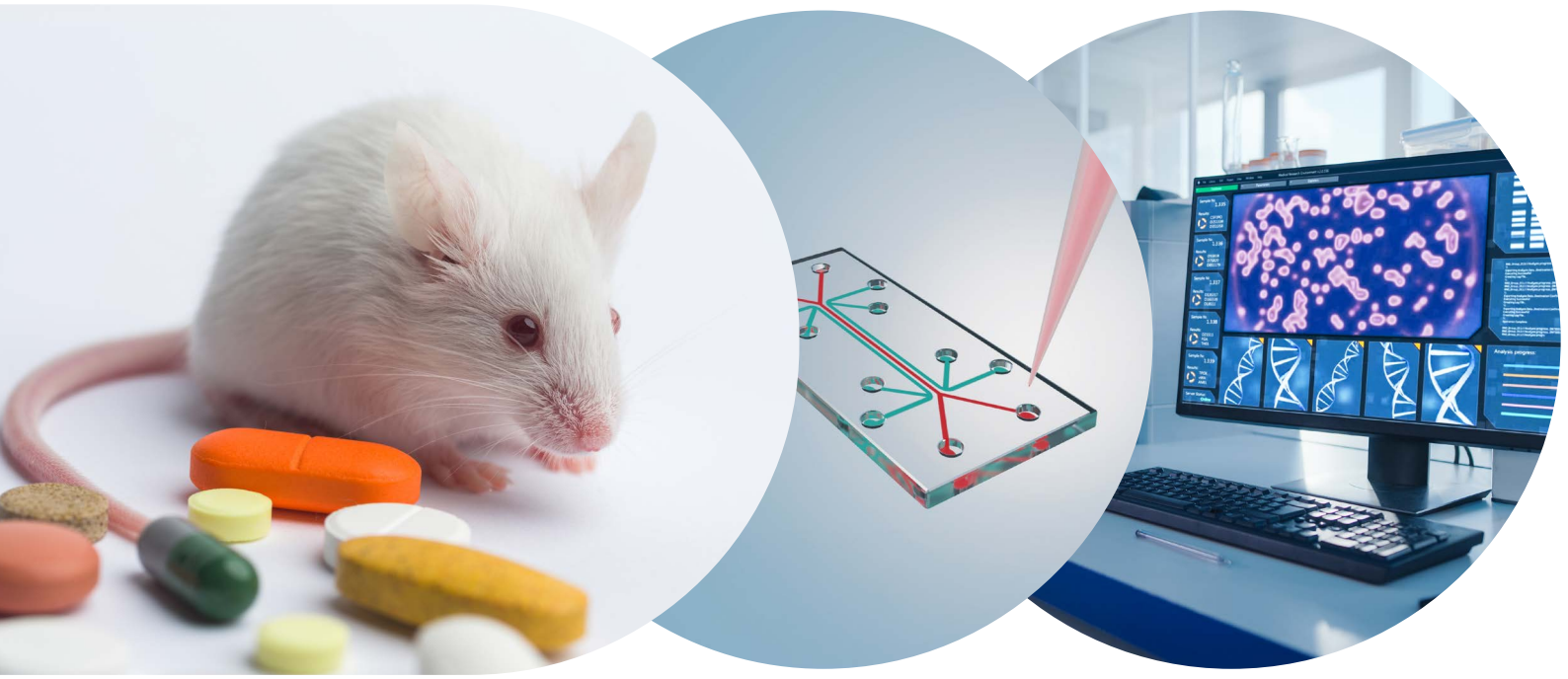


Landscape Analysis

on Alternatives to Animal Testing for Drug Development in India

Dr.Reddy's

BRIC a DBT Organization

inStem इनस्टेम Institute for Stem Cell Science and Regenerative Medicine

Humane World for Animals

Animal Law & Policy Network

Dr. Rajeev Raghuvanshi
Drugs Controller of India

Foreword

The landscape of drug development is at a historic inflection point and undergoing a transformative shift. Scientific advances in human relevant biology, coupled with a growing ethical consensus and progressive regulatory reform, are creating an opportunity to rethink how we evaluate safety and efficacy. This report — a focused landscape study on Non-Animal Methods (NAMs) in the context of India's evolving regulatory environment — arrives at a pivotal moment. It captures both the urgency and the promise of moving toward approaches that better predict human outcomes while reducing reliance on animal testing.

This study is timely for three reasons. First, it aligns with the CDSCO 2025 draft biosimilar guidelines that emphasize analytical rigor, orthogonal characterization, and mechanistic in vitro evidence as central pillars of comparability assessment. Second, it situates India within a global shift toward NAMs — from organ-on-chip and 3D human tissue models to advanced cellular systems and in silico simulations — that together can reduce clinical attrition and accelerate development timelines. Third, it reframes adoption of NAMs as an ethical imperative and a strategic advantage: humane science that also strengthens national competitiveness and public trust.

The analysis that follows is practical and action oriented. It identifies where animal testing is scientifically redundant, maps the regulatory and technical gaps that must be closed, and proposes a phased roadmap for adoption. Key recommendations include establishing clear regulatory acceptance criteria for NAMs, investing in national centers of excellence and accreditation, piloting NAM led dossiers to build precedent, and creating interoperable data standards to accelerate validation and reproducibility.

Realizing this vision will require sustained collaboration across regulators, industry, academia, and civil society. The transition must be evidence driven and phased, balancing innovation with patient safety through pilot programs, conditional pathways, and robust post market surveillance. If pursued with rigor and integrity, India can not only meet global standards but lead in developing and exporting human relevant testing platforms and expertise.

I commend the authors and contributors for assembling a clear, persuasive, and practical blueprint. May this report catalyse the partnerships, investments, and regulatory clarity needed to make humane, predictive, and efficient drug development the norm rather than the exception.

Foreword

The pharmaceutical industry is undergoing a profound transformation, driven by advances in human relevant science and an evolving global regulatory landscape. Although animal models have historically been essential in enabling therapeutic innovation, emerging technologies now provide pathways that are not only more ethical but often more predictive of human responses. Recent policy developments, including the FDA Modernization Act 2.0 and it's subsequent 3.0 updates as well as India's New Drugs and Clinical Trials (Amendment) Rules, 2023, reaffirm regulatory openness to scientifically validated alternatives to traditional animal studies.

The report consolidates Replacement, Reduction, and Refinement (3R) opportunities across all product categories such as generics, differentiated products, new chemical and biological entities to support coordinated stakeholder action.

This report presents a focused landscape analysis of emerging New Approach Methodologies (NAMs), including organ-on-chip platforms, advanced in vitro systems, and AI-driven in silico tools. The integration of NAMs is not only an ethical imperative aligned with the principles of the 3Rs, but also a strategic pathway to enhance translational reliability. By reducing dependence on traditional animal models and leveraging more human-relevant technologies, NAMs have the potential to accelerate the development of safer, more effective therapies.

This report also outlines the scientific and regulatory changes shaping the adoption of NAMs in drug development and highlights the key opportunities, challenges, and strategic actions needed for their responsible use. By embracing these innovations, we can reduce reliance on animal studies while enhancing the rigor, relevance, and compassion of modern research.

I believe this landscape analysis will serve as a valuable resource for stakeholders committed to advancing a future where innovation, responsibility, and patient centered science progress together.

Satish Reddy
Chairman,
Dr. Reddy's Laboratories Limited

Sarfaraz K. Niazi, Ph.D
Adjunct Professor,
University of Illinois, Chancellor,
Chicago University,
Chicago, Illinois, USA.

Foreword

The global pharmaceutical and biomedical research ecosystems stand at a decisive inflection point. For more than half a century, animal-based models have served as the dominant paradigm for non-clinical safety and efficacy evaluation, shaping regulatory expectations, industrial workflows, and academic training. Yet despite their historical role, it has become increasingly evident that animal models often fail to recapitulate the complexity, heterogeneity, and mechanistic specificity of human disease. This translational disconnect is reflected starkly in clinical attrition rates: recent analyses indicate that only 10–14% of drug candidates entering Phase I trials ultimately achieve regulatory approval. Against this backdrop, the emergence and maturation of Non-Animal Methods (NAMs)—including advanced in vitro systems, micro physiological platforms, organoids, and computational modeling—represent not merely a technological evolution but a paradigmatic transformation in how biomedical evidence is generated, evaluated, and translated into patient benefit.

This Landscape Study on Non-Animal Methods arrives at a moment of exceptional global relevance. Across regulatory jurisdictions, scientific communities, and industry sectors, there is an unmistakable shift toward human-relevant, mechanistically grounded, and ethically responsible approaches to drug development. Legislative reforms such as the U.S. FDA Modernization Act 2.0, evolving European Medicines Agency guidance, and India's amendments to the New Drugs and Clinical Trials Rules collectively signal a reorientation of regulatory science away from default reliance on animal testing toward evidence-based flexibility. This report provides one of the most comprehensive, structured, and context-sensitive examinations to date of how these global trends intersect with the Indian pharmaceutical ecosystem—an ecosystem that occupies a uniquely influential position as the global supplier of over 20% of generic medicines and 60% of vaccines, and increasingly, as a source of innovative therapies.

At its core, this report is neither an advocacy document in isolation nor a narrow technical catalogue of emerging methods. Rather, it is a rigorous systems-level analysis that integrates regulatory science, technological readiness, ethical frameworks, and economic considerations into a coherent roadmap for NAMs adoption. By systematically evaluating opportunities for implementation of the 3Rs—Replacement, Reduction, and Refinement—across generics, differentiated products, new chemical and biological entities, cell and gene therapies, toxicity testing, and pyrogen and endotoxin testing, the report establishes a unified evidence base upon which regulators, industry leaders, and researchers can collectively act.

A central strength of this report lies in its sector-specific granularity. In the domain of generics and differentiated drug products, the analysis clearly demonstrates that mandatory in vivo animal studies often provide minimal incremental scientific value when robust human bioequivalence data, established safety profiles, and globally

accepted scientific knowledge already exist. Through detailed comparisons between Indian regulatory requirements under the New Drugs and Clinical Trials Rules 2019 and those of ICH-aligned jurisdictions, the report identifies clear opportunities to eliminate redundant animal testing without compromising patient safety. Importantly, this is framed not as deregulation, but as regulatory modernization—one that aligns ethical responsibility with scientific efficiency and global harmonization.

Equally significant is the report's treatment of innovation-focused pipelines, particularly new chemical entities and new biological entities. Here, the document moves beyond simplistic calls for replacement and instead articulates nuanced, risk-based strategies for reduction and refinement. By mapping global precedents—from the elimination of stand-alone acute toxicity studies to the conditional use of single-species programs and integrated toxicology designs—the report demonstrates that NAMs can be embedded within existing regulatory architectures. This approach preserves scientific rigor while reducing animal use, shortening development timelines, and reallocating resources toward higher-value, human-relevant investigations.

The analysis of cell and gene therapy regulatory frameworks is particularly timely. Advanced therapies challenge traditional toxicological paradigms due to their biological specificity, complex biodistribution, and the often-limited relevance of conventional animal models. By comparing India's National Guidelines on Gene Therapy Product Development and Clinical Trials with the European Medicines Agency's recently released Advanced Therapy Medicinal Products guidelines, the report identifies multiple opportunities for adopting case-by-case, context-of-use-driven non-clinical strategies. These include increased reliance on in vitro systems, integration of pharmacokinetics and toxicology studies, conditional waivers for repeat-dose studies, and reduced reliance on large-animal models. In doing so, the report positions NAMs not as optional adjuncts, but as essential tools for enabling safe, efficient, and scalable cell and gene therapy development.

One of the most technically robust contributions of this study is its extensive mapping of NAMs across toxicity domains. The report catalogues and evaluates 137 commercially available NAM platforms, stratified by organ system and application area. Hepatic toxicity is the most technologically mature domain, with 32 identified platforms that reflect both the clinical importance of drug-induced liver injury and the relative success of liver-on-a-chip and organoid systems in capturing human-relevant metabolic responses. At the same time, the report does not overstate readiness where limitations remain. Renal, cardiac, pulmonary, central nervous system, intestinal, dermal, and ocular NAMs are assessed with appropriate scientific caution, acknowledging the challenges posed by multi-organ integration, immune system representation, and long-term exposure modeling. This balanced assessment enhances the report's credibility and practical utility.

The integration of in silico methodologies—including physiologically based pharmacokinetic modeling, quantitative in vitro–in vivo extrapolation, and artificial intelligence–driven toxicology platforms—further strengthens the report's forward-looking perspective. By situating these tools within global regulatory initiatives such as the FDA's AI4TOX program and the EMA's guidance on model-informed drug development, the report underscores the convergence of computational science and regulatory decision-making. Importantly, it recognizes that in silico approaches are not replacements in isolation, but powerful integrators that connect mechanistic data across NAM platforms, thereby enhancing predictivity and regulatory confidence.

The chapter on pyrogen and endotoxin testing exemplifies how immediate, actionable change can be achieved through the adoption of NAMs. The transition from rabbit pyrogen testing and horseshoe crab–derived Limulus Amoebocyte Lysate assays toward recombinant Factor C and the Monocyte Activation Test represents one of the clearest cases of full replacement. The report meticulously documents global pharmacopoeial acceptance, validation status, and comparative performance, while also addressing practical challenges related to cost, standardization, reagent availability, and training. By doing so, it provides a concrete blueprint for India to accelerate animal-free testing in a quality-critical domain, aligned with global regulatory standards.

Beyond technical and regulatory analysis, the report's ecosystem-level recommendations are among its most enduring contributions. The proposed establishment of a central NAMs agency, modeled on international counterparts such as EURL ECVAM and ICCVAM, addresses a critical structural gap in India's innovation landscape. Such an agency would not only coordinate validation and standardization but also serve as a focal point for data transparency, capacity building, and international alignment. Complementing this, the call for multi-stakeholder consortia, centers of excellence, and indigenous supply chains reflects a sophisticated understanding that NAM adoption is not solely a scientific challenge but also an institutional and economic one.

The emphasis on capacity building—through training, certification, and workforce development—is particularly noteworthy. The successful integration of NAMs into mainstream pharmaceutical research and development will depend as much on human expertise as on technological availability. By advocating structured training pathways for scientists, regulators, and contract research organization personnel, the report anticipates and addresses one of

the most significant barriers to adoption: confidence in the interpretation and use of NAM-generated data.

Looking ahead, the prospects outlined by this report are both ambitious and realistic. In the near term, the strategic application of NAMs in generics, biosimilars, and quality testing offers immediate opportunities for impact, given India's dominance in these sectors. In the medium term, the maturation of organ-on-chip platforms, patient-derived organoids, and integrated computational models promises to reshape early drug discovery and preclinical decision-making. Over the long term, the convergence of NAMs with artificial intelligence, systems biology, and real-world evidence has the potential to fundamentally redefine regulatory science, shifting from prescriptive checklists toward adaptive, evidence-weighted evaluation frameworks.

Crucially, this report positions India not as a passive adopter of global trends, but as a potential leader in the next generation of regulatory innovation. With its scale, scientific talent, and growing emphasis on innovation-driven growth, India is uniquely positioned to demonstrate how NAMs can be implemented responsibly, cost-effectively, and at scale. The roadmap articulated here provides a credible pathway for achieving that vision, grounded in international best practices yet tailored to national priorities and constraints.

It is therefore appropriate, and indeed necessary, to formally recognize the collective effort behind this report. The contributors—from industry, academia, policy, and civil society—have produced a document of exceptional breadth, depth, and methodological rigor. The extensive stakeholder engagement, careful comparative analyses, and transparent articulation of limitations reflect a commitment to scientific integrity and constructive dialogue. This report will undoubtedly serve as a foundational reference for researchers seeking to develop and validate NAMs, for regulators navigating evolving evidentiary standards, and for industry leaders aiming to align innovation with ethics and efficiency.

In congratulating the participants and authors, one must also acknowledge the broader significance of their work. By bridging disciplines, sectors, and regulatory cultures, this report exemplifies the collaborative ethos required to address complex, systemic challenges in modern biomedical research. It demonstrates that ethical responsibility and scientific excellence are not competing objectives, but mutually reinforcing pillars of sustainable innovation.

As the global community continues to grapple with rising research and development costs, unmet medical needs, and ethical imperatives, the insights contained within this report will resonate far beyond national boundaries. They offer a compelling vision of a future in which drug development is more predictive, more human-relevant, and more justifiable—scientifically, economically, and morally. It is my sincere hope that this Landscape Study will catalyze informed action, inspire cross-border collaboration, and accelerate the responsible integration of Non-Animal Methods into the heart of biomedical innovation worldwide.

Foreword

We are now at the forefront of a fundamental change in how safety and efficacy is determined in drugs and vaccines. While animals have always been the go - to model to understand human health and diseases, with the high failure rate of drug development using the current paradigm (90%), this perception is now changing. Regulators and industry are now adopting precise, human cell-based approaches to test new drugs, and looking at human biology-derived data as the new gold standard.

The last decade has seen an accelerated development of non-animal methods (NAMs) that include complex in vitro human-based systems and in silico modelling. Countries, including India, US and UK have now explicitly authorized the use of nonanimal alternatives to test safety and efficacy of new drugs.

However, these legislative changes need to be accompanied with restructuring of research funding for sustainable transition and adoption of NAMs. I am delighted that that we have been an integral part of this report. It provides a timely assessment of global scientific and regulatory landscape of NAMS. It documents the opportune areas where redundant or scientific obsolete animal studies can be waived off and replaced with non-animal methods, backed by thorough scientific justifications and global regulatory decisions. It also provides ecosystem-level changes, such as establishing a Central agency for NAMs and industry consortium to overcome the systemic challenges for adoption of these methods.

These emerging technologies aim to revolutionalise public health by enhancing predictive accuracy of drug development and bringing medicines in a faster and cost-effective manner to patients. I hope this extensive report which has materialized out of collaboration and efforts of multiple stakeholders proves as a guiding document for the funding and regulatory authorities of India and accelerates India's footprint in innovative regulatory science.

Alokparna Sengupta
Managing Director,
Humane World for Animals India

Dr Jitendra Kumar
Managing Director,
Biotechnology Industry Research Assistance Council (BIRAC)
Department of Biotechnology,
Government of India

Foreword

The last decade has seen a paradigm change in drug development and testing with the advancement of scientifically validated non-animal methodologies, such as organ-on-a-chip systems, computational modelling, and advanced in vitro assays. This development reflects a growing realisation that animal models often do not adequately recapitulate the characteristics of human health and disease.

These emerging new technologies that are based on human biology not only pave the way for reducing our reliance on animals in science but can also improve the predictive accuracy and enhance public health by ensuring newer therapies are economical and reach patients faster. The significance of this new frontier is highlighted by the fact that several countries, including the US and UK have come up with strategic Roadmaps in 2025 that focus on development, validation, and adoption these technologies in the regulatory process.

India has also actively supported this transition with the passing of New Drugs and Clinical Trials (Amendment) Rules, 2023 which allows the use of non-animal human-biology based methods to test safety and efficacy of new drug, and several grants to develop indigenous non-animal technologies.

This report comes at an opportune time during this paradigm change where it provides an intensive landscape analysis across different pharmaceutical sectors (generics, differentiated products, new chemical & biological entities, cell & gene therapy, pyrogen & endotoxin testing) and regulatory frameworks. The report identifies key areas of redundant animal testing and opportunities where human-biology based high TRL-methods are replacing animal models in these different sectors in global regulations.

This report provides great insights to bring about regulatory transformation in India by laying down clear recommendations – both technology-specific and systemic – on how India can not just be an adopter but also a global leader in the modern regulatory science and innovation.

Foreword

India's ambition to emerge as a global leader in drug discovery and development is closely tied to how effectively science, regulation, and innovation systems evolve together. As the pharmaceutical sector expands in scale and complexity, there is an increasing need to reassess long-standing development and testing paradigms to ensure that they remain scientifically robust, ethically responsible, and globally aligned. In this context, the growing relevance of Non-Animal Methods (NAMs) marks an important shift in how evidence for safety and efficacy can be generated.

This Landscape Report offers a comprehensive assessment of the current status of NAMs in global drug development ecosystem. It examines their applicability across generics, new chemical and biological entities, and emerging modalities such as cell and gene therapies, while carefully analysing regulatory expectations, global precedents, and scientific readiness. By situating NAMs within the broader challenges of low clinical success rates, duplication of animal studies, and rising development costs, the report provides a clear rationale for why modernisation of non-clinical testing frameworks is both timely and necessary.

A key strength of this report lies in its focus on regulatory alignment and practical pathways for adoption. It highlights where Indian requirements diverge from international guidelines, particularly in the context of generics, and presents evidence-based opportunities for waiving or reducing in vivo studies without compromising patient safety. The emphasis on the principles of Replacement, Reduction, and Refinement reflects a balanced approach—one that recognises ethical imperatives while remaining grounded in scientific rigor and regulatory responsibility.

From the perspective of the Office of the Principal Scientific Adviser to the Government of India, the findings of this report reinforce an important systems-level insight: advancing NAMs is not solely a scientific challenge, but an ecosystem challenge. Progress will depend on coordinated action across regulators, industry, academia, and CRO's, supported by enabling policies, specialised infrastructure, capacity development, and clear demand signals. The report's recommendations on multi-stakeholder consortia, CoE's, indigenous supply chains, and data-sharing frameworks provide a valuable foundation for such coordination.

I hope this Landscape Report serves as a reference for informed dialogue and evidence-based decision-making as India strengthens its regulatory frameworks and innovation capabilities. By thoughtfully integrating NAMs into drug development pathways, India has the opportunity to enhance efficiency, improve translational outcomes, reduce unnecessary animal use, and reinforce its position as a responsible and forward-looking leader in pharmaceutical innovation.

Dr Sapna Poti
Director - Strategic Alliances,
Office of the Principal Scientific Adviser to the Government of India

Dr. Taslimarif Saiyed
Director and CEO,
Centre for Cellular and
Molecular Platforms (C-CAMP)
GKVK Post, Bellary Road
Bangalore 560 065, India.

Foreword

The development of non-animal models for biomedical research and drug discovery represents a transformative shift—one that promises more accurate, ethical, and efficient science. Traditional animal models, while historically valuable, often fail to predict human responses due to fundamental biological differences, leading to high failure rates in clinical trials and prolonged development timelines. These limitations are no longer acceptable when human lives and global health challenges are at stake.

Researchers across academia, industry, and startups are developing these fantastic technologies—organ-on-chip platforms, 3D human tissue models, stem cell-derived organoids, computational biology, and AI-driven simulations—that replicate human physiology with remarkable precision. These enable studying disease mechanisms, testing therapies, and predicting toxicity far more reliably than animal systems.

For India, this moment is uniquely opportune. With world-class talent in biotech, computational sciences, and engineering—bolstered by institutions like inStem—this nation can leapfrog to leadership in next-generation biomedical models. The global scientific community increasingly welcomes and validates these approaches, with regulatory bodies worldwide recognizing their potential to accelerate innovation while reducing ethical concerns.

Non-animal models are not merely alternatives; they are the future of biomedical research. India must invest boldly in their development, scale infrastructure, and foster public-private collaborations to capture this global opportunity. The time to lead is now.

Project Team Structure:

Category	Name	Organization
Project Steering Committee	Parag Agarwal	Dr. Reddy's Laboratories Limited
	Deepak Sapra	Dr. Reddy's Laboratories Limited
	Dr. Arvind Ramanathan	DBT BRiC – Institute for Stem Cell Science and Regenerative Medicine (inStem)
Project Lead	Dr. K V S Narayana Raju	Dr. Reddy's Laboratories Limited
Project Core Team	Dr. Surat Parvatam	Humane World for Animals India
	Dr. Harshita Mittal	Humane World for Animals India
	Dr. Paulami Dey	DBT BRiC – inStem
	Apoorva	Animal Law & Policy Network (ALPN)

List of Authors:

Name of the Author	Associated organization
Dr. K V S Narayana Raju	Dr. Reddy's Laboratories Limited
Dr. Surat Parvatam Dr. Harshita Mittal	Humane World for Animals India
Dr. Paulami Dey Dr. Arvind Ramanathan	DBT BRiC- Institute For Stem Cell Science and Regenerative Medicine (inStem)
Apoorva Vaishnavi Vasanth Anita Varadharajan	Animal Law & Policy Network (ALPN)

Stakeholders Consulted / Contributed

Name of stakeholder	Associated organization
Prof. (Dr.) Yogendra Kumar Gupta	President, All India Institute of Medical Sciences Jammu & Kalyani
Dr. Viraj Mehta	Sai Lifesciences
Dr. Janette Turner	Humane World for Animals
Dr. Nirnith Devireddy, Dr. Pranav Karmwar	InSilico Minds
Dr. Daniel Röshammar	InSilico Trials
Dr. Rekha Pal	Crown Biosciences
Dr. Jugnu Jain	Sapien Biosciences
Dr. Rahul Date, Dr. Abhay Deshpande	JRF Global
Dr. Suresh Poosala	OncoSeek Bio Pvt Ltd
Dr. Prajakta Dandekar Jain	ICT, Mumbai

Name of stakeholder	Associated organization
Dr. Rajarshi Pal	Eyestem
Manoj Kumar S	Biodimension Technology Private Limited,
Dr. C Nirmala Raju	Dr. Reddy's Laboratories Limited
Dr. Pushkar Kulkarni (Co-author)	Dr. Reddy's Laboratories Limited
Dr. Madhu Nag Dr. Arumugham Raghunathan	inSphero
Dr. Sujit Tangadpalliwar	Biolligence
Dr. Sivacharan Kollipara	Dr. Reddy's Laboratories Limited
Dr. Christian Pellevoisin	Mattek Lifesciences
Dr. Lorna Ewart	Emulate Bio
Dr.Varun Ahuja	Syngene International
Dr. Nitin Shetty	Bioneeds
Mr. Venkata Ramana Kommagoni	Dr. Reddy's Laboratories Limited
Dr. Sanjib Benerjee	Veeda Lifesciences
Dr. Rajesh Eswarappa	Aurigene Oncology Limited
Dr. Saravanan Thiyagarajan	Aurigene Oncology Limited
Dr. Navin Rajesh B	Aurigene Pharmaceutical Services Limited
Dr. Upendra Bhatnagar	Vimta labs Ltd.
Mr. Bala Sandala	GV Research Platform Pvt Ltd.
Dr. Subhadra Dravida	TRAN-SCELL Biologics
Dr. MR Balaji Mr. Prasanna Kumar Thenneti Mr. D Mallikarjuna Rao Dr. Ashutosh Ratnakar Joshi Mr. Prasun Kumar Guha Dr. Vikas Kumar	Dr. Reddy's Laboratories Limited
Dr. Murali Addepalli	Lextro Bio Solutions
Dr. Suresh Pothani	National Institute of Nutrition
Dr. Kasturi Mahadik	Centre for Predictive Human Model Systems, Atal Incubation Centre, Centre for Cellular and Molecular Biology
Dr. Dhanesh Kothari Dr. Nimmi Kothari	Pi2Life
Mr. Vikash Agarwalla Dr. Smruthi Suryaprakash	Boston Consulting Group
Dr. Subha Narayan Rath	IIT, Hyderabad
Dr. Anita Krishnan	Biocon Biologics
Dr. Anirban Thakur	Lupin

Name of stakeholder	Associated organization
Dr. Shridhar Narayanan Mr. Hari Pranav K	Indian Pharmaceutical Alliance
Dr. Mukul Jain	Zydus Research Centre
Chaith Kondragunta Dr. Uttara Joshi	Aira Matrix
Dr. Kaushik Chatterjee	Indian Institute of Science
Dr. Suhridh Sundaram	Avay Lifesciences
Dr. Indumathi Mariappan	DBT-inStem
Dr. Madhusudhana Rao	Atal Incubation Centre-CCMB
Dr. Megha K B	Sree Chitra Tirunal Institute for Medical Science and Technology (SCTIMST),
Katrin Pauls	Lonza
VIGILANZA Paola Shahid Shah	Biomerieux

Contents

Executive Summary

1

Background

India's pharmaceutical sector is entering a transformative phase, moving beyond its traditional strength in generics and biosimilars toward innovation in small molecules, biologics, and advanced modalities like cell and gene therapies. As preclinical testing expands, non-animal methods (NAMs) present a strategic opportunity to improve translational relevance, reduce costs, and enhance global competitiveness. Recent regulatory reforms, such as the U.S. FDA Modernization Act 2.0 and India's New Drugs and Clinical Trials (Amendment) Rules, 2023, signal a structural shift toward alternative testing approaches.

Globally, NAMs are gaining traction through technologies like organoids, organ-on-chip systems, microphysiological platforms, and in silico modeling. These approaches directly address challenges in drug development, including high clinical attrition rates and poor species translatability, where only about 10% of Phase I candidates reach approval. India is well placed to benefit from this trend, given its pharmaceutical scale, computational strengths, and growing stakeholder interest. However, barriers such as the absence of a national strategy, fragmented infrastructure, uncertainty around regulatory acceptance, and lack of validation frameworks continue to constrain domestic adoption. Overcoming these challenges through strategic investment, regulatory harmonization, and collaboration across academia, industry, and government could allow India to seize the opportunity and emerge as a global hub for NAMs innovation and services.

Analysis across generics, biosimilars, new chemical entities (NCEs), new biological entities (NBEs), and cell/gene therapies(CGT) highlights concrete opportunities for reduction and replacement of animal studies, alongside immediate cases such as pyrogen/endotoxin testing where NAMs can substitute legacy assays. Globally, jurisdictions such as the United States, European Union, Japan, and the United Kingdom have already established dedicated national centres, validation programs, funding mechanisms, and harmonisation initiatives for NAMs. Without similar structured investment and standards, India risks being a late adopter with limited ability to participate in evolving global regulatory ecosystems.

A national strategy would allow India to capture economic, scientific, and regulatory benefits from NAMs adoption. Five policy lever, national coordination, targeted funding, data standards, regulatory alignment, and biobanking infrastructure, would enable India to move from emerging adopter to a strategic global leader.

Objectives and Methodology

This report was compiled over the span of one year through a collaboration between Dr. Reddy's Laboratories, DBT-InSTEM, Humane World for Animals India, and the Animal Law and Policy Network. It aims to map emerging NAMs and highlight opportunities for their adoption in India. A qualitative assessment was carried out across generics, NCEs/NBEs, and CGT, drawing on existing literature, company websites, and consultations with more than 30 national and international stakeholders. Opportunities to incorporate the 3Rs—Replace, Reduce, Refine—were explored across both regulatory and non-regulatory domains.

Key Messages

NAMs are reshaping preclinical testing.

- NAMs can reduce costly late-stage failures by improving prediction of human safety and efficacy.
- Regulatory acceptance is expanding globally, with the U.S. FDA now allowing NAMs-generated data for certain submissions.
- NAMs address core limitations of animal models, including species differences in physiology, metabolism, and genetics.

India is currently an emerging adopter but could become a strategic contributor.

- India has scale in pharmaceuticals and computational strengths but lacks coordinated national strategy.
- Stakeholders express uncertainty about where NAMs replace, complement, or reduce animal testing.
- Without intervention, India risks lagging behind jurisdictions that are actively building NAMs ecosystems.

Economic and strategic gains from NAMs adoption are significant and time-sensitive.

- NAMs can shorten development timelines and improve success rates across multiple modalities.
- Global competitors are developing NAMs for differentiated regulatory submissions.
- India could build export-oriented preclinical services around high-content in vitro and in silico models.

Sectoral analysis reveals concrete and near-term opportunities for reduction and replacement.

- Redundant animal testing in the development of generic drugs can often be waived because the active pharmaceutical ingredients (APIs) involved are already well-established in terms of safety, and the excipients used are generally recognized as safe (GRAS). NAMs can be applied for impurity qualification and early non-regulatory studies.
- For NCEs/NBEs, NAMs can be integrated into early toxicology studies, mechanism of action investigations, and selective-species workflows. Moreover, NAMs are particularly valuable in assessing organ-specific toxicities, such as drug-induced liver injury (DILI). AI-based approaches can enhance predictive toxicity testing and mechanistic insights.
- For Cell and gene therapies,in vitro pharmacokinetic and pharmacodynamic (PK/PD) models, along with tailored toxicology assays, can provide meaningful data without the need for extensive animal studies.
- Pyrogen/endotoxin testing has immediate replacement potential via Monocyte Activation Test (MAT) and recombinant Factor C (rFC) assays.

