

Human-Relevant Non-Animal Methodologies (NAMs)
Specialised Models Series: Cardiovascular & Respiratory
Respiratory Models
Reference 4.2 in Full Scope Roadmap

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Legal & Scientific Imperative for Universities

Section 2A of the *Animals (Scientific Procedures) Act 1986* imposes a statutory duty on the Secretary of State to ensure compliance with the principles of replacement, reduction, and refinement. The principle of replacement requires that, wherever a specialised, scientifically satisfactory method or testing strategy not entailing the use of protected animals is available, it must be used to replace the use of a regulated procedure.

Respiratory diseases—ranging from asthma and chronic obstructive pulmonary disease (COPD) to lung cancer, pulmonary fibrosis, and airborne viral infections—constitute a massive global healthcare burden. Historically, preclinical drug discovery and safety testing have relied extensively on rodent inhalation models. However, the respiratory tract of rodents exhibits profound anatomical, physiological, and immunological disparities that render these models non-predictive of human clinical responses.

At the physiological and anatomical levels, human and rodent respiratory systems diverge fundamentally:

- **Anatomical Branching Mismatch:** Human lungs exhibit symmetric, dichotomous branching consisting of approximately 23 generations of airways. In contrast, rodents possess an asymmetric, monopodial branching pattern with fewer than 15 generations. This architectural disparity dramatically alters airflow dynamics, shear stress, and the localised deposition patterns of inhaled therapeutics and particulate matter (such as PM 2.5)
- **Cellular Composition Discrepancies:** Human airways are lined with a highly pseudostratified epithelium containing abundant goblet cells and basal cells extending deep into the distal bronchioles. Rodent airways, conversely, are predominantly lined with a simple cuboidal epithelium dominated by Club cells, with basal and goblet cells virtually absent in healthy lower airways, fundamentally altering mucus composition and regenerative responses.
- **Respiratory Dynamics:** Rodents are obligate nasal breathers with an extremely complex turbinate system that filters out inhaled aerosols far more efficiently than the human oral-nasal airway, preventing realistic dose translation.
- **Inhalation Toxicology Severity:** Traditional regulatory and research inhalation studies in rodents require placing animals in highly stressful snout-only or whole-body exposure chambers for hours. This inflicts immense distress, sensory irritation, hypothermia, and restraint-induced metabolic changes, which act as uncontrolled confounding variables.

Transitioning to human-relevant Non-Animal Methodologies (NAMs)—such as differentiated Air-Liquid Interface (ALI) cultures, microfluidic breathing lung-on-chips, and ex vivo human precision-cut lung slices (PCLS)—is a dual scientific and statutory mandate for UK academic research.

4.2 Respiratory & Air-Liquid Interface (ALI) Models

This section covers human-relevant biological, tissue-engineered, and computational platforms designed to model airway mucosal barriers, mucociliary clearance, alveolar-capillary gas exchange, and real-time bronchoconstriction kinetics.

Landmark Regulatory & Scientific Momentum

Significant progress has been made in the development and application of human-relevant respiratory NAMs:

- **Differentiated Air-Liquid Interface (ALI) Systems:** Recreate the human pseudostratified airway mucosal barrier by exposing primary or stem-cell-derived epithelial cells to air on porous membranes, triggering spontaneous differentiation into functional, mucus-secreting goblet cells and beating ciliated cells.
- **Breathing Alveolar Lung-on-Chips:** Simulate the dynamic alveolar-capillary interface by co-culturing epithelial and endothelial cells across a flexible membrane, applying cyclic mechanical stretching to model breathing-induced stretch and microvascular blood flow.
- **Ex Vivo Human Precision-Cut Lung Slices (PCLS):** Maintain viable sections of human donor lung tissue containing intact terminal bronchioles and alveoli, allowing real-time pharmacological measurement of airway contraction and localised inflammatory responses in native human tissue.

