# Aligning nanotoxicology with the 3Rs: What are the short, medium and long-term opportunities?

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**NC 3RS** — National Centre for the Replacement Refinement & Reduction of Animals in Research

## 1. Why focus on the 3Rs in nanotoxicology?

- Increasing uses for nanomaterials (NMs) e.g. cosmetics, medicine, agriculture, food, packaging, industrial chemicals.
- Perceived concerns over NM safety: scientific, governmental and public.
- Each NM may have to be individually tested under appropriate regulations as physical, chemical and biological properties may differ at the nanoscale from the bulk.
- Toxicity testing of NMs for regulatory purposes usually carried out in animal studies (with exception of cosmetic products).
- **Question: Are traditional testing strategies appropriate for NMs?**
- Particular drive from cosmetics sector due to geographical bans on animal testing but alternative methods would benefit multiple industries.
- A working group was established to conduct an analysis of how the application of the 3Rs could improve the science of nanosafety.
- The group identified opportunities where the 3Rs could be applied in short, medium and long-term timeframes and provided recommendations to enable them.
- This analysis was recently published in the journal *Nano Today* (Burden *et al.* (2016) The 3Rs as a framework to support a 21st century approach for nanosafety assessment. *Nano Today*, in press).

## 2. The current landscape

- Some animal toxicity tests continue to be necessary (for non-cosmetic purposes), providing that *in vivo* data is relevant, in the short- to medium-term due to:
  - Limited understanding of NM biokinetics;
  - Need to conduct tests to standard regulatory test requirements;
  - Lack of regulatory experience and confidence in alternative methods;
  - Uncertainty around how well results from non-animal methods compare with *in vivo* data.
- Effects of lung exposure are mainly assessed, to reflect exposure route of concern.
  - Inhalation studies are the preferred testing system of regulators when assessing occupational risk.
  - Refined protocols have been developed e.g. short-term *in vivo* inhalation studies (STIS). Short term studies for dermal and oral routes currently lacking.
- Difficult to extrapolate from *in vitro* or short-term *in vivo* studies.
- Many different nanoforms may need to be tested, potentially leading to many long-term studies.
- Unlikely that the relevant *in vivo* studies can be performed for every single NM, and may not be necessary.
- Cosmetics/personal care products sector must avoid animal testing and other regulations stipulate that animal tests are only carried out as a last resort.

## 3. Opportunities to align the 3Rs with improved safety assessment of NMs

### Short-term (0 to 5 years): Refinement and reduction within existing animal models

- Application of refined short-term inhalation studies as early tier tests
  - Potential screening and grouping tool which could be used to inform read-across and reduce the number of regulatory *in vivo* studies needed.
- Measure several endpoints within studies simultaneously
  - E.g. determine toxicity in multiple organs or measure toxicity and toxicokinetics in the same animal.
- Incorporation of exposure considerations
  - Could provide justification for exposure-based waiving of studies.
  - Toxicokinetic analyses could aid dose setting for toxicity studies and help to avoid use of unnecessarily high doses.
  - Determine relationships between internal dose and systemic effects.
- Evaluate, improve and validate current standard *in vitro* test systems
  - Test item preparation and characterisation.
  - Refined protocols.
  - Mode of action information to support screening early in the NM development process.

### Medium-term (5 to 10 years): Reduction through the leveraging of existing information, development of more robust *in vitro* approaches targeted towards fulfilment of data requirements, and more predictive computational models

- Prioritisation through grouping
  - Complex process as there is a need to categorically identify the most appropriate and relevant factors e.g. physico-chemical characteristics.
  - QSAR models currently under development.
  - Grouping will inform read-across approaches.
- Expanding the use of alternative approaches
  - *In vitro* test platforms that provide an indication of uptake and biological effects of NMs specifically, over range of toxicity endpoints.
  - Integration of data from multiple tests to allow for accurate predictions, as non-animal approaches will not replace *in vivo* studies on a 1:1 basis.
  - Application of currently used test systems (e.g. those applied in traditional chemical toxicity tests) or adaptations thereof.
  - Early screening of candidate NMs.
  - Increased use of innovative technologies e.g. microfluidics.
- Continued investment into refining and reducing the numbers of animals used in mandatory *in vivo* tests

### Long-term (10 years +): Replacement with accepted non-animal methods

- Need for integration and consideration of weight of evidence
- Routine use of more complex *in vitro* models e.g. barrier models, co-cultures and 3D models, human cells and tissues
- Development of adverse outcome pathways specific for NMs
  - Improve mechanistic understanding of NM effects will perpetuate development and implementation of non-animal methods.
  - Incorporation of exposure elements necessary.
- Continued investment into refining and reducing the numbers of animals used in mandatory *in vivo* tests
- Reliable and advanced *in silico* models
- Tiered testing strategy for addressing potential data gaps (vs. "tick-box" approach)

## 4. Working group recommendations for next steps

- Establishment of a framework to enable implementation of non-animal methods into regular NM risk assessment and acceptance.
- Methods developed that accurately predict toxicity, which can be confidently linked to physico-chemical properties and/or bio-physical interactions.
- Need for increased understanding of extrapolation between *in vivo* and *in vitro* models.
- Consensus reached on how best to validate non-animal approaches.
- Adaptation of current standard *in vitro* approaches and improved test item preparation, dosing, and understanding of toxicity mechanisms.
- Raised publication standard so that only high quality, relevant and comparable information is generated in both *in vivo* and *in vitro* studies.
- Incorporation of essential levels of complexity into computational models.

**Drivers to re-evaluate animal-based approaches**

| Legislative/ regulatory | Increased human relevence and predicivity of nanotoxicity tests | Business e.g time, cost and resource | Ethical considerations |

Refine — Reduce — Replace

Short-term — — — — — — — — → Long-term

Predictive and validated *in vitro* and *in silico* models
Regulatory acceptance
Reduced reliance on animal models
More efficient and robust safety assessment of nanomaterials
Safer nanomaterials