International peers are investing systematically in NAMs infrastructure, validation and data acceptance.

- United States, European Union, United Kingdom, and Japan have established national centres and validation programs.
- These jurisdictions provide targeted funding and harmonisation pathways for regulatory acceptance.
- Some have set explicit timelines or targets for reducing or phasing out animal use in regulated applications.

India has foundational strengths that favour NAMs adoption and scaling.

- Strong pharmaceutical manufacturing base and emerging capabilities in organoids and organ-chip.
- Competitive advantages in computational tools, AI-driven toxicology and data science.
- Growing domestic interest from academia, regulators, and industry stakeholders.

Key bottlenecks are structural and addressable through national coordination.

- Absence of validation frameworks and regulatory guidance limits data acceptability.
- Biobanking and tissue infrastructure remain fragmented, reducing access to human-relevant inputs.
- Domestic manufacturing of advanced NAMs platforms and reagents is limited.
- Funding mechanisms do not currently support development, validation, or commercialization stages.

Targeted policy levers could accelerate India's transition from adopter to contributor.

- A national consortium/centre would coordinate infrastructure, validation, and global engagement.
- Dedicated funding could support technology maturation and market readiness.
- Standardised data collection on animal use would inform priority areas for NAMs development.
- Regulatory alignment and acceptance guidance would enable use of NAMs data in submissions.
- National biobanking capacity would provide human-derived materials essential for NAMs platforms.

Strategic execution would position India for participation in globally harmonised preclinical ecosystems.

- India could supply NAMs-enabled preclinical services to sponsors worldwide.
- Domestic regulators could align with global standards and reduce barriers to market entry.
- Pharmaceutical competitiveness could strengthen through reduced attrition and improved translation.
- India could become a technology contributor rather than a downstream adopter.

Key Terms

NAMs (Non-Animal Methods)

In vitro, in silico, and microphysiological platforms that generate preclinical safety and efficacy data without animal models.

Microphysiological Systems (MPS)

Organ-on-chip and related platforms designed to recapitulate human tissue physiology.

Organoids

3D in vitro cultures derived from stem cells that model key structural and functional characteristics of human tissues.

In *Silico* Toxicology

Computational modelling used to predict safety, efficacy, pharmacokinetics, and pharmacodynamics.

Validation

Process of demonstrating that a NAM generates reproducible and scientifically reliable results for regulatory purposes.

Data Acceptability

Conditions under which regulators recognise NAM-derived data as suitable evidence for decision-making.

PK/PD

Pharmacokinetics and pharmacodynamics; critical parameters for toxicology and efficacy assessment.

Replacement/Reduction/Refinement (3Rs)

Framework for shifting away from animal testing through alternative models and approaches.

Biobanking

Systematic storage and distribution of patient-derived tissues, cells, and biological materials essential for NAMs development.

NCE

New Chemical Entity; A novel chemical compound that has never been previously approved or marketed as a drug in any country.

NBE

New Biological Entity; A novel biological molecule (such as a monoclonal antibody, recombinant protein, peptide, vaccine component, or other biologic) that has never been previously approved or marketed as a therapeutic product.

Introduction

2

Several studies have shown the low success rate of current drug development paradigms. A recent study looking at around 20,000 clinical trials conducted by 18 leading pharmaceutical companies from 2006–2022 reported an average likelihood of approval (LoA) rate of 14.3%[1]. The LoA represents the probability that a drug candidate fulfills its efficacy, safety and market benchmarks, and meets the regulatory agency's approval standards. While animal models have been conventionally used in biomedical research and drug development, it is also emerging that they cannot capture the severity and phenotype of human disease in its entirety[2]. In the last two decades, biomedical research has undergone a paradigm change where bioengineered human disease models that can mimic several structural, physiological, and clinical parameters are being developed[3]. This has led to several regulatory, policy and funding-level initiatives from countries across the world. For example, the US FDA recently released a roadmap that discusses strategic and stepwise approaches to reduce animal testing in preclinical safety studies with scientifically validated new approach methodologies (NAMs). This may include organ-on-a-chip systems, computational modeling, and advanced *in vitro* assays[4]. In addition, NIH has announced a new initiative to prioritise human-based science. Towards this, the NIH's Office of the Director intends to establish the Office of Research Innovation, Validation, and Application (ORIVA). Similarly, the European Commission in its European Research Agenda for 2025–27 has recognised "accelerating new approach methodologies to advance biomedical research and testing of medicinal products and medical devices" as a priority[5].

The Ministry of Health and Family Welfare also released the New Drugs and Clinical Trials (Amendment) Rules, 2023, which allows researchers to use human relevant non-animal methodologies (NAMs) technologies such as 3D organoids, organs-on-chip, and computational methods to conduct safety and efficacy testing of new drugs[6].

Despite removal of these legislative barriers to using NAMs as an alternative to drug development, there are still several challenges to its use, adoption and development in the Indian ecosystem. These include lack of awareness amongst end users regarding successful use cases of NAMs, both for regulatory submissions and in the non-regulatory space; lack of structured and strategic investment in NAMs and lack of infrastructure and trained personnel in these new technologies that allows the adoption of NAMs at scale.

In this report, we analyse the regulatory landscape in India and globally to identify two scenarios of NAMs use cases along with challenges, recommendations, and ecosystem-level changes necessary to enable NAMs adoption:

- Scenarios where NAMs have now been accepted regulation-wise globally but still need to be accepted in India.
- Scenarios where NAMs are being developed and are in the advanced stages of regulatory acceptance and technology-readiness levels (TLRs).
- Challenges and recommendations for adoption and development of NAMs in India.
- Possible ecosystem-level systemic changes that may be required to enable the use of NAMs at scale in India.

Currently, these scenarios are being explored across four sectors in this report.

- The first chapter focuses on Generics regulations and identifies the use of NAMs both in the regulatory and non-regulatory space. It provides recommendations on where animal usage can be reduced and replaced using *in vitro* and in silico tools in Generics development.
- The second chapter focuses on New Chemical Entities (NCEs) and identifies possible scenarios where animal usage can be reduced or replaced based on existing global regulations where such changes have already been approved.
- The third chapter covers the cell and gene therapy sector and identifies possible scenarios where animal usage can be reduced or replaced based on existing global regulations where such changes have already been approved or are in advanced stages of TRLs.
- The fourth chapter covers the NAMs and identifies the various Contexts of Use (CoU) where NAMs are currently being developed. It also provides information regarding their regulatory status, commercial availability, scientific and practical limitations, and recommendations to enable their development and adoption in India.
- The fifth chapter covers pyrogen testing that applies across different categories and is done for quality testing of finished pharmaceutical products. Replacement of animal testing with NAMs in pyrogen testing has been identified as one of the priority areas in the recent US NIH-FDA workshop on reducing animal testing[7]. The chapter covers the regulatory status of NAMs, specifically the Monocyte Activation Test (MAT) and recombinant technologies in endotoxin testing, respectively. It also provides challenges and recommendations for adopting these methods in India.
- The last chapter provides an overview of systemic and ecosystem-level changes required to enable the indigenous development, use, and adoption of NAMs at scale in India, both in biomedical research and for regulatory submissions.

References:

1. *Schuhmacher A, Hinder M, Brief E, Gassmann O, Hartl D. Benchmarking R&D success rates of leading pharmaceutical companies: an empirical analysis of FDA approvals (2006-2022). Drug Discov Today. 2025 Feb;30(2):104291.*
2. *Van Norman GA. Limitations of Animal Studies for Predicting Toxicity in Clinical Trials: Is it Time to Rethink Our Current Approach? JACC Basic Transl Sci. 2019 Nov;4(7):845–54.*
3. *Loewa A, Feng JJ, Hedtrich S. Human disease models in drug development. Nat Rev Bioeng. 2023 May 11;1–15.*
4. *FDA. FDA Announces Plan to Phase Out Animal Testing Requirement for Monoclonal Antibodies and Other Drugs. 2025. Available from: https://www.fda.gov/news-events/press-announcements/fda-announces-plan-phase-out-animal-testing-requirement-monoclonal-antibodies-and-other-drugs.*
5. *Council Recommendation of 24 June 2025 on the European Research Area Policy Agenda 2025–2027. 2025. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:C_202503593.*
6. *Central Drugs Standard Control Organization. Use of Induced Pluripotent Stem Cells additional testing methods. New Delhi (India): CDSCO; 2023. Available from: https://cdsco.gov.in/opencms/resources/UploadCDSCOWeb/2022/new_DC_rules/NDCTR_G.S.R.%20175(E)%20dt_09.03.2023_Use%20of%20induced%20Pluripotent%20Stem%20Cells%20additional%20testing%20methods.pdf.*
7. *FDA-NIH Workshop: Reducing Animal Testing - 07/07/2025 [Internet]. FDA. 2025. Available from: https://www.fda.gov/news-events/fda-meetings-conferences-and-workshops/fda-nih-workshop-reducing-animal-testing-07072025.*

Opportunity Assessment for 3Rs for in Vivo Non-Clinical Studies Requirement for Generics / Differentiated Drug Products

3

3.1 Executive Summary

This chapter critically examines the regulatory landscape governing non-clinical in vivo animal studies for generic and differentiated drug products globally and in India. It highlights the global shift toward ethical drug development practices, particularly the 3Rs principle—Reduce, Refine, Replace—in animal testing. The chapter argues for the waiver of mandatory in vivo non-clinical studies for generics in India, citing international precedents, scientific rationale, and regulatory flexibility. It concludes recommendations to align Indian regulations with global standards, thereby accelerating access to affordable medicines. In addition, this chapter also provides information on opportunities for the implementation of 3Rs in the non-regulatory space of drug development.

3.2 Introduction to Generics and Regulatory Definitions

Generics are defined by EMA and FDA as follows:

EMA definition: The European Medicines Agency (EMA) defines a generic medicinal product as one that has the same qualitative and quantitative composition in active substances as the reference product, shares the same pharmaceutical form, and demonstrates bioequivalence through appropriate bioavailability studies[1].

FDA definition: The U.S. Food and Drug Administration (FDA) describes a generic drug as identical to a brand-name drug in dosage form, safety, strength, route of administration, quality, performance, and intended use[2].

Differentiated/Hybrid Medicinal Products: These differ from generics in that they require additional pre-clinical and clinical data due to differences in formulation, route, or therapeutic indication[1,3].

3.3 Role of Non-Clinical Studies in Drug Development

Non-clinical studies provide data on:

- Pharmacology and mechanism of action.
- Absorption, distribution, metabolism, and excretion (ADME).
- Toxicology (acute, sub-acute, chronic, reproductive, carcinogenicity).
- Safety Pharmacology - Core battery (focused on the central nervous system, cardiovascular System, and respiratory system) and supplemental battery (focused on the gastrointestinal tract and renal system)
- Local Tolerance Studies (route specific, e.g., parenteral, dermal)
- Safety for special populations (e.g., Pediatrics).

These studies are traditionally conducted in animals or *in vitro*.

FDA guidance on Generally Accepted Scientific Knowledge (GASK) permits the use of existing scientific literature to meet certain nonclinical safety requirements for New Drug Applications (NDA) and Biologics License Applications (BLA), potentially reducing the need for redundant animal testing[4]. Similar guidance is available in the European Union and other ICH countries.

3.4 Background and Importance of Generics in India

India is a global leader in generic drug production, contributing to approximately 20% of the global supply. Over 40% of India's generics are exported, covering a wide therapeutic range from antibiotics to oncology drugs. The industry aims to move up the value chain and become one of the top five in terms of value and number one in terms of volume by 2047. To achieve this vision, regulatory simplification becomes crucial.

With the worldwide shift towards ethical, science-driven approaches to drug development, the reduction of animal testing has emerged as a critical agenda in the regulatory framework. Anchored in the 3Rs principle (Reduce, Refine, and Replace), India has an opportunity to advance its global leadership in pharmaceuticals by further modernizing its regulatory framework for non-clinical animal testing.

Current requirements for animal studies to assess toxicity and pharmacological activity often go beyond global guidelines (US, EU, Japan). Streamlining animal testing could enhance productivity, drive innovation, and optimize resource use, enabling faster access to generics and differentiated products.

3.5 Regulatory Expectations in India and Global Regulatory Alignment

A comparative analysis (Table 1) highlights that India currently undertakes several additional animal studies across development phases, even where similar data may already exist from international submissions. While case-by-case requirements for certain animal studies remain important, there is now an opportunity to reduce duplication by adopting a more flexible, science-driven approach.

Table 1: The key differences between India and ICH countries

Type of Generic or Differentiated Product	Pre-Requisites	Required In vivo Nonclinical Studies in India*	Required In vivo Nonclinical Studies as per ICH**
Generic Drugs			
New Generic Drug	First-time registration	• Single and repeat-dose toxicity (2 species)	• None
	Approved in other countries (including ICH)	• Skin sensitization (if dermal)	• Impurity qualification (if required)
Follow-up Generic (within 4 years)	Registered within 4 years of first approval.	• Single and repeat-dose toxicity (2 species)	• None
	Approved in other countries (including ICH)	• Skin sensitization (if dermal)	• Impurity qualification (if required)
Any Generic Injectable	Any injectable, even if the same molecule, formulation approved in India	• Repeat-dose toxicity (2 species)	• None • Impurity qualification (if required)

Type of Generic or Differentiated Product	Pre-Requisites	Required In vivo Nonclinical Studies in India*	Required In vivo Nonclinical Studies as per ICH**
Differentiated Products			
New Strength / API Concentration	Same molecule & dosage form registered	• Comparative single and repeat-dose toxicity (2 species)	• Bridging toxicity (1 relevant species)
	Scientific rationale for new strength	• Pharmacological activity (if not in prescribing info)	
New Dosage Form	Same molecule registered	• Comparative single and repeat-dose toxicity (2 species)	• Bridging toxicity (1 relevant species)
	Scientific rationale for the new form	• Pharmacological activity	
New Route of Administration	Same molecule registered	• Comparative single and repeat-dose toxicity (2 species)	• Bridging toxicity (1 relevant species)
	Scientific rationale for new route	• Pharmacological activity	
New Population (e.g., Pregnancy, Paediatrics)	Same molecule and dosage form registered	• Bridging toxicity and pharmacokinetics (2 species)	• Bridging toxicity and pharmacokinetics (1 species)
	Approved in the country of origin for the population	• Juvenile/reproductive/ developmental toxicity (relevant species)	• Juvenile/reproductive/ developmental toxicity (relevant species)
		• Pharmacological activity	
New Indication	Same molecule & dosage form registered	• Comparative toxicity (2 species)	• Bridging toxicity (1 species)
	Originator lacks indication, or data exclusivity applies	• Pharmacological activity	• Pharmacological rationale (if needed)
New Fixed-Dose Combination (FDC)	Same molecules, dosage forms, strengths registered	• Comparative toxicity (2 species)	• Bridging toxicity (1 species)
	Scientific rationale for FDC	• Pharmacological activity	• Pharmacological rationale (if needed)

*As per NDCT, 2019[5]; ** EU Directive, 2001[1]; USFDA Generic Drugs, 2023[2]; USFDA 505(b)(2), 1999[3].*

Apart from the above studies, some additional in vivo nonclinical studies might be required on a case-by-case basis. The above table suggests that several additional animal studies are required in India as compared to ICH requirements, and they apply across all clinical trial phases (I-IV), often duplicating data already available from international sources. By adapting India's NDCT Rules to incorporate such mechanisms, India can further harmonize with global best practices. This would represent not only substantial financial savings for Indian pharmaceutical companies but also a significant ethical advancement towards reduced animal testing.

3.6 Waiver of in Vivo Non-Clinical Studies for Generics

The proposal is to consider the waiver requirements of in vivo non-clinical studies from product development, as well as for securing marketing authorization for generics in India. The justification and scientific rationale for the waiver of in vivo non-clinical studies, as well as the advantages of the proposed approach, are provided below:

1. Science-based rationale: Toxicology studies for generics seldom provide new safety insights, as the APIs involved are already well-established for safety, and the excipients used are generally recognized as safe (GRAS). Robust human bioequivalence studies are sufficient to demonstrate both safety and efficacy. In addition, published literature and globally accepted scientific knowledge bases (e.g., GASK) can effectively substitute the need for animal studies.

2. Evidence-based precedents: Global regulatory authorities routinely approve generics without requiring animal studies, demonstrating the sufficiency of bioequivalence and *in vitro* data. US legislation passed to end mandatory FDA animal testing in 2022. Similarly, India's Rule 75 allows relaxation of animal testing if adequate published safety data exists. Moreover, the increasing adoption of validated *in vitro* and computational alternatives underscores the global shift toward modern, ethical, and scientifically rigorous approaches.

3. Risk-based assessment: Granting a waiver of in vivo studies does not increase patient risk when robust bioequivalence and safety data are already available. Risk is further mitigated by reliance on international regulatory precedents, peer-reviewed scientific literature, and validated *in vitro* or computational models, ensuring scientific rigor.

3.7 Role of Alternative Testing Models

To reduce animal use in areas where global regulations still mandate testing, India can leverage Non Animal Methods (NAMs), in silico tools, and literature-based evidence approaches already recognized by international regulators.

3.7.1. Alternatives Already Feasible

- Skin sensitization for dermal products: Validated *in vitro* assays[6].
- Toxicological assessments: Literature and global databases in place of repeat animal studies.
- Impurity qualification: In silico modeling and read-across approaches.

3.7.2. Alternatives Needing Development

- NAMs for pharmacokinetic studies with new routes of administration.
- NAMs for pharmacodynamic studies in repurposed drugs or route changes.
- Advanced in silico tools for impurity qualification.

3.8 Non-regulatory studies in early stages of product development for generics and differentiated drugs

Non-regulatory research in pharmaceutical companies includes early-stage discovery and preliminary studies that do not need to follow strict guidelines like Good Laboratory Practice (GLP). These "non-GLP" studies are crucial for initial screening, determining drug safety and feasibility, and exploring mechanisms, but they are different from mandatory, formal pre-clinical and clinical trials that are subject to regulatory oversight. Non-regulatory research is for internal decision-making and exploring possibilities using the data from non-regulatory studies to decide whether to continue developing a candidate and to guide further, more regulated studies.

Regulators are aware that sponsors take steps to reduce, refine, or replace animal use in the early stages of product development (e.g., research, discovery, candidate selection), but they generally do not play a role in this stage of product development and thus cannot assess the impact of alternatives in this area.

Alternatives to animal use in non-regulatory drug discovery include in chemico (e.g., protein interactions), in silico (computational modelling, AI), and *in vitro* (cell and tissue cultures, organoids, organ-on-a-chip) methods. These approaches can improve the prediction of drug toxicity and efficacy, offer ethical advantages, and reduce costs. The opportunities for implementation of the 3Rs in product development are provided in Table 2.

Table 2: 3R opportunities in early stages of product development for generics and differentiated drugs (Non-regulatory space)

Drug Products	Type of study	Non-clinical studies	3R Opportunities
Generics	Comparability of formulations (screening assays)	Typically, studies in relevant animal species are carried out for formulation rank ordering (mice, rats, dogs)	Reduction in the number of animals/single relevant species
	Formulation impact (e.g., API form impact crystalline vs amorphous)	Specific cases in vivo studies (rodents) are also conducted to understand the formulation impact	Reduction in the number of animals/single relevant species
	Excipients Impact	To evaluate excipients' impact on absorption, *in vivo* studies are conducted (rodents/non-rodents)	Reduction in the number of animals/single relevant species
	Ex vivo animal models (used mostly for topical generic formulations as a critical tool in justifying formulation differences)	Excised human skin is used and gives a good prediction of *in vivo* bioavailability and bioequivalence, and provides a practical surrogate to clinical bioequivalence studies	Where opportunities exist to use *in vitro* assays for drug screening as part of non-regulatory requirements for generics
Differentiated Drugs/ Modified versions of New Drugs	Pharmacodynamic studies	For differentiated products where a rationale needs to be demonstrated through an *in vivo* pharmacodynamic study (in rodents) to seek approval for clinical trials	Waiver: No pharmacodynamic studies are required (API unchanged) except for repurposing
	Efficacy studies for complex products (where the agency specifically mentions conducting such studies to demonstrate functional biological sameness)	*In vivo* efficacy studies in rodents/non-rodents	Reduction in the number of animals/single relevant species
	Topical applications	Animal studies for absorption, tissue penetration, systemic availability	Reduction in the number of animals/single relevant species

Key Strategies to Reduce Animal Testing in Non-regulatory Drug Development

Assessment and 3R opportunities	**Collaborative Consensus Building**	***In vitro* screening potential**	**Promotion of in *silico* tools**	**AI integration in regulatory science**
Evaluate current reliance on animal testing to identify opportunities for Replacement, Reduction, and Refinement (3Rs).	Share best practices and pool resources to foster regulatory and scientific alignment.	Explore the use of *in vitro* assays for non-regulatory drug screening in generics.	Encourage adoption of robust models like physiologically based pharmacokinetics (PBPK) for predictive pharmacokinetics.	Apply advanced AI tools (e.g., AI4TOX) to enhance FDA safety reviews and regulatory science.

3.9 Impurity Qualification of Generic Products

Impurity qualification refers to acquiring and evaluating data to establish the biological safety of an impurity at specified levels in a drug substance or product. For non-mutagenic impurities (NMIs), ICH Q3A/Q3B guidelines provide basic principles but limited guidance for novel impurities or elevated levels.

NAM-Centric Summary of EMA Reflection Paper on Qualification of Non-Mutagenic Impurities

The EMA reflection paper promotes NAMs as primary tools for qualifying non-mutagenic impurities (NMIs) in pharmaceuticals, reducing reliance on animal studies and aligning with 3Rs principles[7]. Traditional *in vivo* studies often fail to provide impurity-specific insights because impurities are present at levels far below the API, making NOAEL attribution uncertain. Surveys confirm these studies rarely identify toxic NMIs, reinforcing the need for predictive, mechanistic, and non-animal strategies.

Role of NAMs in Risk Assessment

The paper outlines a tiered approach where NAMs are central:

1. **Initial Screening:** Determine if the impurity is API-like or a metabolite. API-like impurities (structurally similar, no new toxicophores) are covered by API toxicology data.

2. **Level of Concern Analysis:** If concern persists, apply NAMs to assess hazard and exposure. Factors include daily dose, route, physicochemical (PC) and pharmacokinetic (PK) properties, degradability, and clinical context.

3. **NAM Integration:** When direct data are unavailable, NAMs provide hazard identification and de-risking strategies before considering *in vivo* studies.

NAMs represent the future of impurity qualification, offering predictive, mechanistic, and ethical solutions. By leveraging RAX, computational toxicology, and *in vitro* systems, regulators and industry can ensure patient safety without defaulting to animal studies. The EMA reflection paper advocates a weight-of-evidence approach combining NAM outputs with exposure and clinical context to qualify NMIs effectively.

1. **Read-Across (RAX)**

   RAX uses surrogate compounds or chemical groups with robust toxicological data. The process involves:

   - Characterizing impurity structure, PC, and PK properties.
   - Identifying toxicophores—structural features linked to toxicity.
   - Demonstrating similarity using metrics like Tanimoto scores. RAX outcomes can support qualitative de-risking or quantitative derivation of an Acceptable Level (AL). Grouping approaches extend RAX by analyzing trends across structurally related compounds.

2. **Computational Toxicology**

   In silico tools predict toxicity endpoints and PC/PK properties using QSAR, machine learning (ML), and AI models. These tools identify toxicophores and assess risks for major targets (liver, kidney, CVS, GIT, CNS, RS). OECD principles guide validation, emphasizing:

   - Applicability domain checks.
   - Performance metrics (ROC-AUC, MCC).
   - Cross-validation and benchmarking. Combining multiple tools and expert review enhances confidence. Frameworks like Adverse Outcome Pathways (AOPs) organize mechanistic data, supporting weight-of-evidence (WoE) assessments.

3. ***In Vitro* Approaches**

   Targeted *in vitro* assays complement computational predictions, addressing bioavailability and potency concerns. Examples include:

   - Transport and metabolism models (e.g., Caco-2 cells, hepatic systems) to refine PK predictions.

- Organotypic and microphysiological systems for endpoint-specific toxicity. While not fully validated for regulatory use, these methods provide critical mechanistic insights. EMA encourages non-standard *in vitro* methods if fit-for-purpose, supported by OECD guidance.

4. **Hazard Characterization and QIVIVE**

   NAMs primarily yield qualitative hazard data. Quantitative risk estimation requires Quantitative *In Vitro–In Vivo* Extrapolation (QIVIVE), translating *in vitro* potency to systemic exposure. Although still evolving, QIVIVE strengthens NAM utility for setting exposure limits.

5. **Acceptable Level (AL) Calculation with NAMs**

   AL represents a product-specific safe exposure level for an impurity. When NAMs inform hazard identification, AL derivation integrates surrogate data (RAX) and NAM-based bioavailability estimates.

3.10 Challenges with Current Indian Regulations

Key Issues

- Redundant animal testing for generics already approved internationally.
- High costs and time delay due to extensive toxicity studies.
- Conflict with ICH guidelines, which promote ethical drug development using the 3Rs.
- Limited scientific value of animal studies in demonstrating bioequivalence for generics.

Comparative Regulatory Flexibility

- FDA and EMA often waive animal studies for generics if bioequivalence is established.
- India's NDCT Rules still require full toxicology data for APIs not used significantly in the country, even if approved elsewhere.

3.11 Recommendations

Aligning India's regulatory framework on animal testing with global practices for generics and differentiated products will enhance efficiency, foster innovation, and accelerate access to affordable therapies. Drawing on international precedents, scientific rationale, and available regulatory flexibilities, the following measures are proposed to modernize India's framework:

1. Global Regulatory Alignment: Build on ICH guidelines and incorporate Generally Accepted Scientific Knowledge (GASK) and literature-based evidence for safety assessment, reducing duplication and fostering efficiency.
2. Waiver of *In Vivo* Non-Clinical Studies for Generics: Allow waivers for mandatory animal studies during development and marketing authorization, with exceptions as justified on a case-by-case basis.
3. Alternative Testing Methods: Encourage adoption of the 3Rs principle and validated modern tools to minimize animal use and strengthen reliance on advanced methodologies.
4. Pre-submission Meetings with Sponsors: US FDA and European Agencies encourage pre-submission (Pre-IND, Controlled Correspondence, etc.) engagements and meetings with sponsors. Such engagements help in rationalizing the study designs and reducing the number of studies and the number of animals for specific products.
5. An industrial consortium can be formed to lead the implementation of 3Rs during drug development and the adoption of in silico, *in vitro*, and generative AI approaches for the reduction of animal usage.
6. Leveraging organizations like the Indian Pharmaceutical Alliance (IPA) to encourage pharmaceutical companies to reduce their dependence on animal testing by making them aware of the potential cost savings.
7. Pre-submission meetings with sponsors to reduce unnecessary experimentation and use of animals and encourage the use of NAMs.

References:

1. *Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. [Internet]. [cited 2025 Dec 12]. Available from: https://health.ec.europa.eu/document/download/6a59e03f-fb86-4cbc-9fca-f8e4a7e938b1_en?filename=dir_2001_83_cons_2012_en.pdf*
2. *USFDA Generic Drugs, 2023. FDA [Internet]. 2025 Sept 16 [cited 2025 Dec 12]; Available from: https://www.fda.gov/drugs/generic-drugs/overview-basics*
3. *USFDA 505(b)(2), 1999. Applications Covered by Section 505(b)(2) [Internet]. FDA; 2021 [cited 2025 Dec 12]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/applications-covered-section-505b2*
4. *Research C for DE and. Generally Accepted Scientific Knowledge in Applications for Drug and Biological Products: Nonclinical Information [Internet]. FDA; 2023 [cited 2025 Dec 12]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/generally-accepted-scientific-knowledge-applications-drug-and-biological-products-nonclinical*
5. *THE NEW DRUGS AND CLINICAL TRIALS RULES, 2019 [Internet]. [cited 2025 Dec 12]. Available from: https://cdsco.gov.in/opencms/resources/UploadCDSCOWeb/2022/new_DC_rules/23NEW%20DRUGS%20AND%20CLINICAL%20TRIALS%20RULES,%202019.pdf*
6. *OECD. Test No. 442E: In Vitro Skin Sensitisation: In Vitro Skin Sensitisation assays addressing the Key Event on activation of dendritic cells on the Adverse Outcome Pathway for Skin Sensitisation [Internet]. OECD; 2024 [cited 2025 Dec 12]. (OECD Guidelines for the Testing of Chemicals, Section 4). Available from: https://www.oecd.org/en/publications/test-no-442e-in-vitro-skin-sensitisation_9789264264359-en.html*
7. *Reflection paper on qualification of non-mutagenic impurities [Internet]. [cited 2025 Dec 12]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/draft-reflection-paper-qualification-non-mutagenic-impurities_en.pdf*

Opportunity Assessment for 3Rs Implementation in Animal Model Testing for New Chemical and Biological Entities (NCEs/NBEs)

4

4.1 Executive Summary

The current Indian landscape explores innovation in the space of New Chemical Entities (NCEs) and New Biological Entities (NBEs) only to a limited extent. However, recent policy changes are encouraging industry players to pursue drug development more actively, creating an opportunity to strengthen the implementation of the 3Rs in animal model testing. This chapter outlines key 3R opportunities, including conditions under which waivers may be granted for single-dose toxicity studies, repeated-dose toxicity studies, female reproductive and developmental toxicity (DART) studies, Fertility and Early Embryonic Development (FEED) studies, male fertility studies, local toxicity studies, phototoxicity studies, and others. We also present a set of recommendations that can be applied within the Indian context to enhance animal model testing practices for NCEs and NBEs.

4.2 Background

While a huge network of pharma companies exists in the country, coupled with many scientists and researchers skilled in the drug development space, India lags behind in the development of New Chemical Entity (NCE)/ New Biologics entities (NBE) when compared to the USA and Europe[1].

Many Indian pharmaceutical companies have conventionally made low investments in NCE R&D due to high cost and longer time. Other contributory factors could be a lack of strong industry–academia collaborations. Moreover, there is a ready dominance of key international players like Pfizer, Novartis, Roche, Eli Lilly, and Bristol Myers Squibb in the country.

While such factors are operational, a report by Bain and Company recently reported that India's top 10 players have a robust pipeline comprising over 40 NCEs/ NBEs, signaling a strong push toward innovation[2].

4.3 Newer policy interventions can transform the innovation landscape

The Indian industry needs to be made relevant by expanding into the New Molecular Entities space, which accounts for 2/3rd of the global value pool, and where India currently has a small presence[3]. With the introduction of "Policy to Catalyse R&D and Innovation in the Pharma MedTech Sector in India", this scenario may undergo a transformation in the next decade[4]. The Indian pharmaceutical industry is the 3rd largest pharmaceutical industry in the world by volume, with a current market size of around USD 50 billion, estimated to grow to USD 120-130 billion over the next decade[4]. One of the key priority areas for research in the policy includes NCEs.

4.4 Potential opportunities for 3Rs – New Drugs and Clinical Trials Rules, 2019, and global regulatory requirements

With the rising trend toward innovation in the country in the coming years, it is anticipated that preclinical testing for NCEs and NBEs will also increase. Therefore, it is essential to identify potential opportunities for implementing the 3Rs within the New Drugs and Clinical Trials Rules, 2019[5], after comparing them with global regulatory frameworks such as the European Medicines Agency guidelines and reflection papers, the U.S. Food and Drug Administration's guidance documents, the comprehensive lists issued by the Center for Drug

Evaluation and Research (CDER), the recent FDA roadmap, and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines[6,7,8,9,10,11,12]. Considering that India has demonstrated a commitment to animal welfare through the recent amendment introduced to the New Drugs and Clinical Trials Rules (2023), there is also significant scope to incorporate further amendments to the NDCT Rules, 2019, to enable waiver and reduction opportunities for NCEs and NBEs[13].