Core Specialised Respiratory Platforms

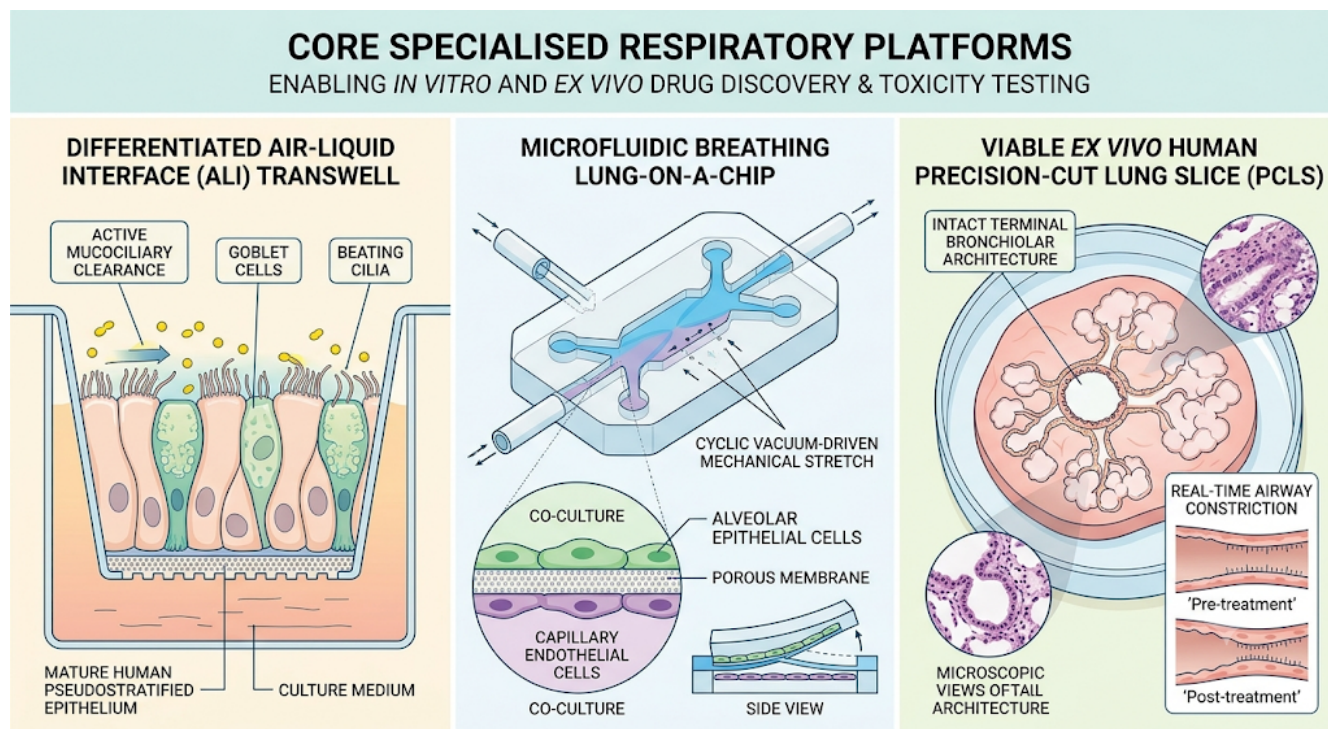


Figure 1: Core Specialised Respiratory Platforms — Differentiated Air-Liquid Interface (ALI) transwell membrane insert showing a mature human pseudostratified epithelium with active mucociliary clearance, goblet cells, and beating cilia (*left*); microfluidic breathing lung-on-a-chip platform with co-cultured alveolar epithelial and capillary endothelial cells under cyclic vacuum-driven mechanical stretch (*centre*); and a viable ex vivo human precision-cut lung slice (PCLS) showing intact terminal bronchiolar architecture used to track real-time airway constriction (*right*).

Table 1: Specialised Biological & Tissue-Engineered (In Vitro) Respiratory Platforms

Platform / Model	Description	Key Applications	Benefits for Universities
3D Biprinted Bronchial Scaffolds	Precisely engineered 3D constructs containing structured microchannels, bio-inks, primary cells, and supporting stromal cells.	Evaluating long-term tracheal tissue regeneration, chronic induced tissue remodelling, and localised drug delivery kinetics.	Bypasses large-animal (sheep) tracheal resection and invasive respiratory biomaterial transplantation studies.
Air-Liquid Interface (ALI) Airway Models	Pseudostratified human airway epithelial cells cultured on permeable membranes with basal media supply and apical air exposure.	Modelling chronic mucus hypersecretion (cystic fibrosis, COPD), ciliary beat frequency, infection kinetics, and aerosol toxicity.	Replaces rodent respiratory infection models and acute inhalation toxicity assays using household and industrial chemical screening.
Breathing Alveolar Lung-on-Chip	Co-culture of human alveolar epithelial and microvascular endothelial cells on a flexible membrane under cyclic mechanical stretch.	Modelling alveolar-capillary permeability, pulmonary oedema, immune cell recruitment, aerosol deposition, and nanoparticle toxicity.	Eliminates the need for stressful rodent acute lung injury models and live-animal nanomaterial inhalation exposure studies.
Precision-Cut Lung Slices (PCLS)	Thin, viable slices of ex vivo lung tissue preserving intact regional micro-anatomy, immune cells, and muscular tone.	Real-time monitoring of bronchoconstriction, drug-induced relaxation, fibrosis development, and surfactant toxicity.	Directly replaces the highly invasive ovalbumin-sensitised rodent asthma and chronic allergen-exposure models.

Cross-Cutting & Enabling Platforms for Respiratory Research

These platforms support multiple respiratory, environmental toxicology, and aerosol pharmacokinetics research areas and are widely applicable across small-molecule design, safety pharmacology, and clinical trial simulations. While physical lung-on-chips capture micro-structural niches, these cross-cutting digital and hybrid systems provide the quantitative, predictive pipelines required to validate high-maturity **human-relevant Non-Animal Methodologies (NAMs)**, ensuring compliance with the **Section 2A statutory duty of the *Animals (Scientific Procedures) Act 1986*** and enabling a transition toward fully predictive, cruelty-free respiratory research.

