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EDITORIAL

Reduction through refinement: bridging animal studies and NAMs through 'omics technologies enriched regulatory studies

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The 3Rs concept proposed by Russell and Burch (1959) marked the beginning of a structured approach to reducing animal use in scientific research. The concept consists of three complementary elements: Replacement, Reduction, and Refinement (3Rs). Nearly fifty years later, the National Research Council report "Toxicity Testing in the 21st Century: A Vision and a Strategy" (Council 2007) proposed a fundamental shift from traditional animal-based toxicity testing towards human-relevant, mechanism-based approaches. The report envisioned the use of in vitro assays, computational models, and systems biology tools to identify chemical perturbations of biological pathways rather than relying primarily on apical endpoints observed in animals. The advantages of such an approach, would be to allow informed testing with systems representative of human physiology instead of having to rely on biological substitutes. However, it faces the challenge that it may not be sufficiently protective of human health which is a substantial regulatory hurdle, particularly for repeated dose endpoints.

The vision has become a major driver of the development and implementation of New Approach Methodologies (NAMs). Today, more than a quarter of the 21st century has passed, and considerable progress has been achieved, particularly in the area of replacement. The development of in silico and in vitro methods has expanded rapidly, providing an unprecedented range of tools for hazard identification and mechanistic investigation. However, the success of NAM development has created new challenges. Validation of NAMs is not keeping pace with NAM development; for regulatory decision-making, elements that are examined in a validation process like reproducibility, sensitivity or specificity of an assay are critical. To be considered under mutual acceptance of data by regulators in OECD member states to foster world-wide use and acceptance, NAMs would also have to be described in OECD test guidelines. Additionally, the number of available assays and platforms continues to grow, making the selection, interpretation, and integration of data increasingly resource intensive. More assays do not automatically translate into improved hazard information and risk assessment. Consequently, strategies are needed to improve the efficiency with which relevant information is generated and integrated into regulatory safety assessments. Lastly, implementation of such methods, as good as they might be, requires the flexibility of regulatory frameworks to do so. Regulations such as REACH often explicitly demand certain animal tests rather than prescribing a required conclusion and level of confidence for an endpoint. This can only be overcome if there is sufficient confidence in any alternative method used. With the current systems strong historical bias towards animal data this will inherently require some bridging between the data regulators are used to and the data new systems deliver. An important part of this bridging will be understand where the new approaches are able to provide equivalent information and where there may be important differences and potentially gaps that in silico and in vitro methods could not recapitulate.

In silico tools can support the rational selection of in vitro methods by identifying likely biological targets, pathways, and toxicological concerns based on chemical structure and other properties. Several organizations, including ECETOC, have proposed tiered approaches that combine in silico and in vitro methods to identify and characterize

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potential hazards (Ball et al. 2022). However, such frameworks recognize that despite significant scientific advances, demonstrating the *absence* of risk or hazard remains challenging when relying solely on current NAMs especially for hazards arising from multi-organs effect.

Demonstrating the absence of risk or hazard remains a fundamental requirement for the safe use of chemicals, pharmaceuticals, and plant protection products. For this reason, as part of the tiered assessment framework, if the preceding in silico and in vitro evidence remains insufficient the ECETOC framework includes a final tier consisting of targeted, improved and information-rich smart in vivo studies. These studies are designed using information obtained from preceding in silico and in vitro investigations and focus specifically on the most relevant toxicological modes of action identified for a given compound.

The distinguishing feature of these "smart studies" is that they are designed not only to satisfy regulatory requirements but also to maximize the scientific value obtained from each animal used. In addition to traditional toxicological endpoints (such as organ weights, pathology or histopathology), such studies may incorporate toxicokinetic measurements, metabolomics, transcriptomics of selected target organs, and other omics technologies. The objective is to generate mechanistic information that can improve hazard characterization, reduce uncertainty, and support assessments of human relevance. Very similar concepts are also emerging within the regulatory community, where enhanced studies incorporating omics technologies are increasingly discussed under the concept of Technical Guideline Plus (TG+) studies (Marx-Stoelting et al. 2023). TG+ would have the advantage of being conducted in accordance with a TG (under mutual acceptance of data) but delivering additional mechanistic data. Such data may also be used to generate a database for mechanistic understanding and direct comparison of new in vitro methods to test guideline studies.