4.5 Intensive growth of the Contract Research Organization (CRO) sector in India offers great potential for NAMs

The CRO sector in India is growing at a CAGR of 10.75% which is expected to reach USD 2.5 billion by 2030. The CRO sector is driven by specialized research and development service providers assisting pharmaceutical and biotechnology companies in drug discovery and development programs[14].

Global pharmaceutical and biopharmaceutical companies often outsource specific services to CROs to leverage their specialized expertise and infrastructure. There is thus a great potential in introducing non-animal methodologies at these CROs.

4.6 Methodology:

To identify potential opportunities for waivers, reduction, refinement, and replacement of animal use during the drug development process, a detailed assessment of non-clinical studies mandated under the New Drugs and Clinical Trials Rules (2019) was conducted. This evaluation was complemented by a comparative analysis with relevant international guidelines, including those from the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), U.S. FDA guidance documents, various roadmap documents, the recently released comprehensive list by CDER on animal-replacing and reducing approaches, as well as reflection papers issued by the European Medicines Agency[6-12].

4.7 Opportunity assessment for waiver/ reduction of animals/refinement

The key 3R opportunities for single and repeated dose toxicity studies are summarised in Table 1.

4.7.1 Single-dose toxicity studies

According to the NDCT Rules 2019, single-dose toxicity studies should be carried out in two rodent species (mice and rats) using the same route as intended for humans. In addition, unless the intended route of administration in humans is only intravenous, at least one more route should be used in one of the species to ensure systemic absorption of the drug.

Global regulatory requirements on single-dose toxicity studies

The ICH guidance for acute toxicity assessment, and the "Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals M3(R2)" [9,15] specify that data needed to assess acute toxicity can be obtained from repeated-dose toxicity studies. Similarly, the ICH Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals guideline (S6(R1)) indicates that acute toxicity information may be derived from study types other than single-dose or lethality studies[16].

The FDA Center for Drug Evaluation and Research (CDER) agrees that acute systemic toxicity data are not needed during the development of pharmaceuticals. The CDER, therefore, does not request nonclinical acute systemic toxicity data[9].

In Europe as well, following discussions within the Safety Working Party of the Committee for Medicinal Products for Human Use, it was agreed to remove the guideline on single-dose toxicity[17].

Table 1: Key 3R Opportunities in Single- and Repeated-Dose Toxicity Studies for NCEs/NBEs

S. No	Type of studies	Animal study objectives	Species	No.of Animals	References/ Guidelines	Key 3R Opportunities
1	Single-dose acute toxicity studies for NCEs	Acute systemic toxicity testing involves an assessment of the general toxic effects of a single or multiple doses of a chemical or product, within 24 hours by particular route (oral, dermal, inhalation) and that occur during a subsequent 14/21 day observation period.	Rodents Non Rodents	Rodent: 5/ sex/group Non Rodent: 1/ sex/group	ICH M3(R2): Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals	When appropriately conducted dose-escalation studies or short-duration dose-ranging studies are available to inform on the acute toxicity risk for small molecules, stand-alone, single-dose acute toxicity studies are not warranted.
2	4-13 weeks Repeat dose sub-chronic Toxicity studies with Toxicokinetics followed by a 2-week recovery period	To study the toxicity potential and Toxicokinetics profile of the drug when administered subchronically over a period of 13 weeks by the desired route (Oral/ Parenteral/Dermal). Also, to understand the reversibility of observed effects or the delayed onset of toxicity. These studies are aimed at determining target organs' drug exposure, kinetics, and determining NOAEL.	Rodents Non Rodents	Rodent: 10/sex/ group Non Rodent: 3/ sex/group	1. ICH M3(R2) 2. Guideline on repeated dose toxicity (CPMP/SWP/1042/99 Rev 1 Corr) 3. EMA/CHMP/ ICH/731268/1998: Guideline on preclinical safety evaluation of biotechnology–derived pharmaceuticals ICH S6(R1) 4. ICH guideline M3 (R2) – questions and answers EMA/CHMP/ ICH/507008/2011	One species could be acceptable on a case-by-case approach, if clearly justified. ICH guideline M3 (R2) - questions and answers document explains in detail the reasons where reversibility is not warranted. If a reversibility study is warranted, it is efficient to conduct it as part of a chronic study so that all toxicities of concern can be assessed in a single study
3	26-52 weeks repeat dose chronic Toxicity studies with Toxicokinetics followed by a 4-8 weeks recovery period (NCEs)	To study the toxicity potential and Toxicokinetics profile of the drug when administered chronically over a period of 26-52 weeks by the desired route (Oral/ Parenteral/Dermal). Also, to understand the reversibility of observed effects or the delayed onset of toxicity. These studies are aimed at determining target organs, drug exposure, kinetics, and to determine NOAEL.	Rodents Non Rodents	Rodent: 20/sex/ group Non Rodent: 4 /sex/group	ICH M3(R2)	Toxicokinetic and pharmacokinetic analyses may be conducted during general toxicity studies.

S. No	Type of studies	Animal study objectives	Species	No.of Animals	References/ Guidelines	Key 3R Opportunities
4	Repeat dose chronic Toxicity studies for NBEs	To study the toxicity potential and Toxicokinetics profile of the drug when administered chronically over a period of 6 months by the desired route	Rodents Non Rodents	Rodent: 20/sex/ group Non Rodent: 4 /sex/group	1. ICH S6 (R1) Guideline on preclinical safety evaluation of biotechnology–derived pharmaceuticals	If both rodents and non-rodents (nonhuman primates or NHPs) are pharmacologically relevant, use a single species if no differences are noted in short-term studies. Reversibility is not warranted

4.7.2 Repeated dose toxicity studies:

Repeat-dose toxicity studies are carried out to evaluate the adverse effects of compounds when administered to experimental animals repeatedly for a period. The duration of repeat-dose studies can be short (up to 4 weeks), sub chronic (up to 3 months), or chronic (longer than 3 months and usually up to 2 years).

As per NDCT Rules, 2019, these studies should be carried out in at least two mammalian species, of which one should be a non-rodent. Dose ranging studies should precede the 14-, 28-, 90-, or 180-day toxicity studies[5]. Duration of the final systematic toxicity study would depend on the duration, therapeutic indication, and scale of the proposed clinical trial. If a species is known to metabolise the drug in the same way as humans, it becomes pharmacologically relevant and should be investigated in toxicity studies.

4.7.2.1 Opportunities post assessment of global regulations:

Comparing different ICH guidelines and key reflection papers by EMA and CDER, FDA's comprehensive list, the following opportunities exist for repeat dose toxicity studies regarding:

1. Waiver

Repeat dose testing is not warranted for microdose radiopharmaceutical diagnostic drugs[18]. There is no need for additional general toxicity studies where a product significantly extends survival or clinical development is extended to oncology indications, borderline to ICH S9. Other toxicology studies should be conducted on a case-by-case basis[8].

For chronic toxicology studies for biologics, information on recovery can be obtained by an understanding that the effect observed is generally reversible/non-reversible; thus, inclusion of recovery animals is not necessarily warranted. When warranted, they only need to be included in one toxicology study[10].

2. Reduction opportunities

Short and long-term repeat-dose toxicology assessments of experimental therapeutic molecules are generally conducted in both a rodent and a non-rodent species. Exceptions can exist, particularly for certain biologics, and these can be applicable in India.

2.a. Restricting the number of species on a case-by-case basis

For biotherapeutic molecules that are pharmacologically active in both a rodent and a non-rodent species, ICH S6 (R1) provides an opportunity to reduce to a single species (preferably rodent), if scientifically justified, largely based on the similarity of toxicity findings identified in each of the two species from early, typically First In Human-enabling, toxicology studies[16,19].

The possibility of using only one species for longer-term repeated dose toxicity testing has also been investigated in a project led by NC3Rs involving 30 pharmaceutical companies, CROs, and regulatory bodies[8,19].

For small molecules and other oncology drugs, a 3-month study instead of a 6-month study in one species (rodent, if relevant) can be sufficient to support continued clinical development of a genotoxic drug targeting rapidly dividing cells (ICH S9 Q&A)[8].

2.b. Assessing pharmacological relevance

Studies involving monoclonal antibodies: Due to the high selectivity of biologics, often only one pharmacologically relevant species can be identified, which is usually the non-human primate (NHP)[19].

Anticancer immunotherapies: There are published examples of nonclinical programmes with no animal toxicology data, particularly for anti-cancer immunotherapies, where no relevant toxicology species were identified[20,21,22].

Other drug modalities like oligonucleotides: Some drug modalities, such as oligonucleotides and other peptides, span the small molecule and biologics space but often follow ICH M3(R2) guidance, and chronic studies are performed in two species, including a 9-month duration study for the non-rodent. Some oligonucleotides have successfully justified single-species programs based on pharmacological relevance, and more flexibility towards ICH S6(R1) rather than ICH M3(R2) approaches for these molecule types may be justified[23,24]. A new ICH guideline (ICH S13) on nonclinical safety studies for oligonucleotide-based therapeutics under development will harmonise non-clinical requirements for oligonucleotide-based therapies, taking 3Rs aspects into account[8].

For genotoxic drugs targeting rapidly dividing cells, a repeat dose toxicity study in one rodent species might be considered sufficient, provided the rodent is a relevant species (ICHS9 guidelines)[10].

2.c. Other examples (drug modality/study design/ metabolites):

Reduction - As per ICHS9 guidelines, there is no need for non-rodent studies for initiation of clinical trials with cytotoxic pharmaceuticals[8,22].

Disproportional metabolites: Disproportional metabolites are drug metabolites present in humans at concentrations significantly higher than in the animal models used for safety testing, or present only in humans. Additional toxicological studies with disproportional metabolites are not needed. They should only be considered if the metabolite is not produced in the test species and human exposure is high[8].

Antibody-drug-conjugate products: ADCs should be studied in at least one species. Studies on the mAb alone are not warranted. If additional characterisation of the payload is needed, it is sufficient to evaluate it in one species, preferably as a separate arm in the toxicology study of the ADC[8].

Other reduction opportunities: Besides restricting the number of species, reduction of animal numbers with one treated group and a control group can be accepted based on scientific justification, as in ICH S6 guidelines[8,16].

2.d. Combining studies with repeated dose toxicity studies to reduce animal usage:

ICH M3(R2) states that consideration should be given to the inclusion of any in vivo evaluations as additions to general toxicity studies, to the extent feasible, to reduce animal use[7,8,15].

These could include the following studies:

Genotoxicity studies: If genotoxicity endpoints are to be incorporated into a general toxicity study, then an appropriate maximum dose should be selected based on a Maximum Feasible Dose (MFD), Maximum Tolerated Dose (MTD), or limit dose of 1000 mg/kg/day.

Safety pharmacology studies: Animal use may be reduced by replacing standalone safety pharmacology studies with integrated toxicology studies, which is consistent with current ICH guidance (ICH, 2009).

Chronic toxicology studies for small molecule drugs (6-9 months; rodent and non-rodent, respectively): Toxicokinetic and pharmacokinetic analyses may be conducted during general toxicity studies[10,16].

For severely debilitating or life-threatening hematologic disorders: Endpoints may be integrated into pivotal toxicology studies. In the absence of a specific risk for patients in clinical trials, such studies will not be called for to support clinical trials or for marketing[25,26].

Local tolerance studies: It is preferable to evaluate local tolerance by the intended therapeutic route as part of the general toxicity studies; stand-alone studies are generally not recommended as per ICH M3(R2).

2.e. 3Rs opportunities

Some opportunities for 3Rs in repeat dose toxicity studies have also been identified in the latest EMA draft reflection paper, which can be incorporated in NDCT rules, 2019[8]:

- Expansion of the concept of integration of additional endpoints in repeated dose toxicity studies if equivalent safety information is supported by retrospective data analysis and/or when sufficient experience has been acquired.
- Reduction in the use of non-human primates (NHP) in regulatory testing is the subject of an EMA reflection paper[27].
- As per the EMA Non-clinical Domain Work plan[28], the development of a reflection paper on the current regulatory testing requirements for medicinal products for more streamlined non-clinical development includes specific guidance regarding duration of toxicity studies that will be considered for other severely debilitating and life-threatening diseases besides cancer.
- A methodology for using virtual control groups to reduce the number of animals in in vivo toxicity testing, the VICT3R project, is led by the eTransafe consortium[10,29].

3. Refinement – Shortening duration of study

3.a. Shortening duration of study from 9 to 6 months in non-rodents:

Small molecules and other drugs, such as oligonucleotides and peptides that generally follow ICH M3(R2) guidance, are usually required to provide data from two species (a rodent and a non-rodent) following dosing for 6 months in a rodent or 9 months

in a non-rodent. A dosing duration of 6 months is accepted for non-rodent data submitted within the EU, although this is not standard practice[8].

3.b. Shortening duration of toxicity studies for monoclonal Abs from 6 to 3 months

To support registration of monoclonal (mAbs) for chronic indications, 6-month toxicity studies have historically been conducted. Whether a 6-month toxicity study is necessary to assess the long-term safety of mAbs was evaluated by 11 companies that submitted data on First-in-Human (FIH)-enabling and chronic toxicity studies for 142 mAbs. Based on these results, the FDA roadmap highlights how the reduction of the routine 6-month primate toxicology testing for mAbs that show no concerning signals in 1-month studies, to 3 months, may be possible[30]. The FDA is also in agreement with adopting a data-driven paradigm (such as a weight-of-evidence model) to remove these extended animal studies for many mAbs[10].

3.c. FDA roadmap considerations for reduction in animal toxicity testing timeframes for other drug categories

The FDA has suggested it may implement a randomized study of new drugs of 3 months of animal testing augmented with AI vs. 6 months of animal testing with AI vs. 3 or 6 months of animal testing alone to evaluate the (human, animal, and economic) benefits and costs of this initiative[10].

3.d. For molecules intended for advanced cancer indications and severely debilitating diseases:

- Following ICH S9 guidance, dosing durations of 3 months in a rodent and a non-rodent species are generally considered sufficient[8,22].
- When the investigational pharmaceutical extends survival or lessens the severity or the frequency of a debilitating event, toxicology studies of six to nine months duration are generally not warranted[26].
- For an individualized antisense oligonucleotide, a single three-month toxicity study is considered adequate to assess safety for initiating human dosing, dose escalation, and chronic treatment[31,32]

4.7.3 Development and Reproductive Toxicology (DART) studies:

As per NDCT Rules, 2019, DART studies need to be carried out for all drugs proposed to be studied or administered to women of childbearing age. Segment I, II, and III studies (Table 2) are to be performed in albino mice or rats, and the segment II study should include albino rabbits also as a second test species. On the occasion, when the test article is not compatible with the rabbit (e.g., antibiotics which are effective against gram-positive, anaerobic organisms and protozoans), the Segment II data in the mouse may be substituted[5].

Table 2 showing Development and Reproductive Toxicology Studies as per NDCT Rules, 2019

S. No	Type of studies	Objective	Species	No.of Animals
1	Fertility and general reproductive performance assessment	To study male and female fertility and reproductive performance	Rats/ Mice	10/ sex/group
2	Teratology or Embryo-fetal toxicity Studies / Segment II	To study the toxicity potential of the drug to cause damage to the developing embryo	Rats/ Rabbits	20 Females/ Group
3	Prenatal and postnatal developmental toxicity study (PPND)/ Segment III	To evaluate drug effects during the last trimester of pregnancy and the period of lactation	Rats	20 Females/ Group

When compared to ICH S5R3 Guidelines, specific opportunities for 3Rs can be considered[7,8,33]:

1.a. Waiver of DART studies

DART studies may not be warranted if the WoE risk assessment suggests an adverse effect on fertility or pregnancy[10].

1.b. Reduction - Combining studies

- Use of the same animals in DART testing: The use of the same species and strain as used in already completed toxicity studies can eliminate the need to use additional animals or conduct additional studies to characterize pharmacokinetics and metabolism, and/or for dose range finding.
- Combination studies can be employed to assess all relevant stages of the reproductive process using fewer animals[10].

2. Fertility and Early Embryonic Development (FEED) studies for biopharmaceuticals:

Waiver of FEED studies:

FEED studies are not warranted in advanced cancer cases: Unless the biopharmaceutical is intended to treat advanced cancer, in which case FEED studies are not warranted, animals should be sexually mature at study initiation for an adequate evaluation of the reproductive tissues to be made. As per ICH S9 guidelines, there is no need for fertility studies[22].

For biologicals where NHP is the only pharmacologically relevant species, the histopathology of reproductive organs in sexually mature animals is sufficient. No dedicated FEED studies are required[8].

3. Segment II (Embryo Foetal Development (EFD) studies in one species):

Pharmacological relevance for biopharmaceuticals-Reduction: The effect of biopharmaceuticals on EFD should typically be assessed in two species (one rodent and one non-rodent) if both are pharmacologically relevant. However, the rodent is often not pharmacologically relevant, in which case EFD assessment in a single pharmacologically relevant non-rodent species can be conducted. Biopharmaceuticals intended for the treatment of advanced cancer typically need only be assessed in a single pharmacologically relevant species (ICH S9).

3.a. Waiver of EFD studies:

If there are no relevant species, genetically modified animals, or surrogates available, in vivo reproductive toxicity testing is not meaningful. In this case, the approach used for risk assessment, or rationale for not conducting studies, should be justified, or in cases where NHP is the only relevant species and WoE clearly indicates risk, an EFD study is not warranted (ICH S9).

EFD studies are not considered essential for pharmaceuticals that are genotoxic and target rapidly dividing cells or belong to a class that has been well characterized as causing developmental toxicity[10].

3.b. Reduction:

When the product is active in rodents and rabbits, sponsors should use these species (instead of NHPs) for EFD assessment. When a study is positive, a study in the second species is not warranted[10].

4. Pre and Post-Natal Development (PPND) Studies:

Waiver of PPND studies:

There is no need for pre-and post-natal development studies if an embryo-foetal development study is positive, and no confirmatory study in a second species is needed (ICH S9).

As per ICH S6(R1) guidelines, there is a reduction of the need for two separate studies (embryo-foetal development and peri-postnatal development studies). An enhanced pre-and postnatal development (ePPND) study can be conducted instead of separate EFD and PPND studies.

Use of a surrogate product to avoid the use of non-human primates, e.g., for reproductive toxicity testing, is possible only if necessary and scientifically justified.

For oligonucleotides, there can be cases where the WoE from existing data may be considered sufficient to communicate the risk to reproduction and embryofetal development, and no additional nonclinical data is required.

For Microdose radiopharmaceutical diagnostic drugs and therapeutics pharmaceuticals, DART may not be warranted10.

5. Waiver of male fertility studies

It is a requirement in the NDCT Rules, 2019, but not mentioned in the ICHS5R3 guidelines[27].

6. Other implemented reduction opportunities:

As per the latest draft EMA reflection paper[8]:

- Defer definitive in vivo testing as part of an integrated testing strategy to support limited inclusion of women of childbearing potential (WOCBP) in clinical trials.
- Inclusion of an exposure-based endpoint for dose-selection.

7. Opportunities for 3R implementation include:

a. Maintenance procedure ICH S5 (R4): Potential updates to Annex 1 to provide additional guidance on in vivo study designs, including:

- Reduction and refinement opportunities.
- Use of the minipig study as a possible replacement for NHP, if sufficient data is available.

4.7.4 Carcinogenicity

Possible introductions in NDCT Rules, 2019 could be the following:

Waiver

- Carcinogenicity studies are not generally needed for endogenous substances given as replacement therapy (i.e., physiological levels). Pharmaceuticals administered infrequently or for a short duration of exposure (e.g., anesthetics and radiolabeled imaging agents) do not need carcinogenicity studies unless there is cause for concern.
- The two-year study in rats may be substituted with a weight-of-evidence risk assessment, which may inform whether a two-year rat study is needed.

Reduction

- An alternative to a two-year mouse study may be a six-month study in transgenic strains of mice, which typically employs fewer animals[34,35,36].

4.7.5 Local toxicity—NDCT Rules, 2019

These studies are required when the new drug is proposed to be used by some special route (other than oral) in humans. Typical study designs for these studies should include three dose levels and an untreated or vehicle control, preferably use of two species, and increasing group size with an increase in duration of treatment. While the NDCT Rules, 2019 include using alternatives for skin and eye before initiating experiments on animals, specific opportunities can be incorporated from EMA guidelines[37], which are:

- **Emphasis on 3Rs:** It is recommended that if animal studies are necessary for an evaluation of local tolerance by the intended clinical route of administration, such an evaluation is included as part of the general toxicity studies whenever possible, and not as a "stand-alone" study.
- **Inclusion of all in vitro methods:** Consideration should be given to internationally validated and regulatory accepted OECD methods, as well as internationally validated methods not yet included in OECD test guidelines. In vitro methods that have not undergone international validation could be considered, if scientifically justified, on a case-by-case basis in a specific context of use.
- **Animal welfare considerations for refinement:** In cases where unexpected local intolerance of animal welfare occurs, the experiment should be terminated before the point where severe adverse reactions are seen and/or the continuation of the experiment is not expected to provide results essential for risk assessment.
- **To be implemented 3R opportunities include:** Guideline on non-clinical local tolerance testing (EMA/CHMP/SWP/2145/2000-Rev.1) is to be revised, where 3Rs aspects will be considered. Newly identified opportunities for consideration include[8]:
  - Increased emphasis on in vitro testing, especially on skin and eye irritation.
  - Integration of NAMs for assessing skin sensitisation potential of topically applied pharmaceuticals in accordance with OECD TG 442C, 442D, 442E, and 497.

4.7.6 Phototoxicity

As per NDCT Rules, 2019, phototoxicity should be tested by the Armstrong or Harber test in a guinea pig. This test should be done if the drug or a metabolite is related to an agent causing photosensitivity or the nature of action suggests such a potential (e.g., drugs to be used in the treatment of uroparma). ICH Guideline S10 suggests that a validated in vitro method should generally be considered before conducting animal testing (see, for example, Directive 2010/63/EU). If the drug developer chooses an in vitro approach, the 3T3 NRU-PT is currently the most widely used assay and in many cases, could be considered as an initial test for phototoxicity. The high sensitivity of the 3T3 NRU-PT results in good negative predictivity and the negative results are generally accepted as sufficient evidence that a substance is not phototoxic. In such cases, no further testing is recommended and no direct phototoxicity is anticipated in humans. In some situations (e.g., poorly soluble compounds), an initial assessment of phototoxicity in an in vitro assay might not be appropriate. In this case, an assessment in animals or in humans could be considered. Alternatively, if drug distribution data is available, it could, on a case-by-case basis, support a decision that no further photosafety assessment is warranted[38].

A Guidance Document (GD) on Integrated Approaches to Testing and Assessment (IATA) has been developed for Phototoxicity Testing. The document introduces an Adverse Outcome Pathway (AOP) for Phototoxicity, an overview of available in vitro/in silico tests, and a decision tree[8].

4.8 Key opportunities for replacement are described in the reflection paper on non-animal methodologies[8]:

- **Pharmacokinetics:**
  1. Use of standard in vitro models for comparison of in vitro metabolism and protein binding across species (including human).
  2. Non-clinical evaluation of drug-drug interactions is limited to in vitro investigations as per ICH guideline M12 on drug interaction studies (EMA/CHMP/ICH/652460/2022).
- **Newly identified opportunities for 3Rs include:**

  Complex in vitro systems like Organ-on-Chip (OoC) have been developed for ADME and could potentially be used to generate data for a regulatory submission or a qualification procedure.

- **Safety pharmacology:**

  1. Integrated test strategy including in vitro tests (e.g., Herg assay) for assessment of QT interval prolongation (ICH S7B). Increased predictive capacity of in vitro assays through implementation of best practices for in vitro IKR/ Herg assays, in vitro ventricular repolarisation assays (e.g., human induced pluripotent stem cell derived cardiomyocyte assays).

  2. EMA is developing regulatory acceptance criteria for the qualification of 3Rs proarrhythmic models, including organ-on-chip models, for use as part of an integrated risk assessment for regulatory purposes. This will be included in the Guideline on the principles of regulatory acceptance of 3Rs testing approaches[39].

  3. Description of generic principles for evaluating the predictivity of proarrhythmic risk prediction models. This can encompass in silico, in vitro, ex vivo, and, where needed, in vivo models.

  4. In vitro assays of anti-tumor activity could be sufficient for characterization of pharmacology, if they generate relevant data (ICHS9).

  5. The ICH E14/S7B Implementation Working Group (IWG) has been reactivated in March 2024 to develop second-round Q&As for the ICH E14 and ICH S7B Guidelines. These will address any outstanding gaps not captured in the first round of Q&As, which were finalised in February 2022. Issues to be resolved with a 3Rs impact are related to

- Clarification of modalities in the scope of ICH S7A, with consideration for assessment needs in line with the pharmacokinetic and dynamic profile of the test material, and aiming for consistency with related ICH guidelines (ICH S7A and ICH S6).
- Clarification of requirements for general toxicology studies with integrated safety pharmacology endpoints when used to inform an ICH E14/S7B integrated risk assessment.
- Clarification of requirements to assess QTc and proarrhythmic liability of novel modalities.
- **Development and reproductive toxicity:**

  Alternative Approaches for Addressing EFD Risk [7,8, 33]

  Several alternative in vitro, ex vivo, and non-mammalian in vivo assays (alternative assays) have been developed to detect potential hazards to embryo-fetal development. They have been used as drug discovery screens for adverse effects on EFD and have assisted in the understanding of the mechanism of toxicity, which can be useful for translating nonclinical data to human risk (especially for human-specific targets). If properly qualified, alternative assays have the potential to defer or replace (in certain circumstances) conventional in vivo studies. ICH S5(R3) Annex 2 identifies where potential use of alternative assays may be possible, including specific scenarios and qualification criteria for NAMs and reference compound lists.

- **Other replacement opportunities include**[8]:

  - Update of qualification criteria based on acquired regulatory experience, update of scenarios under which NAMs can be applied
  - Update of reference compound list, EMA initiatives
  - As per the Non-clinical Domain Work plan, an EMA reflection paper on NHPs is being developed, affecting reproductive toxicological testing.

4.9 Overall recommendations for the implementation of 3Rs in regulated testing of New Chemical and Biological Entities in India

1. Considering key global regulatory changes, single-dose toxicity studies may not be required to be carried out independently and can be part of repeated dose toxicity studies in India. Thus, there could be a provision of waiving off such studies when multi-dose studies have been planned.

2. Based on currently existing regulations and recent studies, use of a single species for different modalities could provide opportunities for potential adoption, which currently fall outside the remit of repeat dose toxicity studies under the NDCT Rules, 2019.

3. It is important to bring industry and regulators together via workshops and meetings to deliberate on opportunities for 3Rs in the current NDCT Rules, 2019. This effort could begin with the formation of an industry consortium to gather consensus on key opportunities for 3Rs and then approach regulators.

4. To accelerate progress in this area, industry champions should be identified who assume responsibility for identifying novel methods and approaches that have the potential to be accepted by regulators. The champions would then lead a group comprising a number of companies that are abreast of the latest science and would provide a consensus opinion on appropriate practice. This group would also provide a link between industry and regulators to enable greater interaction and data-sharing between the two groups.

5. Post deliberations at a workshop comprising key industry players and regulators would recommend amendments to the NDCT rules, which could be envisioned in the form of a circular.

References:

