be confidently made, the nanomaterial may then need to be tested in relevant animal studies (providing it is not intended for use in a cosmetic product). Increased knowledge gained on the release and dispersion and uptake of nanomaterials in this timeframe will help to better understand likely levels of human exposure. This will aid in better determining the most relevant route of administration for animal studies; informing decisions to waive studies; and ensuring that nanomaterials are tested at doses and in models that are reflective of likely human internal doses.

In the long-term, the goal will be the replacement of animal toxicity studies with more predictive, human-relevant methods, coupled with high throughput screening to enable rapid and automated evaluation. This will be supported by the wider utilisation of human cells and 3D cell models that are more representative of human tissues compared with traditional cell-based assays. In vitro systems capable of mimicking physiological processes such as nanomaterial penetration and metabolism will also be essential. Increased understanding of the mechanisms and biochemical pathways that lead to toxic effects gained in the coming years will narrow down which types of biological activity should be examined within the in vitro assays. Relating the effects observed and exposure levels in vitro to the human situation will require the application of elegant computational models. It is clear that it will not be necessary to generate the same types of data for all nanomaterials, as the needs will be dependent on their intended application and their inherent properties. By nature, in vitro and in silico methods will not replace the traditional whole organism tests on a one-for-one basis. Complete replacement will only occur if all the available information on a nanomaterial is considered and integrated. This also relies on the decision-makers within regulatory bodies having confidence in making safety decisions based on types and combinations of data they are not currently familiar with.

There are a number of barriers that will need to be overcome to enable the transition from the status quo towards complete replacement within the timeframe proposed. Firstly, focused scientific endeavours will be required to ensure that the right tools are available and can be successfully harnessed. Key scientific issues that remain to be addressed include, but are not limited to, (a) the need to ensure that new and existing in vitro models are standardised, so that the data generated is comparable and of the highest quality; (b) incorporation of the necessary levels of biological and chemical complexity within integrated approaches, which also encompass exposure considerations, and (c) proving that the new approaches are fit for the purpose they are intended. Secondly, a culture shift is necessary towards an environment where non-animal approaches become an integral part of routine risk assessment that is endorsed by international regulatory agencies. Various global initiatives have started to address some of the issues highlighted here; the biggest challenge for the field will be ensuring that these, and future efforts, are co-ordinated and strategically focused.

In summary, application of the 3Rs principles provides a framework to support and guide the transition towards the vision of a 21st century approach to nanosafety assessment that is exposure-driven, reduces and replaces animal use, increases the efficiency of the process, and is more human relevant. The points raised here are also applicable to all sectors of the chemicals industry. Genuine change will only happen if regulatory bodies are open about which situations they are willing to accept non-traditional data, and ready to compromise on the current reliance on ‘tried-and-tested’ methods of risk assessment. Industry will play a role by specifying their needs and requirements to help steer future academic and exploratory research. Such research will only take place if there is an increase in targeted, strategic funding. The most important aspect of all to move things forward and achieve the goals set out here will be open, face-to-face discussion and collaboration, which incorporates cross-talk between all relevant stakeholders.

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### References


Dr Martin Clift, a Lecturer at Swansea University Medical School, gained his PhD from Edinburgh Napier University in 2009, followed by 7 years’ post-doctoral research experience in Switzerland. Using advanced, next-level in vitro systems, Martin’s research focuses upon the nanoparticle-(mammalian) cell interaction through the use of state-of-the-art microscopy approaches combined with a view towards determining the mechanistic toxicological, immunological and genotoxic effects that nanoparticles, with varying physico-chemical characteristics, may cause at the cellular level. Martin is an editorial board member of Particle and Fibre Toxicology, and to date, has authored over 85 publications in the area of in vitro nanotoxicology.

Shareen is a Professor of Genotoxicology & Cancer, and is Director for the Centre for NanoHealth at Swansea University. Shareen’s research interests are focused on the genotoxicity of engineered nanomaterials, their modes of action and human health implications. She sits on the UK Committee on Mutagenicity, International Genetic Toxicology Technical Committee and the EU Scientific Committee on Consumer Safety Working Group on Nanomaterials in Cosmetics (external expert). Shareen is a UK and European Registered Toxicologist, and is a Fellow of the Royal Society of Biology (FRSB).

Paul Fowler is a toxicologist at Unilever’s Safety and Environmental Assurance Centre (SEAC) specialising in genetic toxicology and cancer with research interests focussing on the use of and generation of human 3D organotypic models in vitro from which pathways-based mechanisms that lead to organ level toxicity can be investigated as well as exposure metrics, free concentration, bioavailability etc. He currently leads a project developing an adverse outcome pathway for lung toxicity after exposure to aerosolised nano phase materials using in vitro models.

Dr Helinor Johnston specialises in the assessment of nanomaterial hazard to human health and the environment. Her research interests include investigating i) the mode of action of nanomaterial toxicity, ii) the physico-chemical properties of nanomaterials which confer toxicity, iii) the development of in vitro systems to screen nanomaterial safety and iv) the safety and efficacy of nanomedicines. Helinor was a scientific advisor (2009–2010) in the Chemicals and Nanotechnologies division of the Department for Environment, Food and Rural Affairs (Defra). Helinor’s PhD was concerned with evaluating the toxicity of nanomaterials to the liver in vitro in 2009 (Edinburgh Napier University).

Dr Robert Landsiedel joined BASF in 1999, where has led an experimental toxicology unit since 2006. His unit performs more than 500 screening and regulatory toxicology studies per year, and develops new alternative methods and investigates the safety of nanomaterials. They have published over 100 scientific papers and received the BASF Innovation Award, the Animal Welfare of the state of Rhineland-Palatinate and the German Federal Research Award. Robert is a member of the European Commission’s Scientific Committee on Occupational Exposure Limit Values (SCOEL), the executive officer of the German Toxicology Society and lecturer in toxicology.

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Vicki Stone is the current holder of the Royal Society of Chemistries Toxicology Award. Vicki’s research investigates the effects of engineered nanomaterials and pollutant particles on human health, including which particle properties drive toxicity and the mechanism of toxicity. Vicki acted as the Editor-in-chief of the journal Nanotoxicology (2006–2011). Vicki has also published over 150 publications pertaining to particle toxicity and was recently recognised by Thomson Reuters as one of the top 1% of all researchers in the world for the most cited publications in the field of Pharmacology and Toxicology.

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