Importantly, this approach provides an opportunity to strengthen the other two elements of the 3Rs: Reduction and Refinement. Traditionally, refinement has focused on minimizing pain and distress. However, refinement can also be viewed from a scientific perspective. By increasing the amount and quality of information obtained from each study, the overall number of studies required to reach a regulatory decision may be reduced. In this sense, refinement becomes a direct driver of reduction — a concept that may be described as "Reduction through Refinement". This concept was initially proposed by van Ravenzwaay et al. (2012) in the context of plasma metabolomics and was further expanded during an ECETOC workshop in 2026 (ECETOC 2026) to include additional omics technologies and systems toxicology approaches.

The essential objective of both Smart Studies and TG+ studies is to increase the value of any in vivo study that remains necessary. The resulting mechanistic data can improve risk and hazard assessment, support human relevance evaluations, facilitate read-across approaches, and potentially contribute to study waiving strategies or, if approved by legislators, reduce the number of animal studies in legal data requirements. Furthermore, these data could provide an important bridge between traditional animal studies and future NAM-based regulatory frameworks. Technical guidance documents for implementing such approaches are also available. For example, recent OECD activities have addressed issues such as biological sample collection for omics analyses (OECD 2025), reporting standards (OECD 2023) and others.

Perhaps most importantly, the integration of omics technologies into targeted in vivo studies offers a unique opportunity to build confidence in the regulatory use of mechanistic data and thus to address the aforementioned confidence hurdle due to historical data bias. Such confidence may ultimately facilitate the acceptance of analogous data generated by in vitro NAMs. In this sense, Smart or TG+ studies should therefore not be viewed as competitors of NAMs, but rather as enabling technologies that can accelerate their validation, interpretation, and regulatory implementation including in vitro testing.

As the number of available in vitro assays continues to increase, the inclusion of information-rich approaches may also help prioritize and streamline in vitro testing strategies. Rather than requiring large batteries of individual assays, future approaches may increasingly rely on a smaller number of highly informative methods capable of generating mechanistic signatures that support robust regulatory decision making.

Conclusion

While replacement of animal studies is progressing some repeated dose animal studies will remain necessary during the regulatory transition period. Therefore, remaining animal studies should become information rich studies and should simultaneously generate bridging evidence needed to accelerate confidence in, and acceptance of, NAMs. Consequently, we believe that Smart/TG+ Studies are an essential facilitator of the wider application of NAMs, as they are building a bridge to accelerate the transition towards a future in which animal studies become increasingly unnecessary for regulatory purposes. This concept needs a broader discussion among regulators, industry – as well as animal welfare representatives –, and NAM developers, and may

represent an important next step in the evolution and application of the 3Rs concept in a regulatory context.

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Declarations

Competing interests The authors declare no competing interests.

References

Ball N, Bars R, Botham PA et al (2022) A framework for chemical safety assessment incorporating new approach methodologies within REACH. Arch Toxicol 96(3):743–766. https://doi.org/10.1007/s00204-021-03215-9

Council NR (2007) Toxicity Testing in the 21st Century: A Vision and a Strategy. National Academies, Washington, DC

ECETOC (2026) Repeat Dose Toxicity testing workshop: From concept to application. In. https://www.ecetoc.org/event/from-concept-to-application-repeat-dose-toxicity-testing-workshop/

Marx-Stoelting P, Rivière G, Luijten M et al (2023) A walk in the PARC: developing and implementing 21st century chemical risk assessment in Europe. Arch Toxicol 97(3):893–908. https://doi.org/10.1007/s00204-022-03435-7

OECD (2023) OECD Omics Reporting Framework (OORF): Guidance on reporting elements for the regulatory use of omics data from laboratory-based toxicology studies. OECD Series on Testing and Assessment, No 390, OECD Publishing, Parishttps://doi.org/10.1787/6bb2e6ce-en

OECD (2025) Guidance on Good Practices and Standardisation of Sample Collection for Omics Analysis. OECD Series on Testing and Assessment, No 409. OECD Environment, Health and Safety, Paris

Russell WMS, Burch RL (1959) The principles of humane experimental technique. Methuen, London

van Ravenzwaay B, Herold M, Kamp H et al (2012) Metabolomics: A tool for early detection of toxicological effects and an opportunity for biology based grouping of chemicals—From QSAR to QBAR. Mutat Research/Genetic Toxicol Environ Mutagen 746(2):144–150. https://doi.org/10.1016/j.mrgentox.2012.01.006

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