1. *Kumar S. Innovative Drug Discovery Research by Pharmaceutical Companies in India and China. J Med Chem. 2024 June 27;67(12):9773–5.*
2. *Healing the World: A Roadmap for Making India a Global Pharma Exports Hub | Bain & Company [Internet]. [cited 2025 Dec 10]. Available from: https://www.bain.com/insights/healing-the-world-a-roadmap-for-making-india-a-global-pharma-exports-hub/*
3. *Sudarshan Jain, Indian Pharmaceutical Alliance, Catalyzing R&D: Industry-Academia Collaboration [Internet]. [cited 2025 Dec 10]. Available from: https://www.education.gov.in/sites/upload_files/mhrd/files/nep/TS9_Catalyzing_IAC_SJ.pdf*
4. *Dr Mansukh Mandaviya launches National Policy on Research and Development and Innovation in Pharma-MedTech Sector in India and Scheme for promotion of Research and Innovation in Pharma MedTech Sector (PRIP) [Internet]. [cited 2025 Dec 10]. Available from: https://www.pib.gov.in/www.pib.gov.in/Pressreleaseshare.aspx?PRID=1960812*
5. *THE NEW DRUGS AND CLINICAL TRIALS RULES, 2019 [Internet]. [cited 2025 Dec 10]. Available from: https://cdsco.gov.in/opencms/resources/UploadCDSCOWeb/2022/new_DC_rules/NEW%20DRUGS%20ANDctrS%20RULE,%202019.pdf*
6. *Non-clinical guidelines | European Medicines Agency (EMA) [Internet]. 2009 [cited 2025 Dec 10]. Available from: https://www.ema.europa.eu/en/human-regulatory-overview/research-development/scientific-guidelines/non-clinical-guidelines*
7. *Reflection paper providing an overview of the current regulatory testing requirements for medicinal products for human use and opportunities for implementation of the 3Rs. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/reflection-paper-providing-overview-current-regulatory-testing-requirements-medicinal-products-human-use-and-opportunities-implementation-3rs-first_en.pdf*
8. *Draft Reflection paper on the current regulatory testing requirements for medicinal products for human use and opportunities for implementation of the 3Rs [Internet]. [cited 2025 Dec 10]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/draft-reflection-paper-current-regulatory-testing-requirements-medicinal-products-human-use-opportunities-implementation-3rs-revision-1_en.pdf*
9. *Strickland J, Clippinger AJ, Brown J, Allen D, Jacobs A, Matheson J, et al. Status of acute systemic toxicity testing requirements and data uses by U.S. regulatory agencies. Regul Toxicol Pharmacol. 2018 Apr;94:183–96.*
10. *Replacing and Reducing Animal Testing at CDER | FDA [Internet]. [cited 2025 Dec 10]. Available from: https://www.fda.gov/about-fda/center-drug-evaluation-and-research-cder/replacing-and-reducing-animal-testing-cder*
11. *Roadmap to Reducing Animal Testing in Preclinical Safety Studies. [cited 2025 Dec 10]; Available from: https://www.fda.gov/files/newsroom/published/roadmap_to_reducing_animal_testing_in_preclinical_safety_studies.pdf*
12. *ICH Official web site : ICH [Internet]. [cited 2025 Dec 10]. Available from: https://www.ich.org/page/ich-guidelines*
13. *Parvatam S. India takes first step to remove animals from drug-testing process. The Hindu [Internet]. 2023 June 29 [cited 2025 Dec 10]; Available from: https://www.thehindu.com/sci-tech/science/animal-trials-drug-development-india-amendment-2019-rules/article67019288.ece*
14. *Patial V. STUDY ON CRO SECTOR IN INDIA Conducted by Department of Pharmaceuticals Ministry of Chemicals & Fertilizers Government of India AUGUST, 2023. Available from: https://pharma-dept.gov.in/sites/default/files/CRO%20Market%20Report_High%20Resolution.pdf*
15. *GUIDANCE ON NONCLINICAL SAFETY STUDIES FOR THE CONDUCT OF HUMAN CLINICAL TRIALS AND MARKETING AUTHORIZATION FOR PHARMACEUTICALS M3(R2). In [cited 2025 Dec 10]. Available from: https://database.ich.org/sites/default/files/M3_R2__Guideline.pdf*
16. *PRECLINICAL SAFETY EVALUATION OF BIOTECHNOLOGY-DERIVED PHARMACEUTICALS S6(R1). In [cited 2025 Dec 10]. Available from: https://database.ich.org/sites/default/files/S6_R1_Guideline_0.pdf*
17. *Questions and answers on the withdrawal of the 'Note for guidance on single dose toxicity, 24 June 2010 EMA/CHMP/SWP/81714/2010 Committee for Medicinal Products for Human Use (CHMP), European Medicines Agency [Internet]. [cited 2025 Dec 10]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/questions-answers-withdrawal-note-guidance-single-dose-toxicity_en.pdf*
18. *Research C for DE and. Microdose Radiopharmaceutical Diagnostic Drugs: Nonclinical Study Recommendations [Internet]. FDA; 2020 [cited 2025 Dec 10]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/microdose-radiopharmaceutical-diagnostic-drugs-nonclinical-study-recommendations*
19. *Prior H, Baldrick P, Beken S, Booler H, Bower N, Brooker P, et al. Opportunities for use of one species for longer-term toxicology testing during drug development: A cross-industry evaluation. Regul Toxicol Pharmacol. 2020 June;113:104624.*
20. *Immunocore pioneers new safety studies [Internet]. [cited 2025 Dec 10]. Available from: https://www.mednous.com/system/files/Immunocore.Apr%202011%20pdf.pdf*
21. *read NB• 10 min. Achilles On Getting 'New Wave' Immunotherapy From Concept To Clinic In Three Years [Internet]. Insights. 2019 [cited 2025 Dec 10]. Available from: https://insights.citeline.com/PS124934/Achilles-On-Getting-New-Wave-Immunotherapy-From-Concept-To-Clinic-In-Three-Years/*
22. *NONCLINICAL EVALUATION FOR ANTICANCER PHARMACEUTICALS. In [cited 2025 Dec 10]. Available from: https://database.ich.org/sites/default/files/S9_Guideline.pdf*
23. *Tessier Y, Achanzar W, Mihalcik L, Amuzie C, Andersson P, Parry JD, et al. Outcomes of the European Federation of Pharmaceutical Industries and Associations Oligonucleotide Working Group Survey on Nonclinical Practices and Regulatory Expectations for Therapeutic Oligonucleotide Safety Assessment. Nucleic Acid Ther. 2021 Feb;31(1):7–20.*
24. *Prior H, Baldrick P, Clarke DO, Passini E, Sewell F, van Meer P. Exploring Greater Flexibility for Chronic Toxicity Study Designs to Support Human Safety Assessment While Balancing 3Rs Considerations. Int J Toxicol. 2024;43(5):456–63.*
25. *Commissioner O of the. Graft-versus-Host Diseases: Developing Drugs, Biological Products, and Certain Devices for Prevention or Treatment [Internet]. FDA; 2023 [cited 2025 Dec 10]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/graft-versus-host-diseases-developing-drugs-biological-products-and-certain-devices-prevention-or*
26. *Research C for DE and. Severely Debilitating or Life-Threatening Hematologic Disorders: Nonclinical Development of Pharmaceuticals Guidance for Industry [Internet]. FDA; 2020 [cited 2025 Dec 10]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/severely-debilitating-or-life-threatening-hematologic-disorders-nonclinical-development*
27. *Reflection paper on non-human primates in safety testing of human medicinal products and opportunities for 3Rs implementation. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/reflection-paper-non-human-primates-safety-testing-human-medicinal-products-opportunities-3rs-implementation_en.pdf*
28. *Consolidated 3-year rolling work plan for the Non-clinical domain [Internet]. [cited 2025 Dec 10]. Available from: https://www.ema.europa.eu/en/documents/other/consolidated-3-year-rolling-work-plan-non-clinical-domain-2026-2028_en.pdf*
29. *Wright PSR, Smith GF, Briggs KA, Thomas R, Maglennon G, Mikulskis P, et al. Retrospective analysis of the potential use of virtual control groups in preclinical toxicity assessment using the eTOX database. Regul Toxicol Pharmacol. 2023 Feb;138:105309.*
30. *Chien HT, Prior H, Andrews L, van Aerts L, Cauvin A, Clarke DO, et al. Re-evaluating the need for chronic toxicity studies with therapeutic monoclonal antibodies, using a weight of evidence approach. Regul Toxicol Pharmacol. 2023 Feb;138:105329.*
31. *Research C for DE and. Nonclinical Testing of Individualized Antisense Oligonucleotide Drug Products for Severely Debilitating or Life-Threatening Diseases Guidance for Sponsor-Investigators [Internet]. FDA; 2021 [cited 2025 Dec 10]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/nonclinical-testing-individualized-antisense-oligonucleotide-drug-products-severely-debilitating-or*
32. *Research C for DE and. Nonclinical Safety Assessment of Oligonucleotide-Based Therapeutics [Internet]. FDA; 2024 [cited 2025 Dec 10]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/nonclinical-safety-assessment-oligonucleotide-based-therapeutics*
33. *DETECTION OF REPRODUCTIVE AND DEVELOPMENTAL TOXICITY FOR HUMAN PHARMACEUTICALS S5(R3) [Internet]. [cited 2025 Dec 10]. Available from: https://database.ich.org/sites/default/files/S5-R3_Step4_Guideline_2020_0218.pdf*
34. *Research C for DE and. S1B Testing for Carcinogenicity of Pharmaceuticals [Internet]. FDA; 2020 [cited 2025 Dec 10]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/s1b-testing-carcinogenicity-pharmaceuticals*
35. *Research C for DE and. S1B(R1) Addendum to S1B Testing for Carcinogenicity of Pharmaceuticals [Internet]. FDA; 2022 [cited 2025 Dec 10]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/s1br1-addendum-s1b-testing-carcinogenicity-pharmaceuticals*
36. *Research C for DE and. S1A The Need for Long-term Rodent Carcinogenicity Studies of Pharmaceuticals [Internet]. FDA; 2021 [cited 2025 Dec 10]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/s1a-need-long-term-rodent-carcinogenicity-studies-pharmaceuticals*
37. *Guideline on non-clinical local tolerance testing of medicinal products. Available from: www.ema.europa.eu/en/documents/scientific-guideline/guideline-non-clinical-local-tolerance-testing-medicinal-products_en.pdf*
38. *PHOTOSAFETY EVALUATION OF PHARMACEUTICALS S10. In [cited 2025 Dec 10]. Available from: https://database.ich.org/sites/default/files/S10_Guideline.pdf*
39. *Concept paper on the revision of the Guideline on the principles of regulatroy acceptance of 3Rs (replacement, reduction, refinement) testing approaches(EMA/CHMP/CVMP/JEG-3Rs/450091/2021 [Internet]. [cited 2025 Dec 10]. Available from: https://assets-us-01.kc-usercontent.com/49d837e7-4c3e-0071-59f0-cb4344fbf121/ba67af0c-9723-4e2e-b025-c21d924e9c10/concept-paper-revision-guideline-principles-regulatory-acceptance-3rs-replacement-7-reduction_en.pdf*

Opportunity Assessment for 3Rs in Cell and Gene Therapy (CGT) Regulatory Frameworks

5

5.1 Executive Summary

India has significantly strengthened its regulatory landscape for cell and gene therapy (CGT) over the past decade, including reforms such as streamlined approval pathways, dedicated oversight committees, and faster review timelines. A comparative review of India's 2019 National Gene Therapy Guidelines and the European Medical Agency's 2025 Advanced Therapy Medicinal Products (ATMP) guidelines identifies multiple 3Rs opportunities to reduce reliance on animals in CGT in India. These include stronger support for in vitro models, combining toxicology and PK studies, conditional use of large animals, allowance of single-species toxicology, case-by-case evaluation of necessity of additional studies, and waivers for repeat-dose toxicity and safety pharmacology.

5.2 Global scenario

The global CGT market size is USD 25.03 billion in 2025, forecasted to reach around USD 117.46 billion by 2034, accelerating at a Compound Annual Growth Rate (CAGR) of 18.7%[1]. Various factors have been attributed to this market growth, including opportunities for advanced therapies in different disease areas, gene delivery technologies, progressive competition, and emerging key players commercialising such therapies.

5.3 India's transforming regulatory framework with respect to Cell and Gene Therapies

The Government of India started the cell therapy initiative in India almost a decade ago[2]. An initiative to accelerate the application of stem cell technology in human diseases was published in 2016. Following this, 2017 saw the publication of revised National Ethical Guidelines for Biomedical and Health Research, and Regulations and Guidelines for Recombinant DNA Research and Biocontainment. National Guidelines on Gene Therapy Product Development and Clinical Trials were published by the Government of India along with New Drugs & Clinical Trial rules in 2019[3].

A dedicated protocol is followed for gene therapies in India. First, approval is required from the Institutional Biosafety Committee, followed by a test license No Objection Certificate (NOC) from the central and local state authority. The Review Committee on Genetic Manipulation (RCGM) then reviews the preclinical application, followed by clinical trials. For new developers seeking advice on clinical development, the Gene Therapy Advisory Evaluation Committee may be approached, which also provides an NOC before initiating clinical trials. Post completion of clinical phases, a marketing authorization application is submitted to the Central Drugs Standard Control Organization (CDSCO), which conducts inspections and issues a test license, followed by a manufacturing license. The RCGM under the Department of Biotechnology handles biosafety and animal studies, while the Indian Council of Medical Research under the Ministry of Health & Family Welfare issues NOCs for trials.

India is currently making remarkable progress towards transforming its regulatory approach to CGT by approving applications more quickly, with the underlying recognition that rapidly approving therapies would engage more industry stakeholders[4]. The government has been supportive of CGT through policy engagement, regulations, and incentives such as separating CGT from other CDSCO branches.

This transformation is occurring in parallel with a growing trend towards domestic innovation, such as India's approval of its first indigenous CAR-T cell therapy, NexCAR19, by ImmunoAct. At the same time, there is increased foreign interest such as from Miltenyi Biotec, a German biotech company with a focus on CGT products[5]. A recent clinical study from the Center for Stem Cell Research, Tamil Nadu used gene therapy with lentiviral-transduced autologous cells and showed promising results in restoring Factor VIII production in haemophilia-A patients. Similarly, another indigenous therapy for sickle cell anaemia with CRISPR-Cas is progressing[6].

CDSCO has formally completed 11 reviews of CAR-T applications to date, and is presently converting CGT submissions to electronic submissions on its SUGAM portal, an online platform where applicants can apply for registration licenses and certificates[7]. Considering a forecasted increase in development of such therapies, guidelines in India need to be in accordance with global regulations to incorporate a better 3Rs framework. The CDSCO is also drafting new guidelines for CGT[8].

Methodology: A detailed assessment of the National Guidelines on Gene Therapy Product Development and Clinical Trials was carried out to identify opportunities for waiver, reduction and replacement of animals. Comparative assessment was done with the recently released Guideline on Quality, Non-Clinical, and Clinical Requirements for Investigational Advanced Therapy Medicinal Products in Clinical Trials by the EMA[9].

5.4 Global regulatory requirements

The Guideline on Quality, Non-Clinical, and Clinical Requirements for Investigational Advanced Therapy Medicinal Products in Clinical Trials was recently released by the EMA. These guidelines present the following opportunities which can be incorporated into currently existing regional guidelines for a better 3Rs framework[9]:

Case-by-case basis evaluation of non-clinical program: As per EMA guidelines, the non-clinical program can be determined on a case-by-case basis depending on the type of respective ATMP, availability of appropriate non-clinical models, and intended clinical use. The extent and duration of exposure to the investigational ATMP also affects the extent of the non-clinical program. For example, if the product is administered locally and/or kept isolated by physical or biological means, the need for evaluation of systemic effects is reduced. This risk-based approach may be applied to identify the necessary non-clinical data on a case-by-case basis.

Table 1 highlights the key 3R opportunities post-comparison of National Guidelines for Gene Therapy Product Development and Clinical Trials 2019 with EMA Guidelines on ATMP 2025.

5.5 Key recommendations to fast track 3R opportunities in this sector would include:

Sharing these opportunities with key academicians, CDSCO, and ICMR to incorporate such recommendations in the Revised Guidelines on Cell and Gene Therapy to be released soon by the CDSCO.

Table 1: Key 3R opportunities post-comparison with National Guidelines for Gene Therapy Product Development and Clinical Trials, 2019 and EMA Guidelines, 2025

Key points	Key recommendations/ opportunities for 3R	National Guidelines for Gene Therapy Product Development and Clinical Trials, 2019	EMA Guidelines, 2025
Selection of non-clinical models while considering the 3Rs	Replacement/ Reduction: Stronger language in favour of 3Rs for *in vitro* technologies can be adopted, in accordance with EMA guidelines.	In the event that no pharmacologically relevant *in vivo* disease models are available, the GTP can be evaluated by combination studies in suitable *in vitro* disease models and in normal strains of animals based on evidence in literature, on a case by case basis, (10.2.1.4)	In vivo animal studies should be carefully planned to ensure generation of robust and meaningful data while considering the 3Rs (reduction, replacement, refinement) principles. Where appropriate, animal testing should be replaced by *in vitro, ex vivo or in silico* studies or a combination thereof. (5.2)

Key points	Key recommendations/ opportunities for 3R	National Guidelines for Gene Therapy Product Development and Clinical Trials, 2019	EMA Guidelines, 2025
Combining toxicology and pharmacoki-netic (PK) studies	Reduction: Combining toxicology and PK studies should be preferred, as per EMA guidelines, and can be adopted in the current National Guidelines/new CGT guidelines.	Not clearly defined in the current guidelines	It is supported to combine relevant safety endpoints and biodistribution analysis in a proof-of-concept study. Safety data can be collected in toxicology studies as well as in proof-of-concept studies conducted in the disease model(s) provided that adequate safety endpoints are included. *In silico*, in vitro and/or *ex vivo* data can be used to substitute or supplement *in vivo* animal data. The overall safety evaluation should take into account cell persistence and biodistribution data. The use of the same animal model in both the toxicology investigations and the pharmacokinetic studies may be beneficial, as it allows correlation of the biodistribution of the investigational ATMP with observed toxicity signals.
Preference for small animals over large animals	Reduction: EMA guidelines have a conditional rationale for including large animals when extrapolation from small animal models becomes challenging, while as per National Guidelines both small and large animals should be included.	10.2.2.3. Pharmacokinetics, dose-response-If the GTP is a first of its kind developed either for preclinical or clinical use, further delineation of biologically active and relevant dose range should be done, wherever possible in multiple animal models, preferably including both small and large animals". 10.2.1.5. For a GTP that is under pre-clinical evaluation for a first in clinical use, a tiered preclinical testing paradigm involving different assays for efficacy / toxicity documentation and both small and large animal models (if available) should be employed to characterize the functional attributes of the GTP.	Small animal models such as rodents are often useful and widely employed since they are readily available and easy to manipulate. However, if extrapolation from small animal models to human becomes challenging due to e.g. differences in the body size and anatomy that may preclude certain administration procedures and devices in small animal models, large animal models may be needed.

Key points	Key recommendations/ opportunities for 3R	National Guidelines for Gene Therapy Product Development and Clinical Trials, 2019	EMA Guidelines, 2025
Preference for single species for toxicology studies:	Conditional basis for conducting animal studies in one species does not exist in the National Guidelines.	Not mentioned in the current guidelines	In the case that animal studies are conducted, one animal species is sufficient if the model is considered predictive. However, multiple animal species or strains may be needed to cover all relevant safety aspects on a case-by-case basis. Both sexes should be included unless justified.
Additional toxicology studies	Reduction: Additional toxicology studies are to be evaluated on a case-to-case basis, as per EMA guidelines, while such studies are to be conducted as per National Guidelines.	If there are differences between the preclinical and clinical vectors, or their production process, additional toxicity studies pertaining to clinical grade vectors may be required. (10.2.5.2)	The need for additional toxicity studies e.g. genotoxicity, tumourigenicity, reproductive and developmental toxicity, and immunotoxicity studies should be determined on a case-by-case basis taking into consideration the risks related to the nature and characteristics of the particular class of ATMPs and the intended clinical use • **Genotoxicity studies:** Standard genotoxicity assays are generally not appropriate for ATMPs. • **Tumourigenicity:** Standard lifetime rodent carcinogenicity studies are usually not required. However, depending on the type of product, the tumourigenic and oncogenic potential shall be investigated in relevant *in vitro/in vivo* models for neoplasm signals, oncogene activation or cell proliferation index. • **Reproductive and developmental toxicity:** If effects on reproductive function and/or development are anticipated, relevant reproductive and developmental toxicity studies should be conducted before exposing larger patient populations.

Key points	Key recommendations/ opportunities for 3R	National Guidelines for Gene Therapy Product Development and Clinical Trials, 2019	EMA Guidelines, 2025
Conditional waiver of repeat dose toxicity	Reduction: Conditional waiver of repeat dose toxicity can be included in the National Guidelines, depending on the dosing interval or where single dosing is anticipated.	Not mentioned in the current guidelines	Repeat-dose toxicity data are needed to support multiple administrations in humans. However, a clinical study with multiple administrations could be initiated without repeat-dose toxicity data provided that such data are available before multiple dosing in humans commences. Omission of repeat-dose toxicity studies may be justifiable if the investigational ATMP has been eliminated from the body before subsequent administrations (e.g., if the dosing interval is very long).
Conditional basis for safety pharmacology studies	Reduction: Conditional basis for conducting safety pharmacology studies are part of EMA guidelines but not National Guidelines.	While Section 10.2.1 states that, selection of a suitable non-human model system appropriate for testing – in vivo and in vitro models; safety pharmacology requirements are not clearly defined in the current guidelines	Safety pharmacology data are not routinely needed for investigational ATMPs. When potential effects on major vital physiological functions i.e. cardiovascular, central nervous system, or respiratory function are anticipated, appropriate safety pharmacology data should be available before human exposure. Safety pharmacology endpoints can be incorporated in the proof-of-concept and /or toxicity studies, if feasible.

References:

1. *Cell and Gene Therapy Market Size to Surpass USD 39.61 Bn By 2034. Available from: https://www.precedenceresearch.com/cell-and-gene-therapy-market*
2. *Pandit KR, Nisar A. Regulatory framework for developing cell & gene therapy products in india. Cytotherapy. 2024 June 1;26(6, Supplement):S186.*
3. *Sinha S, Ghosh A, Rao JG, Jotwani G, Kharkwal G. National Guidelines for Gene TherapyProduct Development and Clinical Trials. New Delhi: Indian Council of Medical Research. 2019.*
4. *Gene Therapy Guidelines: New guidelines for CGTs under process, draft to be out by year-end, ETPharma. Available from: https://pharma.economictimes.indiatimes.com/news/policy-and-regulations/new-guidelines-for-cgts-under-process-draft-to-be-out-by-year-end/123766306*
5. *Miltenyi Biotec opens its first office in India and plans to invest in first of its kind Cell and Gene Therapy Center of Excellence | Miltenyi Biotec | Singapore. Available from: https://spa-sandbox-mithila.miltenyibiotecdev.com/SG-en/about-us/newsroom/press-releases/miltenyi-biotec-opens-its-first-office-in-hyderabad-india-and-plans-invest-in-first-of-its-kind-cgt-coe-for-indian-academia-and-industry.html*
6. *Gopurappilly LP and R. Driving innovation: India's foray into gene and cell therapies. The Economic Times. 2024 Dec 27; Available from: https://economictimes.indiatimes.com/small-biz/entrepreneurship/driving-innovation-indias-foray-into-gene-and-cell-therapies/articleshow/116702561.cms?from=mdr*
7. *Ames Gross. India To Overhaul Cell and Gene Therapy Regulatory Approach [Internet]. 2025. Available from: https://www.pacificbridgemedical.com/news-brief/india-to-overhaul-cell-and-gene-therapy-regulatory-approach/*
8. *New guidelines for CGTs under process, draft to be out by year-end. Available from: https://pharma.economictimes.indiatimes.com/news/policy-and-regulations/new-guidelines-for-cgts-under-process-draft-to-be-out-by-year-end/123766306*
9. *Europeans Medicines Agency. Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials. 2025. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-quality-non-clinical-clinical-requirements-investigational-advanced-therapy-medicinal-products-clinical-trials_en.pdf*

Opportunity Assessment for Integration of Non-Animal Methodologies (NAMs) in Toxicity Testing and Drug Development

6

6.1 Executive Summary

The use of animal models has been traditionally accepted for testing of new drugs. However, globally, latest regulations encourage the use of non-animal methodologies (NAMs) for drug testing quoting the frequent ineffectiveness of animal models in replicating human physiology. The growth of the NAMs market is currently surging, with spin offs from academic institutions driving the adoption. This chapter collates different NAMs for toxicity testing for major organ systems, for genotoxicity testing, mAb testing and applications in rare diseases and cancer. We also stress upon the use of in silico methods for drug development. Although the research on NAMs is picking up in India, we identified several challenges in large scale adoption. Based on our discussions with different stakeholders, we put forth key recommendations for encouraging NAMs adoption in the Indian context.

6.2 Background

Animal testing has been the mainstay of non-clinical drug discovery and development for predicting the efficacy and safety of drug candidates prior to their administration in human clinical trials and eventual use in real-world clinical settings. Both rodent and non-rodent models are used to assess systemic as well as tissue-specific toxicity, efficacy and pharmacokinetics (PK) of a particular drug1. However, despite the rigorous and systematic nature of these non-clinical studies, the current rates of failure in accurately predicting the clinical outcomes is very high, with a substantial number of drug candidates demonstrating unanticipated toxicities or lack of efficacy when tested in human populations[2,3,4]. Data suggests that approximately 10% of drug development projects make it from clinical phase 1 to market approval[5,6,7]. This results in delays in the delivery of safe and effective therapies to patients[8,9,10].

The adoption of Non-Animal Methods (NAMs) is revolutionizing the field of drug discovery and toxicity assessment by offering more human-relevant, ethical and scientifically advanced alternatives to traditional animal testing[11]. NAMs leverage technologies such as 2D in vitro cultures, 3D organoids, 2D and 3D organ-on-chips and computational modelling to address the limitations of animal testing (Fig 1).

Over the last few decades, there have been several global regulatory developments to promote non-animal methodologies. Much attention has been given to the 3R principles (replacement, reduction, refinement of animal use) with a focus on tests that reduce the use of animals and ultimately replace animal use altogether. This has been mandated by the Directive 2010/63/EU, and has been the subject of a recent EU resolution (TA MEF) and similar actions in other countries[12,13,14].

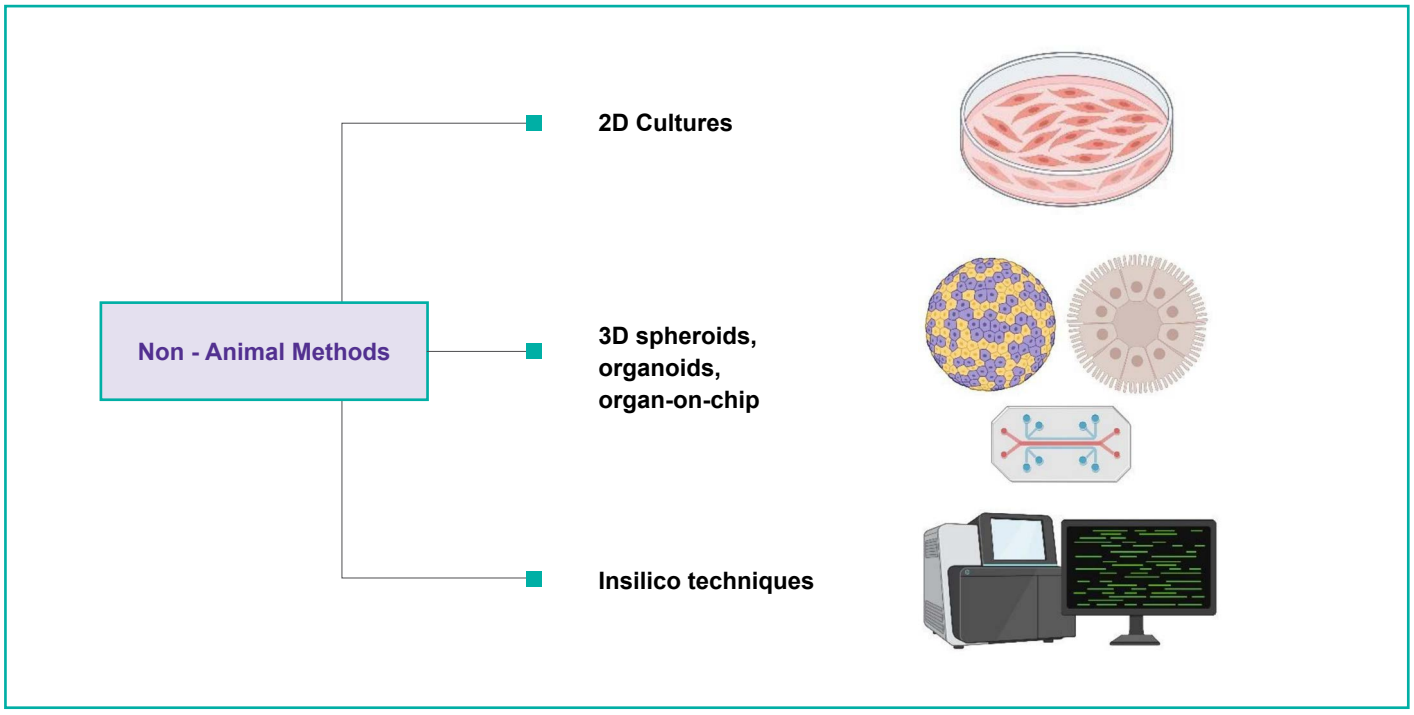


Fig 1: Schematic showing some of the different Non-Animal Methods

6.2.1. NAMs Market

The global NAMs testing market size has grown rapidly in recent years. A report from The Business Research Company published in January 2025 estimated that the NAMs market will grow from $2.33 billion in 2024 to $4.02 billion in 2029 at a compound annual growth rate (CAGR) of 11.6%[15]. Many major NAM developers in the field are spin offs from academic institutions[16]. For example, TissUse from Technische University Berlin, Emulate Bio from Wyss Institute for Biologically Inspired Engineering in Boston, Mimetas from Leiden University, and Nortis from the University of Washington. Other suppliers have licensed Micro Physiological Systems (MPS) technologies from academia, such as CN Bio who has licensed the PhysioMimix platform from the Massachusetts Institute of Technology (MIT) in Cambridge, USA, InSphero who licensed the tissue plate platform from the ETH in Zurich and Hesperos, who used technology developed at Cornell University.

This chapter provides insight into the key global industry players in NAMs, also listing NAMs and their application in tissue-specific toxicity and efficacy studies. This chapter also lists recommendations for accelerating NAMs adoption by the Indian pharmaceutical industry, CROs and academicians.

6.3 Methodology (exact regulations cited and stakeholders consulted)

A list of regulatory initiatives and a detailed list of major industry players in NAMs was created after curating the websites of global industry players (Annexure 1). The detailed methodology listed is as follows:

6.3.1 The regulatory landscape for non-animal methodologies

Regulatory authorities across the world have undertaken initiatives towards developing and validating NAMs in order to improve the prediction of efficacy and safety of drugs in clinical trials as well as real world situations. Major initiatives were referred to for authoring this report (Annexure 2, Table 1).

6.3.2. Analysing company websites, application notes and publications

Detailed information from manufacturers like AxoSim, TissUse, Emulate Bio, inSphero, EPISKIN, etc., about their products/ technologies was analyzed. Documents containing information on how NAMs are applied in real-world scenarios including methodologies, protocols and outcomes for specific use cases like drug toxicity, absorption and metabolism studies, were collected, reviewed and catalogued in specific folders. Publications that serve as validation reports, detailing experimental outcomes and supporting the usage of NAM technologies were analyzed. Over 137 company websites were curated and over 400 publications were reviewed and analyzed.

6.3.3. Compiling a list of industry players in NAMs for tissue-specific toxicity

NAMs based on their application to specific tissues or organs such as the liver, heart, skin, eye, kidney, intestine, lungs, and nervous system are listed in Supplementary Document 1. The models that meet global regulatory guidelines, e.g., OECD, FDA, and ICH guidelines and that are used for studying drug toxicity were identified ensuring relevance for preclinical testing and regulatory submissions. The flowchart for mapping tissue-specific toxicities using NAMs is shown in figure 2.

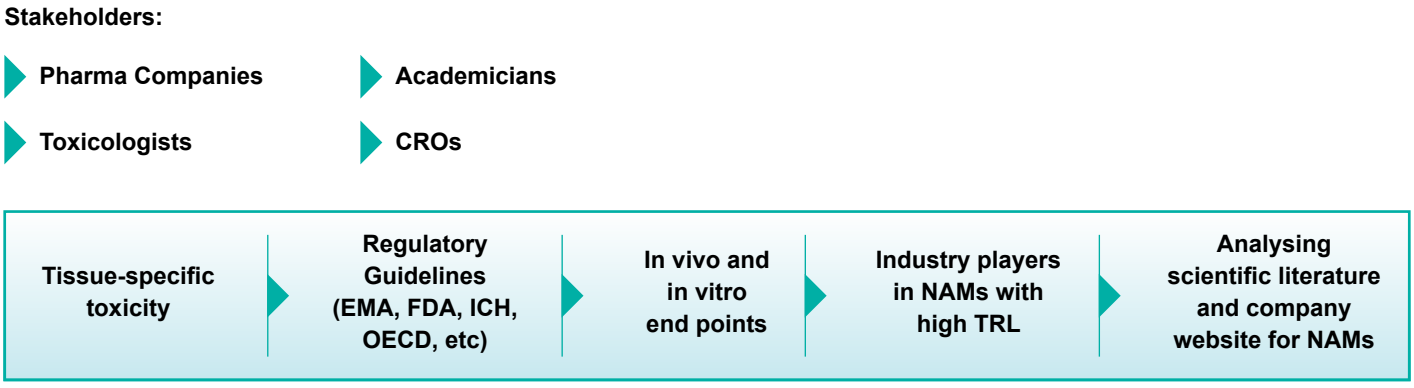


Fig 2. Flowchart for mapping the tissue-specific toxicity using NAMs.

6.3.4. Engaging stakeholders/contributors for input

We engaged in discussions with key stakeholders /contributors to the chapter to validate and refine the list of industry players in NAMs as well as provide recommendations to increase the adoption of NAMs in the Indian market.

6.4. NAMs in toxicity testing and their applications

The applications of NAMs were divided into types of toxicity testing including dermal, hepatic, renal, respiratory, central nervous system (CNS), cardiac, intestinal and ocular. The usage of NAMs in these areas is the most relevant to humans and can also lead to a potential reduction in the number of animals being used for preclinical studies.

6.4.1. Hepatic toxicity testing

Drug-Induced Liver Injury (DILI) is a significant and potentially life-threatening adverse drug reaction caused by certain pharmaceuticals, herbal supplements or chemical agents. DILI is one of the major reasons for drug development failure, market withdrawal, and black box warning due to lack of correlation of responses in clinical trials to real world situations[17]. Additionally, hepatic toxicity studies in animal models are difficult to reproduce in humans, since there are species-specific differences in metabolism between mice, rats, monkeys, dogs and humans[18].

The application of NAMs in hepatic toxicity testing consequently holds great potential for the pharma industry in the drug development field. We identified 32 global commercial technologies of hepatic NAMs (Annexure 2, Table 2).

6.4.2. Renal toxicity testing

About 19–33% of acute kidney injury cases are due to drug nephrotoxicity[19]. In hospitalized patients, drug-induced nephrotoxicity is estimated to account for approximately 20% to 60% of acute kidney injury cases[20].

Animal studies for renal toxicity testing are expensive, have low throughput, are time-consuming, and have lower human reproducibility[21]. Animal studies to predict renal toxicity lead to 8–9% drug failure in preclinical and clinical stages of drug development[19,22].

We identified 9 global commercial technologies in the context of kidney NAMs (Annexure 2, Table 3).

6.4.3. Respiratory toxicity testing

Respiratory toxicity testing is essential for evaluating the safety of drugs, chemicals, and environmental pollutants that may impact lung function. Animals differ from humans in the deposition pattern of drugs and pathways by which the drug is cleared from the lungs, meaning animal models often fail to accurately predict drug toxicity in human lungs[23].

The use of NAMs can provide a more human relevant alternative to animal testing. We identified 13 global commercial technologies in the context of respiratory NAMs (Annexure 2, Table 4).

6.4.4. Central Nervous System (CNS) toxicity testing

CNS toxicity testing is a critical component in evaluating the safety of pharmaceutical compounds particularly those that cross

or interact with the blood-brain barrier (BBB). Animal models do not accurately predict human disease due to specific differences in blood-brain barrier (BBB) permeability, drug metabolizing enzymes and transporters which can lead to differences in drug exposure in the human brain compared to animals[24].

NAMs can be used for replicating CNS-specific toxicity thereby providing alternatives to traditional in vivo methods. In our study, 16 global commercial technologies in NAMs were identified in the context of CNS (Annexure 2, Table 5).

6.4.5. Cardiotoxicity testing

Cardiotoxicity of drugs remains a serious concern in drug development and chemical safety evaluation as adverse effects on the heart can lead to arrhythmias, myocardial dysfunction and heart failure[25]. Species differences of animal models compared to humans lead to an increasing chance of false positive or false negative predictions of patient responses[26]. Therefore, NAMs can be used as an alternative to animal testing offering human-relevant insights into cardiac function, electrophysiology and cellular mechanisms of toxicity.

We identified 14 global commercial technologies for cardiac NAMs (Annexure 2, Table 6).

6.4.6. Intestinal toxicity testing

The intestinal tract plays a critical role in nutrient absorption, metabolism and barrier defense making it highly susceptible to toxicity from pharmaceuticals, chemicals and environmental agents. The *in vivo* testing methods are costly and not translationally relevant due to species differences in metabolism and intestinal physiology leading to the failure of clinical trials.

NAMs can offer human-relevant, cost-effective and high-throughput platforms to test drugs for intestinal barrier-related toxicity. We identified 9 global commercial technologies for intestinal NAMs (Annexure 2, Table 7).

6.4.7. Dermal toxicity testing

The use of animals for testing skin irritation (e.g. the Draize test) has limitations such as large variations in test results and species-difference between rabbits and humans. Dermal toxicity testing for drugs is typically limited unless the drug is intended for topical application. NAMs of dermal toxicity are designed to measure a range of biological endpoints which are critical to understand the effects of chemicals and drugs on human skin. We identified 37 global commercial technologies in dermal NAMs (Annexure 2, Table 8).

6.4.8. Ocular toxicity testing

Evaluating the safety of drugs and chemicals on the eye is essential for the drugs which have this route of administration. Animal models poorly translate to human responses. For example, the Draize test is not reliable because of the fundamental anatomical differences between the human and rabbit eye.

NAMs can act as robust tools for ocular safety assessments. We identified 7 global commercial technologies in the field of ocular NAMs (Annexure 2, Table 9).

Based on the analysis of global commercial technologies involved in NAMs, we have quantified the presence of technologies within the context of tissue-specific toxicity studies. Of the 137 NAMs evaluated (Annexure 2, Table 10), the highest number are utilized for studying dermal toxicity (37) and hepatic toxicity (32). In addition to these, NAMs are also employed for toxicity assessments of other tissues, including ocular, renal, respiratory, intestinal, central nervous system (CNS), and cardiac toxicity, as illustrated in Figure 3.

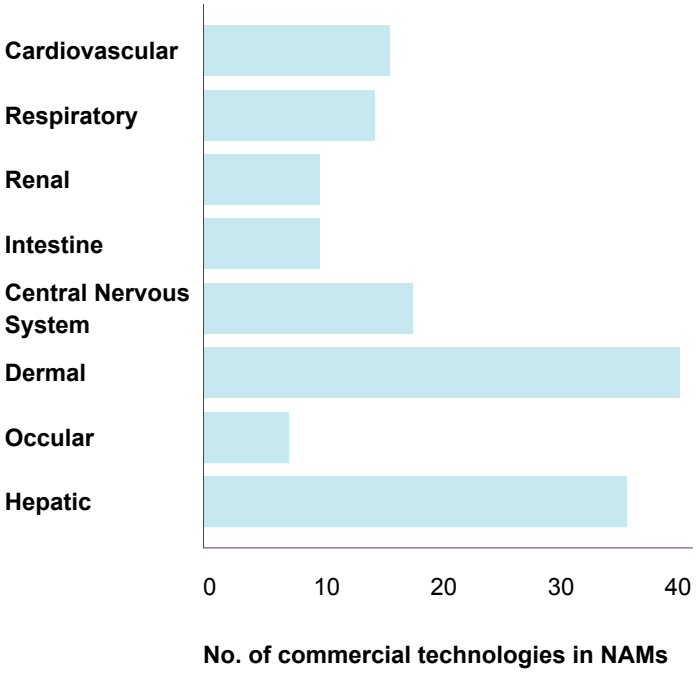


Fig 3: Bar graph showing the number of industry players in NAMs in different tissue-specific toxicity testing

6.4.9. NAMs in genotoxicity testing

Genotoxicity is the ability of a compound to damage the genetic material (DNA or chromosomes) in a cell. Such damage can lead to mutations, chromosomal fragmentation, aneuploidy, or other types of genetic alterations[27]. These changes can occur in somatic cells (potentially leading to cancer or other diseases) or germ cells (potentially causing heritable defects)[27]. Previously, genotoxicity testing was largely based on animal models. But it has disadvantages due to ethical concerns, high costs and limited relevance to human health[28]. Therefore, NAMs including advanced in vitro models, high-throughput screening, computational approaches and in silico models have emerged as promising alternatives that are more ethical, efficient and human-relevant (Annexure 2, Table 11-12).

6.5. NAMs in monoclonal antibody toxicity and efficacy assessment

In addition to tissue-specific NAMs used for toxicity screening, it is important to develop and implement immunologically relevant NAMs to evaluate monoclonal antibody (mAb) safety and efficacy particularly given their growing prominence in modern therapeutics. Immunological NAMs enable mechanistic insights that are difficult to achieve with traditional animal models due to species-specific differences in immune responses.

The recent US FDA roadmap on Reducing Animal use in preclinical research has identified monoclonal antibodies as the first areas of focus[29]. This is due to the fact that animals often mount immune responses to human mAbs which confound the prediction of toxicity. Also, studies show innate differences in immunogenicity between humans and animals which may lead to risk being under or over-estimated. Thus, this is one of the areas where human-relevant methods are proposed to have significant impact.

6.6. Case studies of NAMs in efficacy assessment and precision medicine

6.6.1. Rare diseases

Rare diseases present an interesting area to use human-relevant technologies as several challenges are associated with rare disease therapeutics, with animal models either unavailable or often failing as preclinical test systems, providing limited insights regarding their biological mechanisms. Currently, there are no approved treatments for 95% of rare diseases. NAMs are being used in a number of domains of drug development for rare diseases including: nonclinical research (efficacy testing) and precision medicine (Annexure 2, Table 13).

6.6.2. Cancer

i. Colorectal cancer

Using a large biobank of patient-derived colorectal cancer organoids (CRC), MCLA-158, a bispecific antibody binding epidermal growth factor receptor (EGFR) and leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5), was identified as the most effective antibody as it could reliably discriminate between cancerous and healthy cells. Based on the data from human-derived cancer organoids, the compound went from bench to bedside in just 3 years (Clinical Trial No: NCT03526835[30])

ii. Small cell lung carcinoma

Amivantamab is a bispecific antibody against EGFR and the mesenchymal-epithelial transition receptor, and its efficacy was preclinically tested in patient-derived cells and organoids that harbour EGFR Exon20ins mutation. The results were replicated in clinical trials (Clinical Trial No.: NCT04538664[31]), and amivantamab was eventually approved by the EMA for the treatment of non-small cell lung cancer.

iii. Breast cancer

Currently, 36 trials are underway that are using patient-derived organoids (PDO) to verify their effectiveness (Annexure 2, Table 14), feasibility and consistency in predicting treatment and establish an evaluation system for treatment plans to help precision treatment of advanced breast cancer.

6.7. In silico approaches to NAMs

To improve the treatment of laboratory animals, the 3Rs principle was introduced by Russell and Burch in the late 1950s and focused on replacement, reduction, and refinement of animal use[32]. While the approaches of reduction and refinement have been practiced widely over the last few decades, the concept of replacement is now being increasingly used to promote the complete abolition of all animal-based research[33]. In silico approaches or computational tools in recent times have enabled the simulation of entire drug discovery and development scenarios (Annexure 2, Table 15).

6.7.1. Artificial intelligence (AI) program for toxicology by US FDA

The US FDA has developed an Artificial Intelligence (AI) program for toxicology at the National Center for Toxicological Research (NCTR) known as AI4TOX[34]. It encompasses five key initiatives: AnimalGAN, SafetAI, BERTox, PathologAI and TranslAI. In 2024, EMA started an initiative to include AI in drug approval. The reflection paper on the use of Artificial Intelligence (AI) in the medicinal product lifecycle by EMA outlines how AI and machine-learning (ML) systems can support the entire medicines lifecycle from discovery, non-clinical and clinical development, to manufacturing and post-authorization monitoring[35].

6.8. Global regulatory guidelines on NAMs

Global regulatory agencies like FDA, OECD and EMA support the adoption and usage of NAMs. The FDA regulations under

IND (21 CFR 312.23) and NDA (21 CFR 314.50) allow the use of non-animal methods for pharmacology and toxicology data submissions. The FDA and ICH guidelines encourage the use of alternative methods aligned with the 3Rs principles[36]. OECD guidelines support *in vitro* methods for ocular and skin irritation tests. Specific ICH guidelines such as S5(R3) and S10 describe the use of *in vitro* methods for toxicity evaluations.

EMA has issued guidance on reporting physiologically based pharmacokinetic modeling (PBPK) and simulation results[37,38]. In February 2025, the EMA also came up with a concept paper on reporting mechanistic models used in model-informed drug development[39]. The physiologically based biopharmaceutics modeling (PBBM) guidance issued by the US FDA[40] focuses on general recommendations regarding the development, evaluation and use of PBPK for biopharmaceutics applications.

Most recently, a harmonized guidance has also been issued that details the requirements of model informed drug development[41]. Other guidelines like the ICH S1B(R1) and E14/S7B both encourage the use of NAMs to improve safety testing and reduce animal use. S1B(R1) allows a weight-of-evidence approach using mechanistic, *in vitro*, or other non-animal data to decide whether a traditional 2-year rodent carcinogenicity study is needed[42]. E14/S7B integrates nonclinical (S7B) and clinical (E14) data for assessing cardiac safety, accepting validated *in vitro, in silico* and mechanistic assays[43]. A list of ICH and EMA guidelines for toxicology studies using NAMs (modified from Landscape New Approach Methodologies by National Institute for Public Health and the Environment[44]) is provided in Annexure 2, Table 16-17.

6.9. Current regulatory expectations in India

Clinical trials in India are governed by the Drugs and Cosmetics Act of 1940, the Medical Council of India Act of 1956, the Central Council for Indian Medicine Act of 1970 and the Guidelines for Exchange of Biological Material (MOH order, 1997). Researchers seeking to conduct a clinical trial in the country must obtain permission from the Drugs Controller General India (DCGI), obtain approval from the Ethics Committee established under Drugs and Cosmetics Rules and register on the ICMR-maintained website[45]. The government of India passed the New Drugs and Clinical Trial Amendment Act in 2023, the objective of which is to replace the use of animals in research and in particularly in drug testing. The amendment permits researchers to use non-animal and human-relevant methods to test the safety and efficacy of new drugs. Also, these rules require that any CRO conducting a clinical trial or bioavailability/bioequivalence study of new drugs or investigational drugs in human subjects must first obtain registration from the Central Licensing Authority (CDSCO) before proceeding with the study to ensure ethical standards, data integrity, and subject safety[46].

In relation to NAMs, such registration underscores the importance of validated, ethical and quality-controlled testing methods. For preclinical studies, the RCGM (Review Committee on Genetic Manipulation) under the DBT oversees research involving genetically modified organisms or recombinant products, while the CDSCO evaluates overall safety, efficacy and quality data before human testing. Together, these frameworks ensure that both NAM-based and traditional approaches meet scientific and regulatory standards for drug approval in India.

6.10. Current NAMs landscape in India

Several Indian academic institutions and biotechnology companies are actively engaged in NAM-related research, particularly in the domains of organoids, organ-on-chip platforms, and *in silico* models. Although many of these technologies are currently at lower Technology Readiness Levels (TRLs), they offer considerable potential for scaling and commercialization, provided the appropriate infrastructure and policy support are in place. Institutions such as IIT Bombay, IIT Hyderabad, CCMB, inStem, IISER, IISc, ACTREC, etc., are developing advanced NAMs to study complex diseases such as cancer, diabetes, and wound healing. Concurrently, private enterprises including Dr. Reddy's Laboratories, Sai Life Sciences, OncoSeek Bio, Pandorum Technologies, and JRF Global are also making notable progress. A detailed list of the number of institutions and companies in India working in organoids and organs-on-chips is shown in Annexure 2, Table 18-19.The detailed list containing the names of the scientists, the institution/company names, their contact details, the type of cells used, and the TRL levels of the technologies can be viewed on the CPHMS MPS website[47].

Despite these advancements, most of these NAM platforms remain confined to research settings and have not yet reached levels amenable to commercial scalability. Awareness and technical expertise in NAMs within the Indian scientific community remain limited. Therefore, targeted efforts such as capacity-building workshops, hands-on training programs, and knowledge exchange platforms are necessary to catalyze the integration of NAMs into mainstream pharmaceutical R&D pipelines. Public-private partnerships should be fostered to mobilize financial resources and technical expertise. Moreover, funding support from government agencies such as the Department of Biotechnology (DBT), Council of Scientific and Industrial Research (CSIR), and Biotechnology Industry Research Assistance Council (BIRAC) should be strategically directed towards the development and validation of NAMs. Given the high prevalence of diseases such as cancer, diabetes, and respiratory illnesses in India, leveraging patient-relevant NAMs such as 3D spheroids and induced pluripotent stem cell (iPSC)-derived models can play a pivotal role in enhancing drug efficacy studies and personalizing therapeutic approaches for these complex conditions.

6.11. Challenges

We have identified and outlined the following obstacles and strategies to accelerate the adoption of these NAMs in the Indian context.

1. **Lack of source for scientifically validated cell lines:** Multiple stakeholders whom we consulted from different fields emphasized the importance of having indigenous quality-controlled primary cells, cell lines, or immortalized cell lines. This will ensure rapid deployment and cost-effective construction of both 2D and 3D models subject to appropriate intellectual property considerations.

2. **Quality challenges of NAMs:** One significant obstacle for liver-based NAMs is their potential lack of diversity in cell types and biological variation. This limitation can lead to a reduced ability to accurately simulate the varied responses seen across different genders, ages and genetic polymorphisms. As liver cells in culture may experience changes in phenotype or lose key enzyme activities over time, the long-term predictive value of liver NAMs becomes doubtful. For accurate assessment of inhalation toxicity using in vitro models, it is essential to culture cells under air-liquid interface (ALI) conditions, where cells are exposed directly to air, replicating physiological exposure more effectively[23]. The method is technically demanding, more expensive and requires longer culture times often with primary cells.

3. **Low TRL of NAMs:** Despite advancements in high-TRL NAM platforms, a substantial translational gap persists between their level of complexity and function in vitro compared to human physiological responses observed in clinical contexts. Currently some NAMs lack the integrative capacity to model multi-organ interactions, immune system dynamics and systemic pharmacokinetics and pharmacodynamics accurately.

4. **Lack of guidelines for *in silico* modeling:** Regulatory bodies such as the FDA, have proposed specific frameworks for using in silico data in drug evaluations48. The Indian scientific community must adopt and adapt these guidelines to establish credibility and enable seamless integration of *in silico* models into the drug development pipeline.

5. **Limited strategic application avenues of NAMs:** In the Indian context, the rate of novel drug innovation remains relatively limited. Consequently, the near-term application of NAMs in de novo drug discovery is unlikely to be a primary driver of adoption within India. However, there exists significant potential for the strategic application of NAMs in the development and regulatory assessment of biosimilars and generic drugs, which constitute a major share of the Indian pharmaceutical industry.

6. **Economic challenges in adoption of NAMs:** Cost analysis showed that while some models like spheroids, can be relatively affordable, others, like organ-on-chips, are far more expensive than animal experiments. Indigenization of resources could significantly reduce costs. The high initial costs of importing advanced equipment, licensing proprietary technologies and navigating intellectual property restrictions can make early adoption less attractive to industries and CROs. To make the economic case compelling, India must generate a quantified India-specific cost–benefit analysis that demonstrate how NAM adoption can shorten preclinical timelines and reduce expenditure compared to traditional animal studies.

7. **Need for indigenous culture components:** The procurement of essential laboratory supplies such as culture media, reagents and plasticware from international suppliers poses a financial and operational burden. Promoting indigenous manufacturing of these components would greatly benefit Indian research by reducing costs and ensuring consistent supply chains.

8. **Absence of a centralized governmental body:** Establishing an Indian equivalent of ICCVAM (U.S.), JaCVAM (Japan), or EURL-ECVAM (European Union) would provide the much-needed structure for training, international alignment, and progressive regulatory integration of NAMs.

6.12. Key Recommendations

Globally, several pharmaceutical and biotech companies have already integrated NAMs, particularly *in vitro* and *in silico* systems into their internal pipelines for early-stage screening, efficacy testing and mechanistic understanding. In order to accelerate NAMs regulatory adoption in India, we need to generate a pipeline similar to existing global regulatory criteria. An India-specific roadmap should start with early regulator engagement through scientific advice meetings, followed by compact pilot submissions for single endpoints and bridging ring-trials across Indian CROs and academic labs to demonstrate transferability. A public repository of reference compound data and jointly agreed acceptance criteria (sensitivity, specificity, reproducibility thresholds) would support standardization. Regulators should also receive targeted training to interpret NAM data, while conditional pilot decisions can build confidence before broader adoption.

The key recommendations are as follows:

Key recommendations	Key stakeholders	Implementation Plan	Possible challenges of implementation	Time period for implementation
Indigenous establishment of high quality and well-validated cell repository specifically for: i) HepG2, ii) primary hepatocytes for liver, iii) iPSC derived cardiomyocytes	Pharmaceutical companies, Academic institutions, CROs	• Conduct case studies to analyze the reason behind the failure of previous cell repository initiatives in India • Sourcing of the cells and setting up banks close to hospitals to receive fresh cells. • Setting up well-defined protocols for cell culture and cell handling • Comprehensive training of individuals on proper cell culture SOPs to maintain and freeze the cell lines according to proper protocol. • Work in collaboration with stakeholders with expertise in this area	Maintenance and scaling, QC, workflows of the cell lines in the repository	2–3 years (long-term)
Indigenous production of media components, labware and reagents for cell culture	Pharmaceutical companies, Academic institutions, CROs	• Comprehensive training of individuals to produce the media, and cell culture products. • Work in collaboration with stakeholders with expertise in this area.	Production of reliable, high quality growth factors and other additives for cell culture	6 months–1 year (short-term)
Increasing awareness of the latest NAM technologies	Pharmaceutical companies, Academic institutions, CROs, Regulatory agencies	Organizing workshops, and roundtables with the key stakeholders.	Challenges in adopting disruptive approaches like NAMs over traditional animal- based approaches	6 months–1 year (short-term)
Leveraging public-private partnership grants within India	Funding agencies, Industry and academic partners	Consultations with funding agencies (public and private) for prioritization of NAM development and use in India	Convincing the funding agencies in conjunction with regulators to prioritize funding in this area	2–3 years (medium-term)
The implementation of NAMs in efficacy studies, especially in areas like oncology and diabetes	Pharmaceutical companies, Academic institutions, CROs	Prioritization of discovery and pre-clinical stages of drug development such as liver, cancer in consultation with pharmaceutical companies	-	More than 2 years (long-term)

Key recommendations	Key stakeholders	Implementation Plan	Possible challenges of implementation	Time period for implementation
Adoption of in silico tools like GastroPlus, and SIMCYP. PKSIM	Pharmaceutical companies, Academic institutions, CROs, regulatory agencies	• Use of these tools in the regulatory submissions as well as for internal decision making to reduce the extent of animal testing. • Collaborating with the DCGI to utilize legacy drug data for creating in silico tools to predict drug toxicity • Focus on the indigenous development of software for predicting pharmacokinetics and pharmacodynamics (PK/PD) in partnership with the DCGI	Accuracy of these physiological models to mimic real world scenarios. Model validation is necessary before implementation	1–2 years (medium term)
Developing a centralized, open-access toxicity data repository	Pharmaceutical companies, Academic institutions, CROs	Developing a publicly accessible database by aggregating comprehensive animal and human toxicity data from international sources	-	6 months–1 year (Short-term)
Establishment of robust validation and qualification pathways	Pharmaceutical companies, Academic institutions, CROs, Regulators	• Continuously validating NAMs using historical drug toxicity data with known human outcomes. • Performing retrospective and prospective concordance assessments to determine predictive accuracy. • Conducting parallel testing of new drug candidates using both traditional animal models and NAMs to evaluate comparative accuracy, cost-efficiency, and ethical impact.	-	2–3 years (medium term)
Create Incentive Mechanisms for Industry Adoption	Regulatory bodies, Funding agencies	Introduce regulatory incentives for companies employing NAMs	Lack of resources	2–3 years (medium term)
Establishing dedicated Centres of Excellence (CoEs) for NAMs	Pharmaceutical companies, Academic institutions, funding agencies	• Involve key experts to define CoEs' core objectives and secure initial funding (through ICMR, BIRAC, DBT grants). • Engage with leading academic institutions (IITs, IISc), industry leaders (pharma and biotech companies), and government agencies to formalize partnerships.	Significant upfront investment in cutting-edge equipment, specialized laboratories, and expert personnel.	1–2 years (medium term)
Development of a comprehensive guidance document outlining key priority endpoints	Regulatory agencies		-	2–3 years (medium term)

References:

1. *Prior H, Haworth R, Labram B, Roberts R, Wolfreys A, Sewell F. Justification for species selection for pharmaceutical toxicity studies. Toxicol Res. 2020 Dec;9(6):758–70.*
2. *Marshall LJ, Bailey J, Cassotta M, Herrmann K, Pistollato F. Poor Translatability of Biomedical Research Using Animals — A Narrative Review. Altern Lab Anim. 2023 Mar;51(2):102–35.*
3. *Hackam DG, Redelmeier DA. Translation of Research Evidence From Animals to Humans. JAMA. 2006 Oct 11;296(14):1727.*
4. *Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci. 2013 Feb 26;110(9):3507–12.*
5. *Mullard A. Parsing clinical success rates. Nat Rev Drug Discov. 2016 July;15(7):447–447.*
6. *Arrowsmith J. Phase III and submission failures: 2007–2010. Nat Rev Drug Discov. 2011 Feb;10(2):87–87.*
7. *Schuhmacher A, Hinder M, Brief E, Gassmann O, Hartl D. Benchmarking R&D success rates of leading pharmaceutical companies: an empirical analysis of FDA approvals (2006–2022). Drug Discov Today. 2025 Feb;30(2):104291.*
8. *Van Norman GA. Limitations of Animal Studies for Predicting Toxicity in Clinical Trials. JACC Basic Transl Sci. 2019 Nov;4(7):845–54.*
9. *Akhtar A. The Flaws and Human Harms of Animal Experimentation. Camb Q Healthc Ethics. 2015 Oct;24(4):407–19.*
10. *Issa NT, Wathieu H, Ojo A, Byers SW, Dakshanamurthy S. Drug Metabolism in Preclinical Drug Development: A Survey of the Discovery Process, Toxicology, and Computational Tools. Curr Drug Metab [Internet]. 2017 July 21 [cited 2025 Dec 12];18(6). Available from: http://www.eurekaselect.com/150909/article*
11. *Sewell F, Alexander-White C, Brescia S, Currie RA, Roberts R, Roper C, et al. New approach methodologies (NAMs): identifying and overcoming hurdles to accelerated adoption. Toxicol Res. 2024 Mar 1;13(2):tfae044.*
12. *Avila AM, Bebenek I, Bonzo JA, Bourcier T, Davis Bruno KL, Carlson DB, et al. An FDA/CDER perspective on nonclinical testing strategies: Classical toxicology approaches and new approach methodologies (NAMs). Regul Toxicol Pharmacol. 2020 July;114:104662.*
13. *Turner J. Incorporating new approach methodologies into regulatory nonclinical pharmaceutical safety assessment. ALTEX [Internet]. 2023 [cited 2025 Dec 12]; Available from: https://www.altex.org/index.php/altex/article/view/2592/version/2654*
14. *Save Cruelty Free Cosmetics - Commit to a Europe Without Animal Testing [Internet]. [cited 2025 Dec 12]. Available from: https://citizens-initiative.europa.eu/save-cruelty-free-cosmetics-commit-europe-without-animal-testing_en*
15. *Non-Animal Alternatives Testing Market Size Report 2025 [Internet]. [cited 2025 Dec 12]. Available from: https://www.thebusinessresearchcompany.com/report/non-animal-alternatives-testing-global-market-report*
16. *Marx U. Biology-inspired microphysiological systems to advance medicines for patient benefit and animal welfare. ALTEX [Internet]. 2020 [cited 2025 Dec 12]; Available from: https://www.altex.org/index.php/altex/article/view/1526.*
17. *Atienzar FA, Blomme EA, Chen M, Hewitt P, Kenna JG, Labbe G, et al. Key Challenges and Opportunities Associated with the Use of In Vitro Models to Detect Human DILI: Integrated Risk Assessment and Mitigation Plans. BioMed Res Int. 2016;2016:1–20.*
18. *McGill MR, Jaeschke H. Animal models of drug-induced liver injury. Biochim Biophys Acta BBA - Mol Basis Dis. 2019 May;1865(5):1031–9.*
19. *Ramm S, Todorov P, Chandrasekaran V, Dohlman A, Monteiro MB, Pavkovic M, et al. A Systems Toxicology Approach for the Prediction of Kidney Toxicity and Its Mechanisms In Vitro. Toxicol Sci. 2019 May 1;169(1):54–69.*
20. *Yu P, Zhu H, Bosholm CC, Beiner D, Duan Z, Shetty AK, et al. Precision nephrotoxicity testing using 3D in vitro models. Cell Biosci. 2023 Dec 21;13(1):231.*
21. *Krewski D, Acosta D, Andersen M, Anderson H, Bailar JC, Boekelheide K, et al. Toxicity Testing in the 21st Century: A Vision and a Strategy. J Toxicol Environ Health Part B. 2010 June 17;13(2–4):51–138.*
22. *Cook D, Brown D, Alexander R, March R, Morgan P, Satterthwaite G, et al. Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework. Nat Rev Drug Discov. 2014 June;13(6):419–31.*
23. *Movia D, Bruni-Favier S, Prina-Mello A. In vitro Alternatives to Acute Inhalation Toxicity Studies in Animal Models—A Perspective. Front Bioeng Biotechnol. 2020 June 3;8:549.*
24. *Srinivas N, Maffuid K, Kashuba ADM. Clinical Pharmacokinetics and Pharmacodynamics of Drugs in the Central Nervous System. Clin Pharmacokinet. 2018 Sept;57(9):1059–74.*
25. *Mladěnka P, Applová L, Patočka J, Costa VM, Remiao F, Pourová J, et al. Comprehensive review of cardiovascular toxicity of drugs and related agents. Med Res Rev. 2018 July;38(4):1332–403.*
26. *Pang L, Sager P, Yang X, Shi H, Sannajust F, Brock M, et al. Workshop Report: FDA Workshop on Improving Cardiotoxicity Assessment With Human-Relevant Platforms. Circ Res. 2019 Oct 11;125(9):855–67.*
27. *Phillips DH, Arlt VM. Genotoxicity: damage to DNA and its consequences. In: Luch A, editor. Molecular, Clinical and Environmental Toxicology [Internet]. Basel: Birkhäuser Basel; 2009 [cited 2025 Dec 12]. p. 87–110. (Experientia Supplementum; vol. 99). Available from: http://link.springer.com/10.1007/978-3-7643-8336-7_4*
28. *Pfuhler S, Kirkland D, Kasper P, Hayashi M, Vanparys P, Carmichael P, et al. Reduction of use of animals in regulatory genotoxicity testing: Identification and implementation opportunities—Report from an ECVAM workshop. Mutat Res Toxicol Environ Mutagen. 2009 Nov;680(1–2):31–42.*
29. *Roadmap to Reducing Animal Testing in Preclinical Safety Studies. [cited 2025 Dec 12]; Available from: https://www.fda.gov/files/newsroom/published/roadmap_to_reducing_animal_testing_in_preclinical_safety_studies.pdf*

30. *Cancer Care Northwest: Clinical Trial NCT03526835 [Internet]. [cited 2025 Dec 12]. Available from: https://www.cancercarenorthwest.com/clinical-research/nct03526835/*

31. *Janssen Research & Development, LLC. A Randomized, Open-label Phase 3 Study of Combination Amivantamab and Carboplatin-Pemetrexed Therapy, Compared With Carboplatin-Pemetrexed, in Patients With EGFR Exon 20ins Mutated Locally Advanced or Metastatic Non-Small Cell Lung Cancer [Internet]. clinicaltrials.gov; 2025 Nov [cited 2025 Dec 12]. Report No.: NCT04538664. Available from: https://clinicaltrials.gov/study/NCT04538664*

32. *Tannenbaum J, Bennett BT. Russell and Burch's 3Rs then and now: the need for clarity in definition and purpose. J Am Assoc Lab Anim Sci JAALAS. 2015 Mar;54(2):120–32.*

33. *Rowlett J. ANIMAL FORUM: THE 3RS CONCEPT OF REPLACEMENT: FUTURE TOOLS OR POLITICAL STRATEGY? Drug Alcohol Depend. 2024 July;260:110780.*

34. *Artificial Intelligence | FDA [Internet]. [cited 2025 Dec 12]. Available from: https://www.fda.gov/about-fda/nctr-research-focus-areas/artificial-intelligence*

35. *Fia W. Reflection paper on the use of Artificial Intelligence (AI) in the medicinal product lifecycle_240903.*

36. *FDA/CDER considerations on NAMs for pharmaceutical development, April 2020 [Internet]. [cited 2025 Dec 12]. Available from: https://www.niehs.nih.gov/sites/default/files/research/assets/docs/fdacder_considerations_on_nams_for_pharmaceutical_development_508.pdf*

37. *Committee for Medicinal Products for Human Use (CHMP) | European Medicines Agency (EMA) [Internet]. 2023 [cited 2025 Dec 12]. Available from: https://www.ema.europa.eu/en/committees/committee-medicinal-products-human-use-chmp*

38. *Guideline on the reporting of physiologically based pharmacokinetic (PBPK) modelling and simulation. [cited 2025 Dec 12]; Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-reporting-physiologically-based-pharmacokinetic-pbpk-modelling-and-simulation_en.pdf*

39. *Concept paper on the development of a Guideline on assessment and reporting of mechanistic models used in the context of model informed drug development.*

40. *Research C for DE and. The Use of Physiologically Based Pharmacokinetic Analyses — Biopharmaceutics Applications for Oral Drug Product Development, Manufacturing Changes, and Controls [Internet]. FDA; 2020 [cited 2025 Dec 12]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/use-physiologically-based-pharmacokinetic-analyses-biopharmaceutics-applications-oral-drug-product*

41. *GENERAL PRINCIPLES FOR MODEL-INFORMED DRUG DEVELOPMENT M15 [Internet]. [cited 2025 Dec 12]. Available from: https://database.ich.org/sites/default/files/ICH_M15_EWG_Step2_DraftGuideline_2024_1031.pdf*

42. *TESTING FOR CARCINOGENICITY OF PHARMACEUTICALS S1B(R1) [Internet]. [cited 2025 Dec 12]. Available from: https://database.ich.org/sites/default/files/S1B-R1_FinalGuideline_2022_0719.pdf*

43. *ICH guideline E14_S7B on clinical and nonclinical evaluation of QT_QTc interval prolongation and proarrhythmic potential- questions & answers Step 5 [Internet]. [cited 2025 Dec 12]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-e14s7b-clinical-and-nonclinical-evaluation-qtqtc-interval-prolongation-and-proarrhythmic-potential-questions-and-answers-step-5_en.pdf*

44. *Landscape New Approach Methodologies (NAMs) safety assessment pharmaceutical products. Available from: https://www.rivm.nl/sites/default/files/2024-06/Landscape%20New%20Approach%20Methodologies%20%28NAMs%29%20safety%20assessment%20pharmaceutical%20products%20v2.pdf*

45. *India Amends Rules to Allow Non-Animal Drug Testing [Internet]. IASPOINT. 2023 [cited 2025 Dec 12]. Available from: https://iaspoint.com/india-amends-rules-to-allow-non-animal-drug-testing/*

46. *https://iasscore.in NS: India Takes Historic Step Towards Eliminating Animal Testing in Drug Development [Internet]. [cited 2025 Dec 12]. Available from: https://www.biopharmaboardroom.com/news/14/743/india-takes-historic-step-towards-eliminating-animal-testing-in-drug-development.html*

47. *MPS Researchers Database [Internet]. AIC-CCMB. [cited 2025 Dec 12]. Available from: https://aic.ccmb.res.in/mps-researchers-database/*

48. *Valerio LG. In silicotoxicology models and databases as FDA Critical Path Initiative toolkits. Hum Genomics. 2011 Dec;5(3):200.*

Opportunity Assessment for Transition to Animal-Free Methods in Pyrogen and Endotoxin Testing

7

7.1 Executive Summary

Pyrogen testing ensures the safety of pharmaceutical products and medical devices by detecting fever-causing contaminants. Traditional Rabbit Pyrogen Tests (RPT) and Limulus Amoebocyte Lysate (LAL) tests are being replaced due to ethical concerns, lack of validation, and variability. Newer alternatives such as recombinant Factor C (rFC) and the Monocyte Activation Test (MAT) for endotoxin and pyrogen testing are animal-free, human-relevant, and more reliable. The Indian Pharmacopoeia now allows the use of alternative methods for if shown equivalent or better than validated pharmacopoeial methods, which signals readiness to transition away from animal methods. Challenges to adopting these methods include high costs, limited local reagent production, standardization gaps, and the need for trained personnel. We highlight recommendations such as promoting indigenous reagent production, strengthening supply chains and attracting investment, and clear regulatory guidance for alternative methods.

7.2 Background

Pyrogens are substances that can cause fever or in severe cases, septic shock and death if introduced into the blood stream. Detecting pyrogens is a critical step to assure the quality and safety of parental pharmaceuticals and medical devices. These pyrogens may be endotoxins, such as the lipopolysaccharides (LPS) of the outer cell wall of gram-negative bacteria or non-endotoxins, such as lipoteichoic acid, muramyl peptides, and peptidoglycans of gram-positive bacteria, fungal and viral components, and parasites. Pyrogenic contamination is detected by peripheral blood monocytes and macrophages, which in turn stimulate the release of pro-inflammatory cytokines, such as interleukin 1β (IL-1β), TNF, and IL-6.

Traditionally, the Rabbit Pyrogen Test (RPT) was used to test for pyrogenic contamination. This test involved injecting the test substance into the upper ear vein of at least three rabbits and monitoring body temperature rectally, and a rise indicated pyrogenic contamination. While this test can detect all pyrogenic contamination, the conditions under which the animals are kept, and the rabbit species, can influence results. Also, non-injectable substances cannot be tested. Despite the fact that the test was never formally validated[1], it is still viewed as the 'gold standard' for pyrogen detection.

In 1956, it was discovered that the LPS of gram negative bacteria can cause clotting of horseshoe crab (*Limulus polyphemus*) blood (*haemolymph*). This led to the development of the Limulus Amoebocyte Lysate (LAL) test[2,3]. In the LAL test, contact with endotoxins triggers a coagulation cascade with the activation of three pro-enzymes: Factor C, Factor B, and pro-clotting enzyme. The three approved tests use LAL reagents for endotoxin testing, including gel clot (limit and semi-quantitative), turbidimetric, and chromogenic (end-point and kinetic methods) assays. All three assays are based on the principle that bacterial endotoxins set off a cascade of reactions, resulting in a change of turbidity or color. However, the LAL test is subject to false positives as haemolymph can also coagulate in vitro in the presence of other substances that are harmless to humans, such as salts with divalent cations, chelators, acids, proteins, certain antibiotics, or non-endotoxins such as

β-D-glucan[4,5]. Horseshoe crab blood-based lysates also show high batch-to-batch variability[6]. The capture and bleeding procedures also have an impact on horseshoe crab populations, with a mortality rate of 10-30%, and other impediments, such as a decrease in activity, speed, and haemocyanin levels[7].

A novel version of the LAL assay has been designed using a recombinant form of Factor C (rFC), based on the starting point of the coagulation cascade. With this method, the need for horseshoe crab haemolymph is eliminated. This test specifically recognises bacterial endotoxins and is not influenced by non-endotoxins or substances that can activate the alternate pathway, such as Factor G (which can lead to false positives). Studies that compared the performance of LAL and rFC to detect pyrogens in purified pharmaceutical water matrices and pharmaceutical products found that the rFC test showed better performance, accuracy, repeatability, and had a shorter time-to-results[8,9].

	RPT	**LAL (gel-clot)**	**LAL (chromogenic/ turbimetric)**	**rFC**	**MAT**
Applicability	Parental pharmaceuticals Fluids in general	Parental pharmaceuticals Fluids in general	Parental pharmaceuticals Fluids in general	Parental pharmaceuticals Fluids in general	Parental pharmaceuticals Fluids in general Surfaces of materials Aerosols
Strengths	None	Endotoxin-specific High sensitivity Technically simple	Endotoxin-specific Very high sensitivity Wide dynamic range	Endotoxin-specific Very high sensitivity Wide dynamic range No interference by glucans No animal source Low batch-to-batch variation	All pyrogens Human-specific High sensitivity Fluids and materials testing Full animal replacement
Limitations	Animal-based	Only for endotoxins and glucans Animal material required Batch-to-batch variability may be present	Only for endotoxins and glucans Animal material required	Only for endotoxins Liquid samples	Availability of human blood Cryoblood cold chain Variation in human cell lines Interference from immunogenic drug substances

While the rFC test is specifically to detect bacterial endotoxins, the monocyte activation test (MAT) represents a new generation pyrogen test that detects both endotoxins and non-endotoxins. MAT can be performed using either a) human whole blood (fresh or cryopreserved); b) monocytic cells isolated from whole blood (fresh or cryopreserved); or c) monocytic cell lines. The read-outs for the assay include three well-established proinflammatory cytokines IL-1β, IL-6, and TNF detected using ELISA. As MAT uses whole human blood, it is closest to reproducing the human reaction to pyrogenic substances. While whole blood MAT was initially the only variant available in kit form, MAT kits are currently available based on whole blood, cryopreserved whole blood, peripheral blood mononuclear cells (PBMCs), cryopreserved PBMCs, and cell lines (MonoMac-6, MM-6). The PBMCs and whole blood assay (fresh and cryopreserved) have been completely validated, and cryopreserved PBMCs and cell lines are still pending complete validation (Table 2). Due consideration needs to be given to the challenges for maintaining the cooling chain for cryo-PBMCs while distributing to other labs/testing centres. With respect to cell lines such as MM-6, care needs to be taken towards genetic variability and contamination.

Table 2: Validation status of different MAT kits (Taken from Hartung, 2021[10])

Test	Pre validation transferability	Pre validation non LPS pyrogens	Validation for LPS in products	Validation against rabbit test in products
PBMC (IL 1, IL 6)	Yes	Yes	Yes	?
Cryo PBMC (IL 6)	Missing	Missing	Unpublished	Missing
Whole blood	Yes	Yes	Yes	Yes for albumin, Factor VIII, IgG
Cryo whole blood	Yes	Yes	Yes	?
THP 1 (2 models)	Yes	Yes	Failed	Missing
MonoMac 6	Failed	Failed	Yes	Missing
Others (e.g., TLR transfected)	Missing	Missing	Missing	Missing

7.3 Global regulatory requirements for pyrogen and endotoxin testing

In the table below (Table 3), we discuss the current status quo of rFC and MAT regulatory tests across different pharmacopoeias and countries.

Table 3: MONOCYTE ACTIVATION TEST

Pharmacopoeia	Acceptance status	Details
European Pharmacopoeia	Accepted	Starting 1 January 2026, the rabbit pyrogen test (general chapter 2.6.8. Pyrogens) will disappear from Ph. Eur. texts. Manufacturers will choose methods based on the specific risks of the substance or product containing non-endotoxin pyrogens, using advanced alternatives to animal testing such as the monocyte-activation test or the bacterial endotoxins test (BET).
FDA	Accepted as Alternative	FDA accepts MAT as an alternative method to RPT[12]. Requires product-specific validation to establish whether a particular test substance or material is appropriate for evaluation of the monocyte activation method. Validation should include, but is not limited to, interference testing, accurate detection of pyrogen in individual test samples, and, for devices, the ability of the test system to provide direct contact to the monocytes.
Chinese Pharmacopoeia	Accepted as an alternative Method	MAT added in guideline Ch Ph 9301
Brazilian Pharmacopoeia	Accepted	CONCEA Res. 45 2019 recognizes MAT as a replacement of RPT and mandates its introduction by Oct 2024. The Brazilian Pharmacopoeia is working on a chapter dedicated to the MAT to replace RPT.

Pharmacopoeia	Acceptance status	Details
Indian Pharmacopoeia	Added as an alternative	A general chapter on the Monocyte Activation Test was added

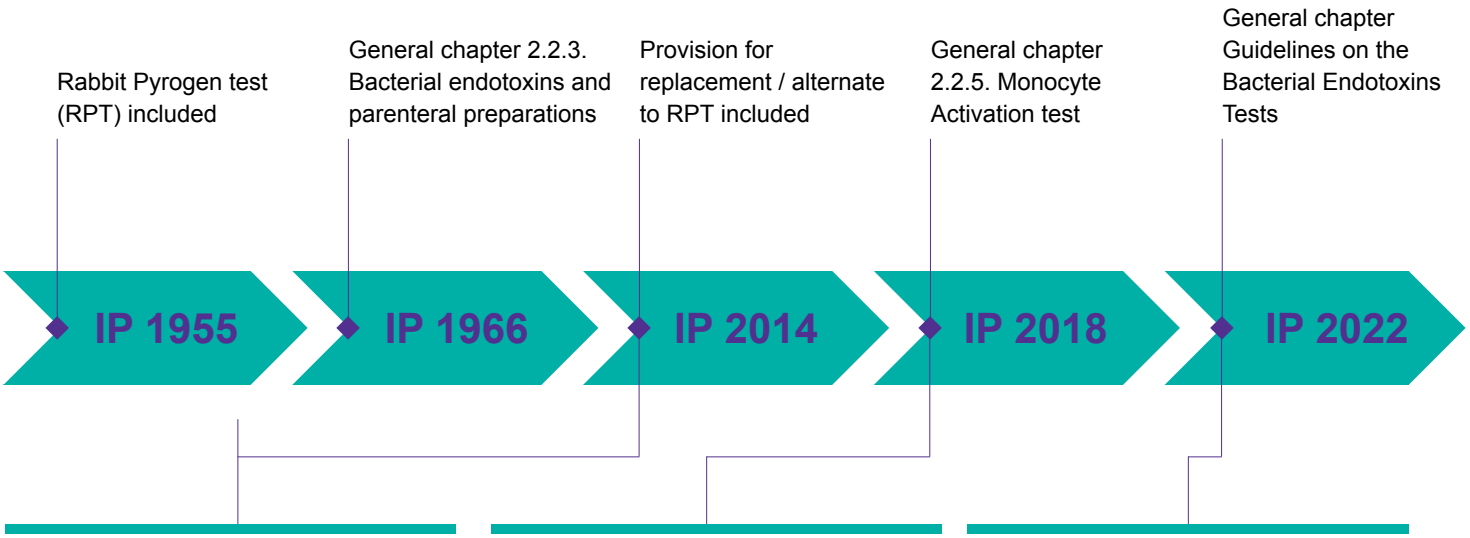
Recombinant Factor C

Pharmacopoeia	Acceptance status	Details
FDA	Accepted as compendial	Guidance for industry, Pyrogen and Endotoxin testing: Q&A; MAPP 5310.7; supports and accepts the use of rFC to replace LAL[11]. "If a manufacturer chooses to use a recombinant factor C-based assay, then method validation should be in accordance with the requirements of USP Chapter <85>, Bacterial Endotoxins Test, as described in the section for Photometric Quantitative Techniques, and USP Chapter <1225>, Validation of Compendial Procedures."
US Pharmacopeia	Accepted as an alternative	USP <1085.1> Use of Recombinant Reagents in Bacterial Endotoxins Test on hold (Jun 2021) SP <1223> Validation of Alternative Microbiological Methods USP General Chapter <86>[12] Bacterial Endotoxin Testing Using Recombinant Reagents.
European Pharmacopoeia EDQM	Accepted as compendial	Starting 1 January 2026, the rabbit pyrogen test (general chapter 2.6.8. Pyrogens) will disappear from Ph. Eur. texts. European Pharmacopoeia Commission (EPC) has announced that recombinant Factor C (rFC) – a synthetic, validated, animal-free solution – will be fully integrated as of Issue 13.1 of the Ph. Eur. as one of the seven methods that can be used to test for bacterial endotoxins (general chapter 2.6.14). Manufacturers will choose methods based on the specific risks of the substance or product containing non-endotoxin pyrogens, using advanced alternatives to animal testing such as the monocyte-activation test or the bacterial endotoxins test (BET).
Japanese Pharmacopeia	Accepted as an alternative	HP G4-4-180 Bacterial Endotoxins Test & Alternative Methods using Recombinant Protein-reagents for Endotoxin Assay[13]
Korean Pharmacopoeia	Accepted as compendial	In 2022 completed a comparison study rFC vs. LAL and confirmed to become part of the KP in 2023
Chinese Pharmacopoeia	Accepted as an alternative	ChP 1143 Bacterial Endotoxins Test ChP 9251 Guideline for BET Application (Introduction of the rFC)
Indian Pharmacopeia	Draft General Chapter Guidelines on the Bacterial Endotoxins Tests allows the use of alternate methodology equivalent to or better than validated pharmacopoeial methods	Draft General Chapter Guidelines on the Bacterial Endotoxins Tests[14]

7.4 Indian regulatory requirements for pyrogen testing

The Indian pharmacopoeia has brought in several changes to the IP in the space of pyrogen testing (Figure 1).

Figure 1: Pyrogenicity testing in the Indian Pharmacopeia



BET:

- Vaccines: 10
- DNA therapeutics: 19
- Chemical and veterinary products: 49
- Blood and blood related products: 01

BET or RPT:

- Blood and blood related products: 09

BET:

- Vaccines: 12
- DNA therapeutics: 06
- Chemical and veterinary products: 85
- Blood and blood related products: 01

BET or RPT:

- Vaccines: 17
- Blood and blood related products: 01

BET:

- Vaccines: 12
- DNA therapeutics: 38
- Chemical and veterinary products: 344
- Blood and blood related products: 02

BET or RPT:

- Vaccines: 24
- Blood and blood related products: 09

In 2023, IPC released the d1 for comments to General Chapter Guidelines on bacterial endotoxin tests[15]. While the draft proposal did not include a specific mention of rFC and MAT, it did include a section titled 'Alternative Test Methods' which included the following: "The methods mentioned in Bacterial Endotoxin (2.2.3) for the detection of bacterial endotoxins (gel-clot limits test, semi quantitative gel-clot, kinetic turbidimetric, kinetic chromogenic and endpoint chromogenic) are considered to be validated. However, a laboratory may choose to use an alternative method that is not mentioned in Bacterial Endotoxin (2.2.3). If such a choice is made, the alternate test for the detection of bacterial endotoxins must be fully validated to ensure that decisions made using the alternate methodology are equivalent to or better than decisions made using the validated pharmacopoeial methods and ultimately approved by the appropriate regulatory authority. This incorporation provides a window of opportunity for using rFC and MAT tests for endotoxin and pyrogen testing.

In addition, an IP General chapter on MAT was introduced in IP 2018: 2.2.25. This provides an indication of the receptiveness of the IPC towards these new methods.

7.5 Challenges

1. Cost

Cost is currently a factor in the adoption of rFC and MAT tests. There are a few commercially available vendors for the production of rFC and MAT kits; however, the cost of the kits compared to the LAL gel clot method is a significant deterrent.

Name of Company	rFC/rCR kit	MAT kit
Lonza	✓	✓
Charles River	✓	
Eurofins	✓	
CapeCod	✓	
Sigma-Aldrich		✓
BioMérieux	✓	
MAT Research		✓

2. Standardisation challenges

Since the MAT assay is based on whole blood or cryopreserved whole blood, donor-specific blood characteristics may lead to donor-to-donor variability. Variability in cell lines can also affect reproducibility and sensitivity.

3. Complexity

While assays based on isolated monocytes or monocytic cell lines would exhibit less donor variability, the process of isolation, culture maintenance, assay set-up, etc., for the MAT test's cytokine analysis requires a controlled laboratory setup, trained personnel, and specialized equipment.

4. Time factor

MAT is more time-intensive than LAL due to its cytokine release and detection steps.

5. Training

Adopting the MAT test requires hands-on training, which is not currently provided by any private or government lab in India. Also, there is a lack of clarity in the use of different technologies that are available under MAT.

7.6 Key Recommendations

1. **Production of indigenous reagents associated with the rFC and MAT tests to reduce costs**

   Currently, reagents such as IL-6 and recombinant proteins are not manufactured in India. Importing these reagents raises the cost of the kit and assay in general.

2. **Ensuring a continuous supply chain for kits/reagents**

   As there are few vendors in this space, an uninterrupted supply of reagents/kits/products and more players/suppliers in the market would be critical to build confidence in adopting these methods.

3. **Mapping the market size of pyrogen testing in India**

   Ultimately, higher demand would assist in bringing down costs. This would help in tapping this sector and incentivising Indian start-ups and companies to invest in this space.

4. **Documentation of the global standardisation efforts of these tests**

   Standardisation of the assays, including those that use whole blood and cell lines for MAT assays, would be essential to reduce variability and increase consistency of production. Documenting and increasing access to the global efforts in this direction, including guidelines by WHO, EDQM, etc., could help in this direction.

5. **Clear regulatory guidance and incentives**

   Several stakeholders and end-users show hesitance for adoption of these methods due to lack of clear regulatory guidance. Clarifying the regulatory language in terms of acceptance of these methods could increase uptake, and thus scale and hereby reduce the associated costs.

References:

1. *Fennrich S, Hennig U, Toliashvili L, Schlensak C, Wendel HP, Stoppelkamp S. More than 70 years of pyrogen detection: Current state and future perspectives. Altern Lab Anim. 2016 July;44(3):239–53.*
2. *Levin J, Bang FB. A description of cellular coagulation in the limulus. Bull Johns Hopkins Hosp. 1964 Oct;115:337–45.*
3. *Levin J, Bang FB. The role of endotoxin in the extracellular coagulation of limulus blood. Bull Johns Hopkins Hosp. 1964 Sept;115:265–74.*
4. *Dawson, M. Interference with the LAL test and how to address it. LAL Update. 2005;22(3):1–5.*
5. *Morita T, Tanaka S, Nakamura T, Iwanaga S. A new (1 → 3)-β-D-glucan-mediated coagulation pathway found in limulus amebocytes. FEBS Letters. 1981 July 6;129(2):318–21.*
6. *Bolden JS, Warburton RE, Phelan R, Murphy M, Smith KR, De Felippis MR, et al. Endotoxin recovery using limulus amebocyte lysate (LAL) assay. Biologicals. 2016 Sept;44(5):434–40.*
7. *Anderson RL, Watson WH, Chabot CC. Sublethal behavioral and physiological effects of the biomedical bleeding process on the American horseshoe crab, Limulus polyphemus. Biol Bull. 2013 Dec;225(3):137–51.*
8. *Marius M, Vacher F, Bonnevay T. Comparison of bacterial endotoxin testing methods in purified pharmaceutical water matrices. Biologicals. 2020 Sept;67:49–55.*
9. *Bolden J, Smith K. Application of recombinant factor C reagent for the detection of bacterial endotoxins in pharmaceutical products. PDA J Pharm Sci Technol. 2017;71(5):405–12.*
10. *Hartung T. Pyrogen testing revisited on occasion of the 25th anniversary of the whole blood monocyte activation test. ALTEX. 2021;38(1):3–19.*
11. *FDA. Guidance for Industry: Pyrogen and Endotoxins Testing: Questions and Answers. 2024. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-pyrogen-and-endotoxins-testing-questions-and-answers*
12. *Bacterial Endotoxins Test Using Recombinant Reagents. USP-NF. 2023. Available from: https://www.uspnf.com/notices/86-bet-using-recombinant-tests-gen-annc-20230822*
13. *Pharmaceuticals and Medical Devices Agency. Bacterial Endotoxins Test and alternative methods using recombinant protein reagents for endotoxin assay. Available from: https://www.pmda.go.jp/files/000231653.pdf*
14. *Indian Pharmacopoeia. Draft proposal for comments and inclusion in the Indian Pharmacopoeia: General chapter guidelines on the bacterial endotoxins tests. 2023. Available from: https://www.ipc.gov.in/images/Guidelines_on_Bacterial_Endotoxins_Tests_Version_2.0_20.01.2023.pdf*

Ecosystem-level Recommendations for NAMs Adoption through Institutional, Regulatory, and Capacity-Building Initiatives

8

8.1 Executive Summary

At the level of the ecosystem involving the adoption of NAMs, several steps can be taken to raise awareness, improve collaborations between different stakeholders and standardise the process of adoption of NAMs. This chapter details five such approaches, primarily concerning the set up of a central NAMs agency, a multi-stake holder consortium with an industry chapter, establishing centres of excellence, training and skill building and focus on an indigenous supply chain. A systematic implementation of these recommendations has been outlined, aiming to aid the expedition of NAMs application in the Indian context.

8.2 Introduction

The ecosystem-level recommendations for all the stakeholders concerning the adoption of NAMs in India can be broadly classified into the following:

1. Establishment of a Central NAMs agency
2. Multi-stakeholder consortium with an industry chapter
3. Setting up Centres of Excellence (CoEs)
4. Training and Skill Building
5. Setting up an indigenous supply chain

8.3 Key recommendations

8.3.1 Establishment of a Central NAMs agency

Rationale:

The FDA's proposed central NAMs office represents a strategic shift from a fragmented approach towards methodical validation and implementation with respect to NAMs.

Key functions:

1. Establishment of validation centres modelled on EURL ECVAM (European Union Reference Laboratory for Alternatives to Animal Testing and JaCVAM (Japanese Center for the Validation of Alternative Methods).
2. Establishment of a knowledge database, acting as a central repository for NAMs efforts and tests, following FAIR principles (Findable, Accessible, Interoperable, Reusable), similar to the FDA's approach (FDA ISTAND, 2021).
3. Ensuring data transparency and metrics tracking functions by establishing a centralised, FAIR-compliant database to track NAMs adoption rates, adoption rates, reductions in animal use, and industry uptake, analogous to systems implemented by EURL ECVAM.
4. Promote increased adoption of NAMs by pharmaceutical companies through consistent dissemination of studies, programmes and workshops highlighting the importance of adoption of NAMs.
5. Encourage indigenous development and manufacturing of NAMs through developing more financial pathways in the private sector in partnership with the Central Agency to develop a phased approach (such as Make in India and the Draft National Deep Tech Startup Policy) in creating a sustainable indigenous manufacturing ecosystem for NAMs technologies.

Proposed structure:

The proposed Central Agency could be set up as an autonomous institution under the CDSCO.

8.3.2 Establishing a multi-stakeholder consortium with an industry chapter

Rationale:

Globally, consortia have played a significant role in propelling NAM technologies further.

Key functions:

1. Provide a common platform for industry, academia, and regulatory players to collaborate for catalysing the development and adoption of NAM technologies.
2. Facilitate public-private and industry-academia partnerships for NAMs development.
3. Creation of an **industry consortium chapter** to bring together key industry players in these sectors to collectively advocate for the 3Rs.

   This chapter will have the following functions:

   a. Awareness generation about the benefits of moving away from animal testing: reduction of costs; increased ability to invest in more human-relevant research methods, etc., within the pharmaceutical industry.

   b. Focus on creating more awareness about 3R opportunities within the pharmaceutical industry in India by collating and disseminating best practices that have already been/ can be adopted to reduce the number of animal tests.

   c. Leverage the Indian Pharmaceutical Association (IPA) to encourage more and more pharmaceutical companies to reduce their dependence on animal testing by making them aware of the potential cost savings that the shift could entail.

   d. Collectively approach regulators to advocate for reduction and replacement opportunities by seeking meetings, submitting representations, and inviting them to workshops and webinars organised by the Consortium.

4. Validation of NAMs: Through the identification of labs working on NAMs in India, the consortium can collate information and priority areas of technologically ready NAMs and begin validation efforts by encouraging the development of technologies meeting global scientific and regulatory standards.
5. Mobilisation of funding for the development of NAMs by academia and industry through philanthropic funding agencies like BMGF.
6. Encourage adoption and development of NAMs by CROs and the pharmaceutical industry: Through engagement with multiple stakeholders, barriers to adoption can be identified. Post this, CROs can be incentivised through awareness programmes like High-throughput Screening methods as incorporated by NCRIS in Australia. Programmes like the National Tech Startup Policy under the Office of the Principal Scientific Advisor can be leveraged to provide a robust starting point to encourage CROs in adopting NAMs.
7. Identify labs that have the potential to develop competitive NAMs technologies and promote the indigenous development and manufacturing of NAMs technologies by facilitating global partnerships and providing funding.
8. Civil society groups (like Humane World for Animals) could advocate for greater data transparency from agencies like the Committee for Control and Supervision of Experiments on Animals (CCSEA) as far as the extent and nature of experimentation on animals is concerned.
9. Advocacy for policy reform: Advocate for the revision of testing guidelines and for regulatory acceptance of data generated through validated NAMs. The Consortium can lead the movement in India towards the release of guidelines, guidance documents, etc., by engaging with the regulatory bodies like CDSCO and DCGI.

Proposed structure:

General Body - composed of all members.

Governing Body - elected by the general body. Functions: overseeing operations, monitoring progress, resource allocation to Working Groups, and decision-making

Working Groups - set up by the Governing Body for specific purposes. Example: Validation Working Group.

8.3.3 Setting up Centres of Excellence (CoEs)

Rationale:

Establishing dedicated Centres of Excellence (CoEs) can accelerate the development and adoption of NAMs by offering specialised infrastructure, training, and validation services.

Key functions:

1. **Creation of basic infrastructure for catalysing NAMs:** The CoEs can accelerate the transition towards NAMs by offering infrastructure such as cell repositories, state-of-the-art resources with high-throughput screening systems, organ-on-chip devices and subscription to industry-verified computation models and data systems.
2. **Encourage innovation:** Globally, many key players in the NAMs space have emerged from academic settings.
3. **Provision of services:** Academic labs (designated as CoEs) can adopt a service-provider model to ensure their financial viability and reduce dependence on grants. They can provide fee-based testing services, form contract research modules, and consulting services to the industry to monetise their research.
4. Facilitate industry-academia and government-academia partnerships.

5. Serve as validation centres.

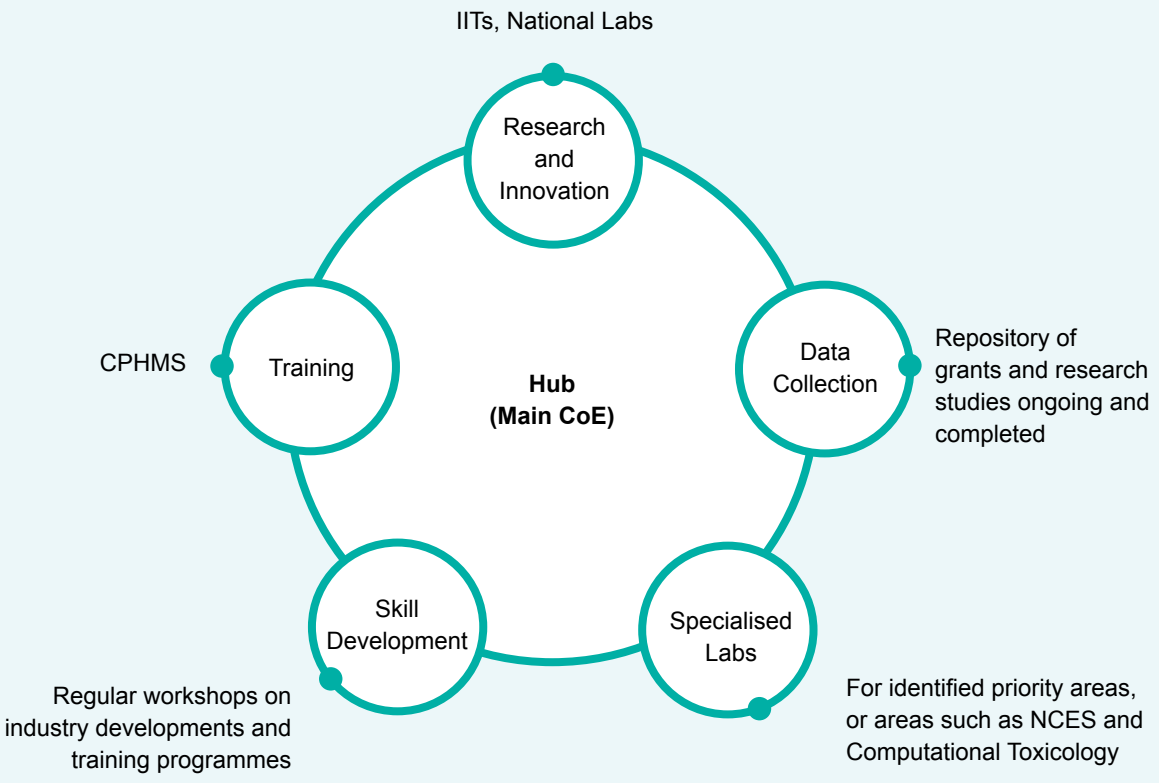


Fig 4. Proposed Hub and Spokes of the Centre of Excellence

In the proposed CoE, the following iteration of the Hub and Spoke Model is proposed:

Central Hub (Core CoE):

State-of-the-art resources: Large-scale laboratories equipped with high-throughput screening systems, organ-on-chip devices, and data repositories. The hub could define research agendas, manage collaborations (with industry, academia, regulators), and ensure quality control.

Spoke Institutions (Specialised Labs):

a. Research and innovation spokes: Located in IITs or national labs focusing on specialised NAMs research, e.g., computational toxicology.

b. Training and education spokes: Offer workshops, certification courses, and skill development programs for both academic and industry professionals.

8.3.4 Training and Skill Building

Rationale:

The adoption of NAMs needs to be encouraged by raising awareness and providing necessary training for their application in existing systems for drug discovery and development.

Key functions:

1. **CoEs can generate awareness on NAMs:** Organise sectoral and cross-sectoral events through awareness programs to highlight the benefits of using NAMs technologies in drug development and build industry-academia partnerships to facilitate technology transfer and validation of NAMs. The programmes can provide valuable data on expectations, barriers and opportunities for each stakeholder involved. Awareness of NAMs to the general public and other relevant stakeholders can be done through strategic dissemination of information through media campaigns, collaborations and engaging the public.
2. **Enable skill development:** CoEs can conduct workshops and short-term certificate courses on NAMs to create a skilled workforce.

8.3.5 Setting up an indigenous supply chain

Rationale:

As detailed in earlier chapters, the field of NAMs faces a bottleneck due to the absence of well-established cell repositories and indigenous availability of culture components and other amenities.

Key function:

Indigenous establishment of high quality and well-validated cell repository at CoEs for primary cells for liver, hepatocytes, and cells from different genetic backgrounds, with the cell repository being a hub with multiple spokes.

ANNEXURE - 1

Table 1: General Tox Studies for New Chemical Entity(NCEs)

S. No	Type of studies/ tissue type	Objective	Species	No.of Animals	Total No. of animals in Standard study	Parameters / End points of In vivo studies	Parameters/ in vitro end points	Alternatives if any	References/ Guidelines
1	Single dose toxicity testing	Acute systemic toxicity testing involves an assessment of the general toxic effects of a single or multiple doses of a chemical or product, with in 24 hours by particular route (oral, dermal, inhalation) and that occur during a subsequent 14/21 day observation period.	Rats/ Mice/ Dogs/ monkeys	Rodent: 5/ sex/group Non Rodent: 1/ sex/group	Rodents - 50 Non Rodents-10	Signs of intoxication, Effect on body weight,Gross pathological Changes	Murine fibroblast cell line, BALB/c 3T3 cells, clone 31 cytotocity for UDP, the ATC method, or FDP methods. Based on IC50 with 3T3, LD50 can be predicted and used.	in vitro alternative : GUIDANCE DOCUMENT ON USING CYTOTOXICITY TESTS TO ESTIMATE STARTING DOSES FOR ACUTE ORAL SYSTEMIC TOXICITY TESTS : OECD GD 129	1.ICH M3(R2)) CHMP/ ICH/646107/2008ICH M3 (R2) Non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals - Scientific guideline 2. OECD GD 129Guidance Document on Using Cytotoxicity Tests to Estimate Starting Doses for Acute Oral Systematic Toxicity Tests
2	7-14 day repeat dose DRF studies with Toxicokinetics	To study the toxicity potential and Toxicokinetics profile of the drug when administered over a short period of time by desired route (Oral/ Parenteral/Dermal). These studies serveto decide the right doses for the 4 week definitive studies	Rats/ Mice/ Dogs/ monkeys	Rodent: 6-10 /sex Non Rodent: 3/ sex	Rodents - 60-100 Non Rodents-30	Identification of Maximum Tolerated dose, Signs of intoxication, Effect on body weight,Gross pathological Changes, Microscopic examination of Affected organs, Toxicokinetics		No alternatives to test the systemic toxicity	1. ICH M3(R2)) CHMP/ ICH/646107/2008ICH M3 (R2) Non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals - Scientific guideline 2. ICHS3A NOTE FOR GUIDANCE ON TOXICOKINETICS: THE ASSESSMENT OF SYSTEMIC EXPOSURE IN TOXICITY STUDIES
3	4 weeks repeat dose Toxicity studies with Toxicokinetics followed by 2 weeks recovery period	To study the toxicity potential and Toxicokinetics profile of the drug when administered over a period of 4 weeks by desired route (Oral/ Parenteral/ Dermal). Also to understand the reversibility of observed effects or delayed onset of toxicity. These studies are aimed to determine target organs drug exposure, kinetics and to determine NOAEL which is crucial to select the first in human dose.	Rats/ Mice/ Dogs/ monkeys	Rodent: 10/ sex/group Non Rodent: 3/ sex/group	Rodents - 120 Non Rodents-32	Clinical signs, body weight changes, food or water intake, blood biochemistry, hematology,gross and histopathology of all viscera and tissues & Toxicokinetics		No alternatives to test the systemic toxicity	1.Note for Guidance on Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals (CPMP/ICH/286/95; ICH M3(R2)) 2. Guideline on repeated dose toxicity (CPMP/SWP/1042/99 Rev 1 Corr) EMA/CHMP/ICH/731268/1998 3.ICH M3(R2)) CHMP/ ICH/646107/2008ICH M3 (R2) Non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals - Scientific guideline 4.ICH S9 EMA/CHMP/ ICH/126642/2008 NONCLINICAL EVALUATION FOR ANTICANCER PHARMACEUTICALS 5. ICH S4 :Duration of Chronic Toxicity Testing in Animals (Rodent and Non Rodent Toxicity Testing) 6.Note for Guidance on Non-clinical Evaluation for anticancer Pharmaceuticals (EMEA/CHMP/ICH/646107/2008; ICHS9) 7. ICHS3A- NOTE FOR GUIDANCE ON TOXICOKINETICS: THE ASSESSMENT OF SYSTEMIC EXPOSURE IN TOXICITY STUDIES
4	13 weeks repeat dose Toxicity studies with Toxicokinetics followed by 4 weeks recovery period	To study the toxicity potential and Toxicokinetics profile of the drug when administered subchronically over a period of 13 weeks by desired route (Oral/ Parenteral/Dermal). Also to understand the reversibility of observed effects or delayed onset of toxicity. These studies are aimed to determine target organs drug exposure, kinetics and to determine NOAEL.	Rats/ Mice/ Dogs/ monkeys	Rodent: 10/ sex/group Non Rodent: 3-4 /sex/group	Rodents - 120 Non Rodents-40	Clinical signsof toxicity, body weight changes, food or water intake, blood biochemistry, hematology,Organ weights,urine analysis, gross and microscopic study of all viscera and tissues & Toxicokinetics		No alternatives to test the systemic toxicity	
5	26-52 weeks repeat dose Toxicity studies with Toxicokinetics followed by 4-8 weeks recovery period	To study the toxicity potential and Toxicokinetics profile of the drug when administered chronically over a period of 26-52 weeks by desired route (Oral/ Parenteral/Dermal). Also to understand the reversibility of observed effects or delayed onset of toxicity. These studies are aimed to determine target organs drug exposure, kinetics and to determine NOAEL.	Rats/ Mice/ Dogs/ monkeys	Rodent: 20/ sex/group Non Rodent: 4 / sex/group	Rodents - 200 Non Rodents-40	Clinical signsof toxicity, body weight changes, food or water intake, blood biochemistry, hematology,Organ weights,urine analysis, gross and microscopic study of all viscera and tissues & Toxicokinetics		No alternatives to test the systemic toxicity	

S. No	Type of studies/ tissue type	Objective	Species	No.of Animals	Total No. of animals in Standard study	Parameters / End points of In vivo studies	Parameters/ in vitro end points	Alternatives if any	References/ Guidelines
6	Carcinogenicity studies	This study provides information on the possible health hazards likely to arise from repeated exposure for a period lasting up to the entire lifespan of the species used.The study will provide information on the toxic effects of the substance, including potencial carcinogenicity indicate target organs and the possibility of accumulation. It can provide an estimate of the NOAEL for toxic effectsand , in the case of non-genotoxic carcinogens, for tumour responses, which can be used for establishing safety criteria for human exposure.	Rats/ Mice	Rodents : 50 / sex / Group	Rodents - 400	Target organs neoplasms/ Histopathology of target organs/ Clinical biochemistry including hormones	WoE factors: 1) HistopathologyCellular hypertrophy o Cellular hyperplasia o Persistent tissue injury and/ or chronic inflammation o Pre-neoplastic changes o Tumors 2) Hormonal effects hormone receptor binding, regulation of hormone levelsAtrophy o Hypertrophy o Hyperplasia 3) Secondary PharmacologySelectivity o Off-target potential o Carcinogenic risk (e.g., binding to nuclear receptors) 4) Additional investigative studies or analyses of specimens collected from prior studies (e.g., special histochemical stains, molecular biomarkers, serum hormone levels, immune cell function, in vitro or in vivo test systems, data from emerging technologies) 5) Clinical data generated to inform human mechanistic relevance at therapeutic doses and exposures (e.g., urine drug concentrations and evidence of crystal formation, targeted measurements of clinical plasma hormonal alterations, human imaging data)	"REDUCTION: In vitro Carcinogenicity tests are available - Syrian hamster embryo (SHE) cells - Cell Transformation assay (TCA) These should not be used as stand alone methods. They should always be used in combination with other complementary information sources. REPLACEMENT EFFORTS: ""WoE evidence to assess the relevance of 2 -yr rat study"	Note for Guidance on Carcinogenicity: Testing for Carcinogenicity of Pharmaceuticals (CPMP/ICH/299/95; ICH S1B (R1)) In vitro: ADDENDUM to ICH S1B (R1): https://www.fda.gov/media/152777/download
7	In vivo Micronucleus test	This mammalian in vivo micronucleus test is especially relevant to assess mutagenic hazard , in that it allows consideration factors of in vivo metabolism, pharmacokinetics and DNA repair processes although these may vary among species,among tissues and among genetic end points. An in vivo assay ia also useful for further investigation of a mutagenic effectdetected by an in vitro system	Rats/ Mice	Rodents : 5 / sex / Group	Rodents - 50	Bone marrow- immature erythrocytes or in peripheral blood to detect clastogens/aneuploidy inducers		REDUCTION: In vitro Mutagenicity : Bacterial mutagenicity test (Ames test) with Salmonella typhinurium and Eschericia coli; Mouse lymphoma assay; the hypoxanthine phospho ribosyl tranferase (HPRT) gene is on the X chromosome of the mammalian cells, and it is used as a model gene to investigate gene mutations in mammalian cell lines. Invitro Micronucleus Test using human peripheral blood lymphocytes is available and is being used extensively.In vivo study is used to confirm any positive findings.	ICH Guideline S2(R1) on genotoxicity testing and data interpretation for pharmaceuticals intended for human use (EMA/CHMP/ICH/126642/2008)
8	In vivo Chromosomal aberration test	This test is used to detect chromosomal aberrations that may result from clastogenic events	Rats/ Mice	6/ sex/ group	Rodents - 60	Chromosomal aberrations in peripheral lymphocytes / metaphase cells in bone marrow)		REDUCTION: In vitro chromosomal aberration test using human peripheral blood lymphocytes or V79 cells is available and is being used extensively	
9	Fertility and general reproductive perfomance assessment	To study male and female fertility and reproductive performance	Rats/ Mice	10/ sex/ group	Rodents - 100	Male: weights of each testis,epididymis, sperm motility and morphology, Histopathology of testis Female:Mating behaviour, progress of gestation,parturition, gross and histopathology of affected organs		No alternatives to test the systemic toxicity	Note for Guidance on the Detection of Toxicity to reproduction for Medicinal products & Toxicity to Male Fertility (CPMP/ICH/386/95; ICH S5(R2))
10	Teretology or Embryo-fetal toxicity Studies / Segment II	To study the toxicity potential of drug to cause damage to the developing embryo	Rats/ Rabbits	20 Females/ Group-Rats 6 females/ group -Rabbits	Rats - 100 Rabbits-30	Dams: signs of toxicity,examination of uterus,overies.No of corpora lutea,implantation sites Foetuses: Total number,gender,body length, weight gross or visceral or skeletal abnormalities		No alternatives to test the systemic toxicity	ICH S5 (R3) Guideline on detection of reproductive and developmental toxicity for human pharmaceuticals - Scientific guideline

S. No	Type of studies/ tissue type	Objective	Species	No.of Animals	Total No. of animals in Standard study	Parameters / End points of In vivo studies	Parameters/ in vitro end points	Alternatives if any	References/ Guidelines
11	Prenatal and postnatal deveopmental toxicity study (PPND)/ Segment III	To evaluate drug effects during the last trimester of pregnancy and the period of lactation	Rats	20 Females/ Group	Rats - 100	F1 litter:Growth till sexual maturity,pairing, gestation,patrurition and lactation F2 Pups: Clinical signsTotal number,gender,body length, weight gross or visceral or skeletal abnormalities		No alternatives to test the systemic toxicity. DevTox NAMs to complement weight of evidence (WoE) assessments are available but not accepted officially by regulators - https://doi.org/10.1016/j.reprotox.2024.108686	
12	Photo toxicity testing	To determine the skin/dermal irritation potential of drug for treatment of inflammation diseases such as Psoriasis, atopic dermatitis in Guinea pigs	Guinea pigs	3/sex/group	G. Pigs- 30	Erythema, edema,early inflammatory markers in skin or lymph node reactions	In vitro end point: relative reduction in viability of cells exposed to the chemical in the presence versus absence of light.	REDUCTION: In vitro 3T3 NRU photo toxicity test. In vivo study is conducted to confirm the results from in vitro study REPLACEMENT: Tied strategy	ICH guideline S10: Guidance on photosafety evaluationof pharmaceuticals (EMA/CHMP/ ICH/752211/2012)
13	Central Nervous system safety evaluation	To evaluate the toxicity potential of drug on the central nervous system	Rats/ Mice	5/sex/group	Rodents-50	Motor activity, neurobehavioural changes,coordination,sensory/ motor reflexes, FOB tests (Grip strenth,stereotypy,Startle response, pain response, irritability)/ Irwin test(undisturbed behavioral observationsand invasive tests such as reflex testing)	OECD 377 Neurodevelopmental process at cellular level: - proliferation -migration -neurite growth -synaptogenesis -myelination -apoptosis Nervous system connectivity -Structural -Neurochemical -Neurophysiological	REPLACEMENT: A battery of in vitro tests to test developmental neurotoxicity for chemicals published by oecd Reference: https://one.oecd.org/document/ENV/CBC/MONO(2023)13/en/pd In silico tests available but not in regulatory practice see https://pmc.ncbi.nlm.nih.gov/articles/PMC9281386/pdf/nihms-1795109.pdf	ICH S7A- SAFETY PHARMACOLOGY STUDIES FOR HUMAN PHARMACEUTICALS
14	Cardiovascular system safety evaluation	To evaluate the toxicity potential of drug on the cardiovascular system	Rats/dogs/ minipigs/ monkeys	Rodents : 3/ sex/group, Non rodents: 1/ sex/group	Rodents: 30 Non rodents:10	Blood pressure, Heart rate, ECG; Follow up parameters Cardiac output, ventricular contractility, vascular resistance	Delayed ventricular potential (pro-arrythmia risk) measured via the following: 1) Ionic currents in cardiomyocytes 2)Action potential parameters in isolated cardiac preparations 3)Proarrhythmic effects measured in isolated cardiac preparations (QT interval) Reference: ICH S7B	REDUCTION: In vitro HERG assay is used to assess the HERG chanel inhibition potential, but to assess the heart rate,ECG,BP stand alone in vivo study is required REPLACEMENT: ICH S7B for pro-arrythmia	1. ICH S7A- SAFETY PHARMACOLOGY STUDIES FOR HUMAN PHARMACEUTICALS 2. Note for Guidance on the Non-clinical Evaluation of the Potential for Delayed Ventricular Repolarisation (QT Interval Prolongation) by Human Pharmaceuticals (CPMP/ICH/423/02; ICH S7B)
15	Respiratory system safety evaluation	To evaluate the toxicity potential of drug on the respiratory system	Rats/dogs/ monkeys	Rodents : 5 /group, Non rodents: 3 / group	Rodents: 30 Non rodents:15	Respiratory rate,tidal volume, hemoglobin oxygen saturation; Follow up study parameters such as airway resistance,pulmonary arterial pressure, blood gases, Blood pH		Airway resistance,compliance, pulmonary arterial pressure, blood gases, Blood pH Various commercially available respiratory NAMs used in pharma (MucilAir, EPiAlveolar, EpiAirway etc but nothing approved by ICH	ICH S7A- SAFETY PHARMACOLOGY STUDIES FOR HUMAN PHARMACEUTICALS
16	In vivo Pharmacokinetics	To assess absorption, distribution, metabolism and excretion of drug and its time course	Rats/Mice/ dogs/ monkeys	6-10 /sex/ group- Rodents 3 per sex per group- Non rodents	Rodents-80 Non Rodents-24	Pharmacokinetic paramters such as Cmax, Tmax, t1/2, AUC etc., Tissue distribution studies Absorption, distribution,Metobolism and excretion		REDUCTION: Standard in vitro models for comparison of in vitro metabolism across species, effect on enzyme P450 activity, protein binding, absorption using Caco-2 cells Standard in vivo models for single dose pharmacokinetic studies in rodent and non-rodent. Guideline on the investigation of drug interaction (CPMP/EWP/560/95-Rev.1 Corr.): in vitro approaches are preferred.	Note for Guidance on Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals (CPMP/ICH/286/95; ICH M3(R2)) Note for Guidance on Pharmacokinetics: Repeated Dose Tissue Distribution Studies (CPMP/ICH/385/95; ICHS3B)
17	Local Tolerance studies (Single/ repeated)	To support human exposure to a compound (both active substance and excipients) at contact sites of the body	Rabbit	3-5 per group	Rabbits-25	Draize score at the site of injection for inflammation,Macroscopic and microscopic evaluation of injection site		It is prefered to evaluate local tolerance by the intended therapeutic route as part of the general toxicity studies. Stand alone studies are not recommended	Guideline on non-clinical local tolerance testing (EMA/CHMP/SWP/2145/2000-Rev.1); Updated in 2016 (effective 01/05/2016).
18	Skin sensitization studies	Identifies the potential for a substance to cause allergic contact dermatitis	Guinea pigs	10-20 animals for test 5-10 animals for control	Guinea pigs-90	Magnusson and Kligman grading scale evaluation of challenge patch test reaction, Histopathological examination	1) Activation of dendritic cells 2) Activation of keratinocytes OECD 442C,D,E	REPLACEMENT: Defined Approached to skin sensitsation published by oecd https://www.oecd-ilibrary.org/environment/guideline-no-497-defined-approaches-on-skin-sensitisation_b92879a4-en	OECD 406 (Skin Sensitization)

Table 2: Non clinical practices for assessing potential hepatotoxicity in human and alternatives

Current Non clinical practices for assessing potential hepatotoxicity in human								Alternatives (early specific screening of hepatotoxicity)				
S. No	**Type of studies**	**Objective**	**Species**	**No.of Animals**	**Total No. of animals in standard study**	**Parameters / End points of In vivo studies**	**Parameters / End points of In vitro studies**	**In Vitro cytotoxicity screening assays REDUCTION**	**Invitro Covalent binding assays REDUCTION**	**Toxico genomics/ proteomics REDUCTION**	**References/ Guidelines/ Concept paper**	**REPLACE-MENT EFFORTS**
1	Tier-1 Toxicity studies	Standard screening studies and tests to dtect hepatic change	Rodents- 6 months study Dog/ Monkey- 1 year study	Rodents : 10 /group, Non Rodents : 3-5/group Multi doses are selected to produce both limit-ing toxicity and a NOEL	Rodents-50 Non Rodents-25	Broad range of parameters are evaluated at various time points to insure detection and characterization of hepatic changes (clinical chemistry,In life findinmgs,Enzymatic induction, Liver macroscopic and histopathology)	Albumin production rate, urea synthesis rate, and drug metabolizing relevant gene expression levels	Cultured hepatocytes; Precission cut liver slices (Beta -oxidation if the compound induces mitochondrial toxicity) Hepatocellular Necrosis(cultured hepatocytes, cell death end points(enzymes,leakage/ dye exclusion) Cell viability and function, mitochondrial impairment, oxidative stress, bile acid excretion etc - all available but not regulated. E.g. https://www.eurofinsdiscovery.com/solution/hepatotoxicity	For drug induced liver injury binding of drug to liver proteins (immuno histo chemical techniques- selective binding to microsome-42-44 kDa protein,cytosolic 56-58 Kda	Microarray techniques- detection of changesin gene expression Nucleotide array plaform cDNAs/ RNAs harvested from hepatocytes Proteomics(2D gel electrophoresis, protein arrays or laser desorption techniques)	FDA concept paper 2000- Nonclinical assessment of potential hepatotoxicants	Liver spheroids Liver organoids Liver organ on chip
2	Tier-2 toxicity tests	Specialized studies for further characterize a hepatic change in non clinical isuuses				Ultra structural pathology, Morphometrics, Histological special strains & antiboy detection						
3	Systemic toxicity studies	For reporting hepatic changes/ toxicity	Rodents Non rodents	Rodents : 10 /group, Non Rodents : 3-5/group	Rodents-50 Non Rodents-25	Clinical signs of toxicity, Clinical chemistry- AST, ALT,Bilurubin; Organ weights- Liver, Macroscopic and microscopic evaluation of Liver					ICH S6 (R1), ICH M3 (R2), NDCT rules	

Table 3: ADME/ PK studies -Commercially available NAMs

S. No	Commercially available NAMs	Name of the company	Website link
1	HEPATOMUNE® Cultures (2D invitro), HEPATOMUNE® Cultures (2D invitro), HEPATOPAC Cultures (2D invitro)	BioIVT	bioivt.com
2	Gut-liver Multi-chip Dual-organ plates by the PhysioMimix®, LiverChip-3D model cultured in Multi-chip Liver plates by the PhysioMimix® OOC range of microphysiological systems	CN Bio	https://cn-bio.com/
3	3D liver on chip	Emulate Bio	http://www.emulatebio.com/
4	3D kidney on chip	Emulate Bio	http://www.emulatebio.com/
5	3D colon-intestine chip	Emulate Bio	http://www.emulatebio.com/
6	Duodenum intestine chip	Emulate Bio	http://www.emulatebio.com/
7	Liver on chip	Tissuse (HUMIMIC)	https://www.tissuse.com/en/about-us/
8	3D Intestine – Liver – Kidney – Neuro on chip	Tissuse (HUMIMIC)	https://www.tissuse.com/en/about-us/
9	3D Intestine – Liver – Kidney – Neuro + Blood-Brain-Barrier on chip	Tissuse (HUMIMIC)	https://www.tissuse.com/en/about-us/
10	3D Lung – Liver on chip	Tissuse (HUMIMIC)	https://www.tissuse.com/en/about-us/
11	3D Intestine – Liver on chip	Tissuse (HUMIMIC)	https://www.tissuse.com/en/about-us/
12	Kidney (glomerulus) – Liver on chip	Tissuse (HUMIMIC)	https://www.tissuse.com/en/about-us/
13	2D- invitro liver hepatocytes	Primacyt	primacyt.com
14	Liver, kidney, intestine, bile duct on a chip	AZAR	https://azar-innovations.com/
15	3D heart-liver-neuron-skeletal muscle on chip	Hesperos	https://hesperosinc.com/
16	Human primary hepatocytes	AnaBios	https://anabios.com/
17	3D InSight™ Liver Safety solutions- spheroids	InSphero	https://insphero.com/
18	DILI mechanistic panel	Eurofins	https://www.eurofinsdiscovery.com/solution/hepatotoxicity
19	EpiKidney™ (beta)-3D	MatTek Life Sciences	http://www.mattek.com/
20	EpiIntestinal™- 3D	MatTek Life Sciences	http://www.mattek.com/
21	3D Proximal TubuleaProximate™, aProximate™	Newcells Biotech	https://newcellsbiotech.co.uk/
22	human telomerized cells from different tissues and organs (e.g. skin, cornea, lung, vascular system, kidney, placenta), Kidney RPTEC/TERT1, PODO/SVTERT152, PODO/TERT256	Evercyte	https://evercyte.com/
23	2D human primary intestinal epithelial cell model systems, RepliGut Systems	Altis Biosystems	https://www.altisbiosystems.com
24	Colon-on-a-plate®- in vitro simulation of the physiology and microbiology of the colon, SHIME®, Diamod®	Prodigest	https://prodigest.eu/en

ANNEXURE - 2

Table 1: List of initiatives by agencies that have been approved within the framework of regulatory requirements

Regulatory Agency	Initiatives & Activities	Description	References
Food and Drug Administration (FDA), USA	AI4TOX program which has the following sub-programs: AnimalGAN & SafetAI	AnimalGAN aims to create virtual animal models or digital twins powered by generative AI to predict the toxicological effects of untested substances. SafetAI is designed to build a collection of advanced AI models that assess key toxicological endpoints essential for evaluating the safety of drug candidates before they enter clinical trials.	https://www.fda.gov/about-fda/nctr-research-focus-areas/artificial-intelligence
National Toxicology Program, USA	Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)	ICCVAM is a permanent committee composed of representatives from U.S. federal regulatory and research agencies. These regulatory and research agencies require, use, generate, or disseminate toxicological and safety testing information.	https://ntp.niehs.nih.gov/whatwestudy/niceatm/iccvam
European Commission, EU	European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL)	EU-NETVAL is an EU Reference Laboratory for alternatives to animal testing network of specialized laboratories set up in response to EU Directive 2010/63/EU on the protection of animals used for scientific purposes. It has worked on validation of NAMs for endocrine disruption.	https://joint-research-centre.ec.europa.eu/projects-and-activities/reference-and-measurement/european-union-reference-laboratories/eu-reference-laboratory-alternatives-animal-testing-eurl-ecvam/alternative-methods-toxicity-testing/european-union-network-laboratories-validation-alternative-methods_en
National Institute of Health Sciences (NIHS), Japan	Japanese Center for the Validation of Alternative Methods (JaCVAM)	JaCVAM promotes the development, validation, and regulatory acceptance of non-animal testing methods.	https://www.jacvam.go.jp/en/
National Medical Products Administration (NMPA), China	Zhejiang Institute for Food and Drug Control (ZJIFDC) working in collaboration with the Institute for In Vitro Sciences (IIVS)	ZJIFDC's work in alternative testing is a key component of China's efforts to implement alternative methods and support future changes in regulations regarding animal testing.	https://www.zjyj.org.cn/
Food and Drug Administration (FDA), USA	Innovative Science and Technology Approaches for New drugs (ISTAND) Program	ISTAND pilot program is designed to support the qualification of novel drug development tools (DDTs) that fall outside the scope of existing qualification pathways but hold potential to enhance drug evaluation and regulatory decision-making.	https://www.fda.gov/drugs/drug-development-tool-ddt-qualification-programs/innovative-science-and-technology-approaches-new-drugs-istand-program?hss_channel=lcp-9389491#:~:text=The%20Innovative%20Science%20and%20Technology%20Approaches%20for%20New,but%20may%20still%20be%20beneficial%20for%20drug%20development

Regulatory Agency	Initiatives & Activities	Description	References
Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)	ICCVAM's Method Developers Forum	ICCVAM's Method Developers Forum (MDF) is a recurring event held virtually to facilitate collaboration between developers of NAMs and regulatory agencies. The first ICCVAM Method Developers Forum was held virtually in August 2024 and focused on new approach methodologies NAMs for carcinogenicity testing. The webinar featured presentations by selected method developers describing their methods and proposing how they may be useful for regulatory and/or industry stakeholders.	https://ntp.niehs.nih.gov/whatwestudy/niceatm/resources-for-test-method-developers/method-developers-forums/2024
National Institute of Health (NIH)	Complement Animal Research In Experimentation (Complement-ARIE) Program	The Complement-ARIE program will help to improve the ongoing efforts related to NAMs, while identifying opportunities for innovation and coordination. Complement-ARIE will advance understanding of human health and disease by providing a range of ready and standardized biomedical research models. Developing these models will require expertise in disease research, personalized medicine, and in screening therapeutics for safety and effectiveness. Complement-ARIE will bring these expertise together through a consortium of researchers.	https://commonfund.nih.gov/complementarie
European Medicines Agency (EMA)	3Rs working Party (3RsWP)	The 3Rs Working Party (3RsWP) is a joint working party of the Committee for Medicinal Products for Human Use (CHMP) and the Committee for Veterinary Medicinal Products (CVMP). It advises these committees on all the matters concerning the use of animals in the regulatory testing of medicines with particular focus on the application of the so called 3Rs principles- replace, reduce and refine.	https://www.ema.europa.eu/en/committees/working-parties-other-groups/chmp/3rs-working-party
European Medicines Agency (EMA)	Non-clinical Working party (NcWP)	The Non-clinical Working Party (NcWP) was set up by the Committee for Medicinal Products for Human Use (CHMP) to provide advice and recommendations directly or indirectly related to non-clinical issues.	https://www.ema.europa.eu/en/committees/working-parties-other-groups/chmp/non-clinical-working-party

Table 2: Table listing the global commercial technologies in NAMs used for studying hepatic toxicity

In vivo endpoints	In vitro endpoints	Guidelines	Commercially available NAMs	Type of NAM	Name of the company and location
Detection of DILI: Detection and characterization of hepatic changes (clinical chemistry, In life findings, enzymatic induction, Liver macroscopic and histopathology)	**Detection of DILI:** 1) Albumin production rate, urea synthesis rate 2) Alanine aminotransferase (ALT), Lactate dehydrogenase (LDH) activity 3) miR122, cytokines production and stability over time period of 14 days 4) Hepatocellular Necrosis, cell death end points (enzymes, leakage/ dye exclusion) 5) ATP production assays like CTG assay 6) Liver phase I/II metabolizing enzymes capability (measure of CYP450 enzymatic capacity and induction)	FDA guidance on in vitro methods	HEPATOMUNE® Cultures	2D	BioIVT (Westbury,NewYork)
			Gut-liver Multi-chip_	3D	CN Bio (Cambridge, UK)
			liver on chip	3D	Emulate Bio (Boston, Massachusetts)
			Liver on chip	3D	Quris Ai (Boston)
			Liver spheroids	3D	Sai Lifesciences (Malkajgiri District, Telengana)
			Liver organoids	3D	Sai Lifesciences (Malkajgiri District, Telengana)
			Liver on chip	3D	Vivodyne (San Fransisco, USA)
			Liver on chip	3D	Tissuse (HUMIMIC) (Berlin, Germany)
			InSight™ Liver Safety solutions- spheroids	3D	InSphero (Schlieren, Switzerland)
			invitro liver hepatocytes	2D	Primacyt (Schwerin, Germany)
			HUREL® Micro Livers- self-assembling co-cultures (SACCs) of primary hepatocytes	3D	Visikol (Hampton, New Jersey)
			Liver- kidney- intestine- bile duct- on chip	3D	AZAR innovations (Utrecht, Netherlands)
			heart-liver-neuron-skeletal muscle on chip	3D	Hesperos (Orlando, Florida, USA)
			Intestine – Liver – Kidney – Neuro on chip	3D	Tissuse (HUMIMIC) (Berlin, Germany)
			Intestine – Liver – Kidney – Neuro + Blood-Brain-Barrier on chip	3D	Tissuse (HUMIMIC) (Berlin, Germany)
			Lung – Liver on chip	3D	Tissuse (HUMIMIC) (Berlin, Germany)
			spheroid (GenuineSelect-TOX)	3D	PredictCan Biotechnologies (Grabels, Languedoc Roussillon, France)
			HepG2	2D	acCELLerate (Hamburg,Germany)
Ultra structural pathology, Morphometrics, Histological special strains & antibody detection	1) Histology of MPS 2) Drug metabolizing relevant gene expression levels (mRNA expression levels of ADME genes and their stability over time) 3) Measuring mitochondrial metabolism	FDA guidance on invitro methods	HEPATOPAC Cultures	2D	BioIVT (Westbury, NewYork)
			iPSC derived hepatocytes (OptiHEP)	2D	Definigen (Cambridge, United kingdom)
			invitro liver hepatocytes	2D	Primacyt (Schwerin, Germany)
			3D Intestine – Liver on chip	3D	Tissuse (HUMIMIC) (Berlin, Germany)

In vivo endpoints	In vitro endpoints	Guidelines	Commercially available NAMs	Type of NAM	Name of the company and location
	4) Measuring mitochondrial mass, potential and number of mitochondria 5) Measuring ROS production 6) Imaging based assays 7) Measures of daily rates of transporter substrate and bile acid uptake, metabolism, conjugation, and export in media				
Clinical signs of toxicity, Clinical chemistry- AST, ALT,Bilurubin; Organ weights-Liver, Macroscopic and microscopic evaluation of Liver	1) Oxygen gradients and metabolic zonation for studying zone specific toxicities 2) Regulated fluidic flow for sampling of media flow-through for metabolites and biomarkers 3) Study of multicellular biological interactions, including role of innate and adaptive immune function on hepatobiliary BA transport. Study of immune mediated idiosyncratic DILI	FDA guidance on invitro methods	iPSC derived hepatocytes (OptiHEP)	2D	Definigen (Cambridge, United kingdom)
			InSight™ Liver Safety solutions- spheroids	3D	InSphero (Schlieren, Switzerland)
			HUREL® Micro Livers- self-assembling co-cultures (SACCs) of primary hepatocytes	3D	Visikol (Hampton, New Jersey)
			LiverChip model cultured in Multi-chip Liver plates by the PhysioMimix® OOC range of microphysiological systems	3D	CN Bio(Cambridge, UK)
			Human primary hepatocytes	2D	AnaBios (San Diego, California, USA)
			Javelin Liver Tissue Chip	3D	Javelin Biotech (Woburn, Massachusetts)
			Liver-on-chip	3D	Dynamic 42 (Winzerlaer, Germany)
			DILI mechanistic panel, hepatocytes	3D	Eurofins (Luxembourg)
			Hepatocytes	2D	JRF Global (Gujarat, India)
			Primary Human Hepatocytes or 3D hLiMTs	Primary Human -2D hLiMTs-3D	Evotec – Cyprotex (Massachusetts, USA and Manchester, England)

Table 3: Table listing the global commercial technologies in NAMs used for studying renal toxicity

In vivo endpoints	In vitro end points	Guidelines	Commercially available NAMs	Type of NAM	Name of the company and location
Urinary volume, specific gravity, osmolality, pH, fluid/electrolyte balance, proteins, cytology, evaluation of biomarkers of nephrotoxicity and blood chemistry determinations such as blood urea nitrogen, creatinine and plasma proteins	Renal toxicity biomarkers including total urinary protein, kidney injury molecule-1 (KIM-1), clusterin, β2-microglobulin (β2-MG), Cystatin C. Trefoil factor-3, renal papillary antigen-1 (RPA-1) and albumin	ICHS7A	kidney on chip	3D	Emulate Bio (Boston, Massachusetts)
			Kidney on chip	3D	Nortis Bio (Washington, USA)
			EpiKidney™ (beta)	3D	MatTek Life Sciences (Ashland, Massachusetts, USA)
			Proximal TubuleaProximate™, aProximate™	3D	Newcells Biotech (Newcastle upon Tyne, England)
			Liver- kidney-intestine-bile duct on a chip	3D	AZAR innovations (Utrecht, Netherlands)
			human telomerized cells from different tissues and organs (e.g., Kidney) RPTEC/TERT1, PODO/SVTERT152, PODO/TERT256	2D	Evercyte (Vienna, Austria)
			Intestine – Liver – Kidney – Neuro on chip	3D	Tissuse (HUMIMIC) (Berlin, Germany)
			Intestine – Liver – Kidney – Neuro + Blood-Brain-Barrier on chip	3D	Tissuse (HUMIMIC) (Berlin, Germany)
			Renal proximal tubule epithelial cells RPTEC	2D	Evotec (Hamburg, Germany)

Table 4: Table listing the global commercial technologies in NAMs used for studying respiratory toxicity

In vivo endpoints	In vitro end points	Guidelines	Commercially available NAMs	Type of NAM	Name of the company and location
Respiratory rate, tidal volume, hemoglobin oxygen saturation; Follow up study parameters such as airway resistance, pulmonary arterial pressure, blood gases, Blood pH	Inhalation toxicology endpoints like percentage cytotoxicity and release of cytokines, chemokines and trans epithelial electrical resistance (TEER)	ICH S7A	AXLung-on-Chip System	3D	AlveoliX (Bern, Switzerland)
			MucilAir™- in vitro tissue model of the human upper airway epithelium	3D	Epithelix (Plan-Les-Ouates, Swizterland)
			MucilAir™-Pool nasal- in vitro tissue model reconstituted with a mix of nasal cells from 14 different healthy human donors	3D	Epithelix (Plan-Les-Ouates, Swizterland)
			SmallAir™	3D	Epithelix (Plan-Les-Ouates, Swizterland)
			ImmuPHAGE™ - an *in vitro* cell model of human alveolar macrophages	2D	ImmuONE (Hertfordshire, England, UK)
			ALIsens- human alveolar *in vitro* models	3D	Invitrolize (Belvaux, Luxembourg)
			EpiAirway™	3D	MatTek Life Sciences (Ashland, Massachusetts, USA)
			Human Lung Fibroblasts	3D	Newcells Biotech (Newcastle upon Tyne, England)
			Coculture models	3D	Sabeu (Germany)
			In vitro cells	2D	Evercyte (Vienna, Austria)
			Lung – Liver on a chip	3D	Tissuse (HUMIMIC) (Berlin, Germany)
			Lung on a chip model	3D	SynVivo (Hunstville, Alabama, USA)
			Lung organoid	3D	Thermo Fisher Scientific (Waltham, Massachusetts, USA)

Table 5: Table listing the global commercial technologies in NAMs used for studying CNS toxicity

In vivo endpoints	In vitro end points	Guidelines	Commercially available NAMs	Type of NAM	Name of the company and location
Motor activity, neuro-behavioural changes, coordination, sensory/ motor reflexes, undisturbed behavioral observations and invasive tests such as reflex testing	Neurodevelopmental process at cellular level: 1) proliferation 2) migration 3) neurite growth 4) synaptogenesis 5) myelination 6) apoptosis: 7) Differentiation 8) Neural network formation	ICHS7A, OECD 426- DNT invitro testing battery	Organized individual neurons, neurons-on-chip, NeuroHTS™	Organized individual neurons- 2D, neurons-on-chip, NeuroHTS™- 3D	Ananda Devices (Montreal, Quebec, Canada)
			Neuron on chip	3D	Netri (Lyon, France)
			Vascularized Micro-Brain (VMB™)	3D	Aracari Bio (Irvine, California, USA)
			brain-on-chip, brain organoids (BrainSim, microBrain, NerveSim)	3D	AxoSim (New Orleans, Louisiana, USA)
			neural spheroids, neurons, astrocytes, microglia	neural spheroids- 3D, neurons, astrocytes, microglia- 2D	BrainXell (Madison, USA)
			brain on chip	3D	Creativ Biolabs (New York, London, Frankfurt)
			neurospheres, hNPC (human neural progenitor cells)	3D	DNTOX (Germany)
			NeuroExir™	3D	ExirBio (Sunnyvale, California, USA)
			MEPS-BBB- blood brain barrier on chip	3D	MEPSGEN (Seoul, South Korea)
			BBB-on-a-chip	3D	MIMETAS (Leiden, Netherlands)
			In vitro iPSC culture	2D	Neurosetta (Madison, Wisconsin, USA)
			Human neural organoids	3D	Stem Pharm (Madison, Wisconsin, USA)
			Intestine – Liver – Kidney – Neuro on chip	3D	Tissuse (HUMIMIC) (Berlin, Germany)
			Blood-Brain-barrier – Neuro – Liver on chip	3D	Tissuse (HUMIMIC) (Berlin, Germany)
			heart-liver-neuron-skeletal muscle on chip	3D	Hesperos (Orlando, Florida, USA)
			NMJ on chip	3D	Hesperos (Orlando, Florida, USA)

Table 6: Table listing the global commercial technologies in NAMs used for studying cardiac toxicity

In vivo endpoints	In vitro endpoints	Guidelines	Commercially available NAMs	Type of NAM	Name of the company and location
Blood pressure, Heart rate, ECG; Follow up parameters: Cardiac output, ventricular contractility, vascular resistance	Delayed ventricular potential (pro-arythmia risk) measured via the following: 1) Ionic currents in cardiomyocytes 2) Action potential parameters in isolated cardiac preparations 3) Proarrhythmic effects measured in isolated cardiac preparations (QT interval).	1. ICH S7A 2. ICH S7B	Cardiac organoid	3D	4DCell (Montreuil, France)
			heart on chip (uHeart)	3D	BiomimX (Milano, Italy)
			Engineered heart tissue (EHT), 2D heart model, Celo.Cardiomyocytes	Engineered heart tissue (EHT)- 3D, 2D heart model, Celo. Cardiomyocytes- 2D	Curi Bio (Seattle, Washington, USA)
			Cardiac organoid	3D	Foresee Biosystems (Genova, Liguria, Italy)
			FLEXcyte 96 technology- growing invitro iPSC derived cardiomyocytes	2D	Innovitro (Gothenburg, Sweden)
			CardioExcyte 96- growing invitro non-contractile, such as hepatic or cancer cells, alongside contractile cardiac cells.	2D	Innovitro (Gothenburg, Sweden)
			heart-liver on a chip	3D	Hesperos (Orlando, Florida, USA)
			heart-liver-neuron-skeletal muscle on chip	3D	Hesperos (Orlando, Florida, USA)
			Cardio – Liver on chip	3D	Tissuse (HUMIMIC) (Berlin, Germany)
			The Maestro Pro and Edge MEA systems		Axion biosystems (Atlanta, USA)
			Atrial cardiomyocytes	2D	Axol Bioscience (Edinburgh, UK)
			Ventricular cardiomyocytes	2D	Axol Bioscience (Edinburgh, UK)
			TrueCardium® Cardiac Organoids	3D	inSphero (Schlieren, Switzerland)
			hERG channel clamp assay employing The QPatch HTX and the SyncroPatch 384PE	2D	Evotec (Hamburg, Germany)

Table 7: Table listing the global commercial technologies in NAMs used for studying intestinal toxicity

In vivo endpoints	In vitro endpoints	Guidelines	NAMs	Type of NAM	Name of the company and location
Gastric secretion, gastrointestinal injury potential, bile secretion, transit time in vivo, ileal contraction in vitro, gastric pH measurement	Permeability, barrier function, Drug absorption, metabolism, drug transport	ICH S7A	Human primary stem-cell derived ntestinal epithelial cell model systems, RepliGut Systems	2D	Altis Biosystems (Durham, North Carolina, USA)
			Colon-intestine chip	3D	Emulate Bio (Boston, Massachusetts)
			Duodenum-intestine chip	3D	Emulate Bio (Boston, Massachusetts)
			EpiIntestinal™	3D	MatTek Life Sciences (Ashland, Massachusetts, USA)
			OrganoReady^{R} Colon Caco-2	3D	Mimetas (Leiden, Netherlands)
			OrganoReady^{R} Colon Organoid	3D	Mimetas (Leiden, Netherlands)
			Intestine – Liver on chip	3D	Tissuse (HUMIMIC) (Berlin, Germany)
			Intestine – Liver – Kidney – Neuro on chip	3D	Tissuse (HUMIMIC) (Berlin, Germany)
			Intestine – Liver – Kidney – Neuro + Blood-Brain-Barrier on chip	3D	Tissuse (HUMIMIC) (Berlin, Germany)

Table 8: Table listing the global commercial technologies in NAMs used for studying dermal toxicity

Parameters / End points of In vivo studies	Parameters/ in vitro end points	Guidelines	Commercially available NAMs	Type of NAM	Name of the company and location
Skin irritation/ corrosion	Skin irritation/ corrosion	OECD TG 404, 431, 439 (Skin corrosion/ irritaion)	Reconstructed human epidermis	3D	Scantox (Denmark)
			Reconstructed human epidermis	3D	SenzaGen (Lund, Sweden)
			Skin models using reconstructed human tissues	3D	XCellR8 (Daresbury, Halton, United Kingdom)
			organoids (Skimune® AD, Skimune® Epi AD)	3D	Alcyomics (Newcastle Upon Tyne, United Kingdom)
			EpiDermTM	3D	MatTek Life Sciences (Ashland, Massachusetts, USA)
			Xenoskin H discs - ex vivo full thickness skin explants	3D	Xenometrix (Gewerbestrasse, Switzerland)
			skin on chip	3D	Tissuse (HUMIMIC) (Berlin, Germany)
			SURFACE (skin ubiome Reconstruction for Assessment of Cutaneous Effects),	3D	Draper (Massachusetts, USA)
			Labcyte EPI-MODEl-24	3D	J-TEC (Japan)
			epiCS®	3D	SkinInVitro GmbH (Germany)

Parameters / End points of In vivo studies	Parameters/ in vitro end points	Guidelines	Commercially available NAMs	Type of NAM	Name of the company and location
Skin sensitization studies	Skin sensitization studies	OECD 406 (Skin Sensitization)	3D skin models	3D	Keratify (London, UK)
			keratinocyte cell lines KeratinoSens® and LuSens	2D	acCELLerate (Hamburg,Germany)
			skin-on-a-chip	3D	Ten Bio (Dundee, Scotland, UK)
			Skin-on-chip	3D	Vitrocell (Waldkrich, Germany)
			Reconstructed human epidermis	3D	Scantox (Denmark)
			organoids (Skimune® AD, Skimune® Epi AD)	3D	Alcyomics (Newcastle Upon Tyne, United Kingdom)
			Reconstructed human epidermis	3D	XCellR8 (Daresbury, Halton, United Kingdom)
			Reconstructed human epidermis, GARDskin	3D	SenzaGen(Lund, Sweden)
			Skin including dermal papillae on chip	3D	Tissuse (HUMIMIC) (Berlin, Germany)
			SURFACE (skin ubiome Reconstruction for Assessment of Cutaneous Effects)	3D	Draper (Massachusetts, USA)
Skin absorption	Skin absorption	OECD 428 (Skin absorption)	in-vitro skin tissues, skin discs and dermatomed skin,	in-vitro skin tissues-2D; skin discs and dermatomed skin-3D	Primacyt (Schwerin, Germany)
			Xenoskin H discs - ex vivo full thickness skin explants	3D	Xenometrix (Gewerbestrasse, Switzerland)
			invitro skin models	2D	DTL (Dermal tech lab) (United Kingdom)
			Reconstructed human epidermis	3D	SenzaGen (Lund, Sweden)
			frozen full thickness skin	3D	Xenometrix (Gewerbestrasse, Switzerland)
			in vitro skin tissues	3D	PKDERM (Grasse, France)
			Reconstructed human epidermis, GARDskin	3D	SenzaGen (Lund, Sweden)
			SkinEthic RHE / Human Epidermis	3D	EPISKIN (Lyon France)
Photo toxicity testing	Photo toxicity testing	ICH guideline S10: Guidance on photosafety evaluation of pharmaceuticals (EMA/CHMP/ ICH/752211/2012)	EpiDermTM	3D	MatTek Life Sciences (Ashland, Massachusetts, USA)
			skin-on-a-chip	3D	Tenskin ((Dundee, Scotland, UK))
			SkinEthic RHE / Human Epidermis	3D	EPISKIN (Lyon France)
			Reconstructed human epidermis	3D	Scantox (Denmark)
			EpiDermTM FT	3D	MatTek Life Sciences (Ashland, Massachusetts, USA)
			skin tumor on chip	3D	Tissuse (HUMIMIC) (Berlin, Germany)
			FibroExir™	3D	ExirBio (Sunnyvale, California, USA)
Photoallergy or dermal phototoxicity	Photoallergy or dermal phototoxicity	NDCT guidelines	Phenion® FT Skin Model	3D	Phenion (Germany)

Table 9: Table listing the global commercial technologies in NAMs used for studying ocular toxicity

In vivo endpoints	Invitro endpoints	Guidelines	Commercially available NAMs	Type of NAM	Name of the company and location
Ocular corrosion and irritation assay	Ocular corrosion and irritation assay	OECD TG 405, 496	SkinEthicTM HCE - Human Corneal Epithelium, Statens Seruminstitut Rabbit Cornea (SIRC) cells	2D	EPISKIN (Lyon France)
			EpiOcularTM	3D	Mattek Life Sciences (Ashland, Massachusetts, USA)
			Retinal organoids, Retinal Pigment Epithelium	3D	Newcells Biotech (Newcastle upon Tyne, England)
			OptiSafeTM EIT	3D	LEBRUN labs (Anaheim, California, USA)
Ocular Sensitization assay	Ocular Sensitization assay	NA	Reconstructed Human Cornea -like epithelium	3D	XCellR8 (Daresbury, Halton, United Kingdom)
Ocular Absorption assay	Ocular Absorption assay	OECD TG 437	3D skin tissue	3D	Scantox (Denmark)
Ocular Phototoxicity test	Ocular Phototoxicity test	OECD TG 498	Reconstructed Human Cornea-like epithelium	3D	XCellR8 (Daresbury, Halton, United Kingdom)

Table 10: Table quantifying the number of global commercial technologies in NAMs used for studying tissue-specific toxicity

Tissue-specific toxicity	Number of global commercial technologies in NAMs
Dermal	37
Hepatic	32
Central nervous system	16
Cardiovascular	14
Ocular	7
Respiratory	13
Intestine	9
Renal	9

Table 11: Table listing the NAMs for evaluating genotoxicity

Type of tissue/organ	Invitro end points	Guidelines	Type of NAMs	NAMs
Lungs	DNA strand breaks and DNA oxidation lesions (Alkaline and Fpg-modified Comet assay)	OECD TG 473	3D	Advanced 3D Lung Model at ALI (human alveolar epithelial cells) (A549)
	DNA strand breaks and DNA oxidation lesions (Alkaline and Fpg-modified Comet assay)	OECD TG 473	2D	Endothelial (EA.hy926) cells and differentiated monocytes (dTHP- 1)
	DNA strand breaks (Comet assay) and oxidative stress (MDA release)	OECD TG 473	2D	Triple-culture model (epithelial cells, macrophages and fibroblasts)
	DNA strand breaks (Alkaline Unwinding method)	OECD TG 473	2D	BEAS- 2B Monocultures, Cocultures (BEAS- 2B/ dTHP- 1) and Triple cultures (BEAS- 2B/ dTHP- 1/CCD-33Lu)
	DNA damage (Comet assay), Chromosomal damage (Micronucleus assay)	OECD TG 473, TG 487	2D	BEAS- 2B and A549 Monocultures, Cocultures with EA.hy926
	Chromosomal damage (Micronucleus assay), Oxidative stress (NAC inhibition study)	OECD TG 473, TG 487	2D	TT1 Monoculture and TT1/d. THP- 1 Co-culture
	Gene expression changes associated with oxidative stress and inflammation		2D	Mono-culture (A549) and Co-culture (A549 + differentiated THP- 1 macrophages)
	Chromosomal damage (micronucleus assay)	OECD TG 473, TG 487	2D	Mono-culture (16HBE14o-), Co-culture (16HBE14o- +dTHP- 1 macrophages)
	DNA strand breaks and DNA oxidation lesions (Alkaline and enzyme modified comet assay), Oxidative stress (HMOX1 expression)	OECD TG 473,	2D	Triculture model (A549, dTHP- 1 macrophages, EA.hy926 endothelial cells)
	DNA strand breaks (Comet assay), IL- 8 expression (inflammation)	OECD TG 473	2D	Mono-culture (A549), Coculture (A549 + THP- 1a macrophages
Skin	Chromosomal damage (OECD TG 487-based cytokinesis block micronucleus assay, evaluation by high content analysis, DNA strand breaks (Alkaline comet assay), Chromosomal damage (Micronucleus assay), DNA fragmentation and cell cycle (G2/M arrest)	OECD TG 473, TG 487	2D	HaCaT keratinocytes
	DNA damage (phosphorylated γH2AX) only with Ker-CT cells)	OECD TG 473	2D, 3D	Human keratinocytes Ker-CT, skin explants
	8-hydroxy- 2'-deoxyguanosine (8-OHdG) by an enzyme-linked immunosorbent assay (ELISA) kit		3D	EpiDerm™
	DNA strand breaks (alkaline comet assay)	OECD TG 473	2D	LC-like dendritic cell (DC) line XS52

Type of tissue/organ	Invitro end points	Guidelines	Type of NAMs	NAMs
Intestine	DNA strand breaks and DNA oxidation lesions (Alkaline and Fpg-modified comet assay)	OECD TG 473	2D	Caco- 2/HT29-MTX, differentiated, non-differentiated
	DNA strand breaks and DNA oxidation lesions (Alkaline and Fpg-modified comet assay), Micronucleus assay	OECD TG 473	2D	HT29-MTXE12 (mucus secreting)
	CometChip		2D	Triculture small intestinal epithelium (SIE) model
Liver	**DNA strand breaks and DNA oxidation lesions (Alkaline and Fpg-modified comet assay)**	OECD TG 473	3D	**Human liver microtissue model (InSphero system)**
	DNA strand breaks and DNA oxidation lesions (Alkaline and Fpg-modified comet assay), Micronucleus assay	OECD TG 473, TG 487	3D	**HepG2-based spheroid model**
	DNA strand breaks and DNA oxidation lesions (Alkaline and Fpg-modified comet assay)	OECD TG 473	2D, 3D	HepaRG based models

Table 12: Table listing the number of NAMs available for evaluating genotoxicity

Tissue-type	Number of NAMs available to evaluate genotoxicity
Lungs	12
Skin	5
Intestine	4
Liver	4

Table 13: Table listing the NAMs in efficacy testing in nonclinical research and precision medicine

Nonclinical Research				
Name of the rare disease	**NAM used**	**Name of the drug**	**Description of the usage**	**Reference**
Chronic inflammatory demyelinating polyneuropathy (CIDP)	Human-on-a-chip model of peripheral motoneuron conduction velocity.	Sutimlimab (SAR-445088; formerly BIVV-020), a humanized anti-C1s monoclonal antibody	**Efficacy testing** This was one of the first cases in 2023 where an IND application was approved for conducting clinical trials based on efficacy data purely generated from CIVM.	Rumsey et al 2022
Cystic fibrosis	In vitro models of the different CFTR mutations in Fisher rat thyroid cell lines. These cells were then treated with ivacaftor and assessed with Ussing chamber electrophysiology	ivacaftor	**Extension of pharmaceutical approval** In 2017, the FDA granted expanded approval to Vertex Pharmaceuticals' cystic fibrosis (CF) drug ivacaftor on the basis of in vitro data. The change adds 23 mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene to the existing 10 mutations previously covered by the drug label, representing a further ~900 patients in the United States	Kingwell, 2017

Clinical Research (Efficacy testing and precision medicine)					
Rare disease	**ClinicalTrials.gov ID/ EudraCT Number**	**Name of the Project**	**Start Date/ Status**	**Interventional/ Observational**	**Usage of organoids/iPSCs in the clinical trials**
CF	NCT06468527	Clinical Trial to Evaluate the Efficacy and Safety of Dirocaftor/ Posenacaftor/ Nesolicaftor in Adults With CF (CHOICES)	Ongoing	Interventional: Dirocaftor/ Posenacaftor/Nesolicaftor	(i)Performing a two-step approach, where in the first step, novel CFTR modulators/ combinations were tested on organoids from over 500 European and Israeli CF patients with rare CFTR mutations to identify patients predicted to clinically benefit from these treatments. The second step will evaluate the predicted clinical effect in subjects identified by their organoid response.
CF	2016-001619-19 (Netherlands - Competent Authority)	Genistein as an add-on treatment for CF	Ongoing	Interventional: Genistein	(i)Assessing correlations between individual Ivacaftor genistein induced CFTR function in vitro (organoid-based measurements) and in vivo treatment affect
CF	NCT05100823 (University Hospital, Montpellier, France)	Validation of Therapeutic Efficacy Targeting the Splicing Variants in Cystic Fibrosis and CFTR Pathologies (ONB-CFTR)	Ongoing	Interventional: Oligonucleotide blockers (ONB)	(i)Assessing ONB (named ONB-CFTR) to be performed using an air-liquid interface model of airway epithelium, developed from nasal cells of patients, without or with a combination of existing CFTR modulators, depending on the patient' genotype. (ii)Building a local biobank of rectal organoids from CF patients

Table 14: Table listing the clinical trials using patient-derived organoids

Clinical Trial No.	Trial Description	Description of the usage
NCT06702800 (https://clinicaltrials.gov/study/NCT06702800)	Clinical Exploratory Study on Predicting Drug Sensitivity for Breast Cancer Treatment Using Simulated Organoid Models	Assess drug sensitivity in PDO and compare with clinical characteristics.
NCT06102824 (https://clinicaltrials.gov/study/NCT06102824)	Organoid-based Functional Precision Therapy for Advanced Breast Cancer	Assess drug sensitivity in PDO and compare with clinical characteristics
NCT06438055 (https://www.careacross.com/clinical-trials/trial/NCT06438055)	Clinical Treatment of Refractory Breast Cancer Based on Organoid Drug Sensitivity Results	Assess drug sensitivity in PDO and compare with clinical characteristics.
NCT06468124 (https://clinconnect.io/trials/NCT06468124)	Sensitivity of Organoids to Predict Treatment Outcome in Breast Cancer Metastases	Assess drug sensitivity in PDO and compare with clinical characteristics.
NCT06268652 (https://cdek.pharmacy.purdue.edu/trial/NCT06268652/)	Patient-Derived Organoid-guided Personalized Treatment Versus Treatment of Physician's Choice in Breast Cancer	Assess drug sensitivity in PDO and compare with clinical characteristics.

Table 15: Table listing the commercial technologies in in-silico modeling

Type of study	Objective	NAMs	Name of company and location
In-silico prediction of cardiotoxicity	Predicts a compound's proarrhythmic risk using electrophysiology and machine learning	Cardiac Drug Safety Suite (QT/TdP Risk Screen)	InsilicoMinds (Hyderabad, India)
In-silico	Predict the behavior of a drug in the human body, identify potential safety concerns, and optimize dosing regimens	505b2 insilsico support	InsilicoMinds (Hyderabad, India)
In-silico	Quantitative Systems Pharmacology (QSP) is a mathematical modeling approach that integrates biological, pharmacological, and physiological knowledge to understand and predict the behavior of drugs in the body.	Quantitative Systems Pharmacology (QSP)	InsilicoMinds (Hyderabad, India)
In-silico to predict PBPK of drugs	Inter-species PBPK models are used to predict how a drug or chemical will be distributed, metabolized, and eliminated in the bodies of different animal species as well as humans.	Inter-species PBPK Modeling	InsilicoMinds (Hyderabad, India)
In-silico to predict PBPK of drugs	PK/PD modeling and simulation can be a valuable tool for assessing the safety and efficacy of drugs or chemicals in animal populations. insilico solutions can be used to estimate exposure levels and potential risks associated with the use of these substances in different animal species.	PopPK/PD Modeling (Non-Clinical)	InsilicoMinds (Hyderabad, India)
In-silico to predict PBPK of drugs	IVIVE models are used to predict the in vivo performance of a drug based on its in vitro characteristics. in silico solutions help optimize drug formulations and predict the effect of formulation & process changes on the drug's performance.	IVIVE models	InsilicoMinds (Hyderabad, India)
In-silico prediction for carcinoma	QSP model for mammary carcinoma	InSilico ONCO	InsilicoMinds (Hyderabad, India)
In-silico	In silico Quantitative Structure-Toxicity Relationship (QSTR) Model for Predicting Toxicity of Aromatic Aldehydes using Extended Topochemical Atom (ETA) Indices.	Insilico QSTR model	InsilicoMinds (Hyderabad, India)

Type of study	Objective	NAMs	Name of company and location
In-silico	Simcyp® provides a seamless PBPK modeling environment for all stages of drug development, reducing the reliance on extensive in vivo studies.	Simcyp® Simulator	CERTARA (Pennsylvania, USA)
In-silico prediction of ADME	Provide high quality estimates of human ADME/PK (absorption, distribution, metabolism, excretion, pharmacokinetics) directly from chemical structure	ANDROMEDA	Prosilico (Huddinge, Sweden)
In-silico	The In Silico World project aims at accelerating the uptake of modelling and simulation technologies used for the development and regulatory assessment of medicines and medical devices, by lowering seven identified barriers: development, validation, accreditation, optimisation, exploitation, information, and training	InSilico Trials (IST)	InSilicoWorld (Trieste, Italy)
In-silico PBPK modeling	GastroPlus simulates intravenous, oral, oral cavity, ocular, inhalation, dermal, subcutaneous, and intramuscular absorption, biopharmaceutics, pharmacokinetics, and pharmacodynamics in humans and animals.	GastroPlus®	SimulationsPlus (Lancaster, California, USA)
In-silico absorption, distribution, metabolism, excretion and toxicity predictor	ADMET Predictor that accurately predicts more than 175 properties of a compound including solubility, logP, pKa, sites of CYP metabolism and Ames mutagenicity.	ADMET Predictor®	SimulationsPlus (Lancaster, California, USA)
In-silico	It is a Quantitative systems toxicology (QST) software for predicting drug-induced kidney injury.	RENAsym®	SimulationsPlus (Lancaster, California, USA)
In-silico	It is a mathematical model of idiopathic pulmonary fibrosis.	IPFsym®	SimulationsPlus (Lancaster, California, USA)
In-silico	It predicts the efficacy of drugs for the metabolic dysfunction-associated steatotic liver disease (MASLD) also known as nonalcoholic fatty liver disease (NAFLD).	NAFLDsym®	SimulationsPlus (Lancaster, California, USA)
In-silico	It can predict Drug-Induced Liver Injury (DILI)	DILIsym®	SimulationsPlus (Lancaster, California, USA)
In-silico	BioTox supports better decision-making, ultimately bringing safer, more effective drugs to market faster.	BioTox	Biolligence (Sydney, Australia)
In-silico	It unravels the relationship ranging from genomic data, QSP and bio-marker outcomes	DeepPumas™	PumasAI (Dover, Delaware)
In-silico (**ML and AI Predictive Models**)	It analyzes the amino acid sequence of an antibody's variable region to predict whether the mAb is likely to have high or low immunogenicity	AbImmPred	GenScript Co., Ltd(Piscataway, New Jersey, USA)
In-silico (**ML and AI Predictive Models**)	It predicts toxicities (like acute systemic toxicity, off-target binding, or cytokine release potential) by learning patterns from molecules that caused certain adverse events.	CATMoS	-
In-silico	It analyzes whether the drug might bind to similar epitopes in the human proteome, highlighting potential safety concerns	**Bioinformatics and *Insilico* Off-target Screening**	-
Insilico	It predicts whether the drug candidate can penetrate the blood-brain-barrier	**QSAR model**	Multicase (Cleveland, Ohio, USA)

Table 16: A table containing list of ICH and EMA guidelines for toxicology studies using NAMs (modified from Landscape New Approach Methodologies by National Institute for Public Health and the Environment)

ICH and EMA guidelines	Toxicological endpoints
M3	Toxicokinetics and pharmacokinetics, chronic toxicity
S1A, B, C CPMP/SWP/2877/00 CHMP/SWP/2592/02 CPMP/SWP/372/0	Carcinogenicity
S2	Genotoxicity, chronic toxicity
S3	Toxicokinetic and pharmacokinetics, and biodistribution
S4, S6, S9 CPMP/SWP/1042/99	Chronic toxicity
S5 EMEA/CHMP/SWP/169215/2005 CPMP/SWP/2600/01, EMEA/273974/2005	Reproduction toxicity (fertility and developmental toxicity)
S7A	Cardiac toxicity, neurotoxicity, respiratory toxicity
S7B	Cardiac toxicity
EMEA/CHMP/SWP/94227/2004	Neurotoxicity
S8	Immunotoxicity
S10	Phototoxicity
EMA/CHMP/SWP/2145/2000	Local tolerance
EMEA/CHMP/SWP/150115/2006 (reflection paper)	Hepatotoxicity
S11	Multiple
S12	Biodistribution

Table 17: Table showing the list of OECD guidelines for toxicology studies (modified from OECD work on international acceptance of NAMs by Patience Brown)

OECD guidelines	Toxicological endpoints
TG 420, 423, 425	Oral toxicity
TG 402	Dermal toxicity
TG 403, 433, 436	Inhalation toxicity
TG 496, 498	Eye Irritation and damage
TG 431, 439	Skin Irritation and corrosion
TG 442C, 442D, 442E, TG 497	Skin sensitization

Table 18: Table showing the number of different institutions and companies working in the field of organoids in India (data curated from CPHMS MPS database)

Type of organoid	Total	Institution	Company
Brain	17	12	5
Tumour	32	24	8
Liver	20	11	9
Corneal	13	10	3
Lung	14	9	5
Skin	26	18	8
Kidney	6	3	3
Pancreas	8	4	4
Gut	12	8	4
Placenta	7	5	2
Dental	4	3	1
Cardiac	3	2	1
Retina	2	1	1
Gallbladder	1	1	0
Multi-organ	13	8	5
Endometrium	1	1	0

Table 19: Table showing the number of different institutions and companies working in the field of organ on chips in India (data curated from CPHMS MPS database)

Type of organ-on-chip	Total	Institution	Company
Liver	16	11	5
Lung	11	9	2
Tumor	22	19	3
Kidney	4	3	1
Skin	3	3	0
Retina	2	2	0
Heart	5	4	1
Brain	8	6	2
Gut	7	5	2
Placenta	5	4	1
Pancreas	6	4	2
Cornea	7	5	2
Vessel on a chip	5	4	1
Breast	1	1	0
Ovary	1	1	0
Prostrate	1	1	0
Cervix	1	1	0
Wound	1	1	0
Multi organ	8	6	2
Uterus	1	0	1

Dr.Reddy's

8-2-337, Road No.
3, Banjara Hills, Hyderabad,
Telangana 500034, India

BRIC a DBT Organization
inStem इनस्टेम Institute for Stem Cell Science and Regenerative Medicine

GKVK - Post, Bellary Rd, Rajiv
Gandhi Nagar, Vignana Kendra,
Bengaluru, Karnataka 560065

Humane World for Animals

8-2-332/5/1, Rd Number 3, Green
Valley, Banjara Hills, Hyderabad,
Telangana 500034

Animal Law & Policy Network

43, Residency Road, Shanthala
Nagar, Ashok Nagar, Bengaluru,
Karnataka 560025

Concept & Design (info@wyatt.co.in)