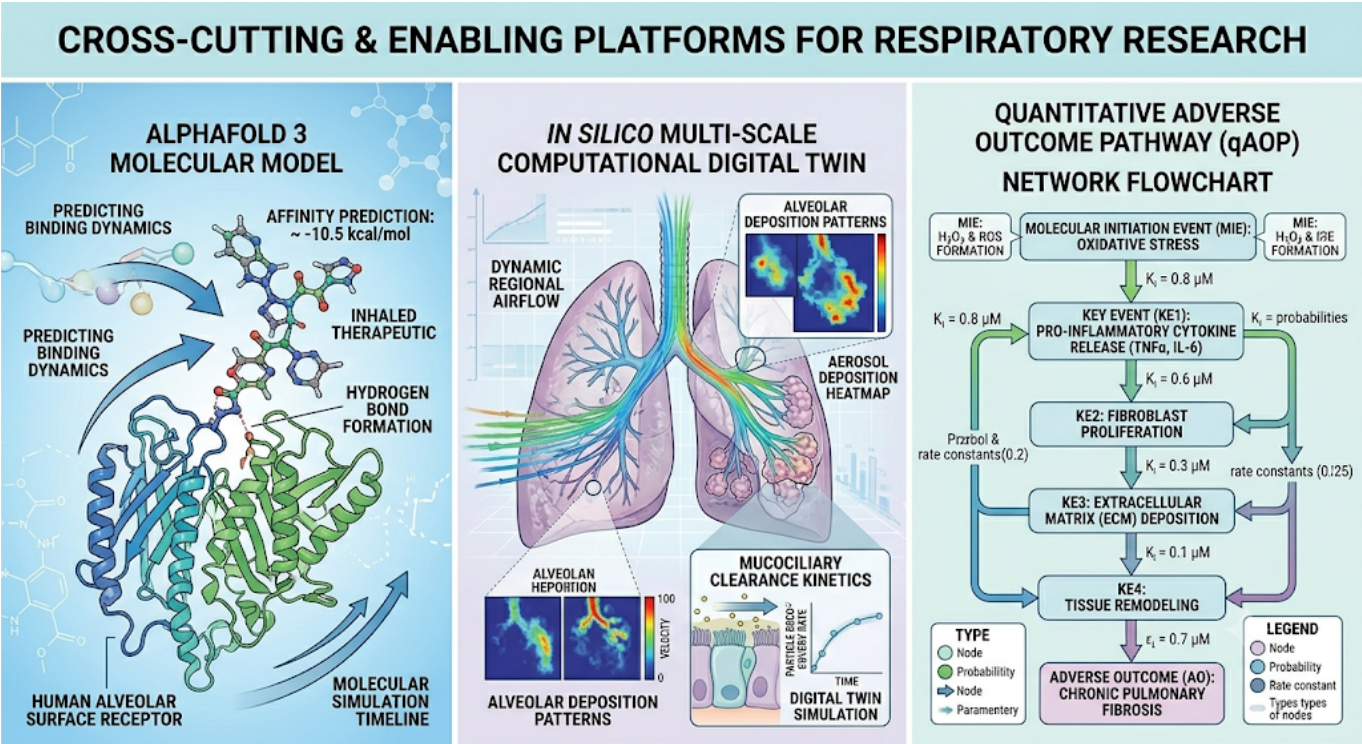


Figure 2: Cross-Cutting & Enabling Platforms for Respiratory Research — AlphaFold 3 molecular model predicting the binding dynamics of inhaled therapeutics interacting with human alveolar surface receptors (*left*); in silico multi-scale computational digital twin simulating dynamic regional airflow, mucociliary clearance kinetics, and localised aerosol deposition patterns (*centre*); and a quantitative Adverse Outcome Pathway (qAOP) network flowchart linking molecular initiation events (such as oxidative stress) to organ-level chronic pulmonary fibrosis (*right*).

Table 2: Genetic, Computational & Framework-Driven Platforms

Platform / Model	Description	Key Applications	Benefits for Universities & 3Rs
In silico Aerosol Deposition Simulators	Computational fluid dynamics (CFD) models mapping human airway geometry, airflow turbulence, and aerosol settling velocities.	Predicting regional deposition of inhaled particles (e.g., PM _{2.5}), optimising dry-powder inhalers, and calculating local tissue doses.	Bypasses ex vivo animal tracheal deposition assays and radiolabelled aerosol exposure studies in live primates or rodents.
Virtual Lung Digital Twins	Multi-scale mathematical models simulating whole-lung airflow dynamics, gas exchange efficiency, and localised FEV ₁ decline.	Simulating systemic respiratory disease progression, predicting clinical outcomes in virtual patient cohorts, and optimising drug dosing.	A system-wide in silico framework that replaces highly distressing chronic tobacco smoke and pollutant exposure models in rodents.
qAOP-Integrated Respiratory Screeners	Quantitative Adverse Outcome Pathways linking molecular initiation events (e.g., cell stress) to chronic pulmonary fibrosis.	Regulatory inhalation safety assessment, environmental pollutant screening, and high-throughput respiratory target prioritisation.	Integrates multiple in vitro data points into a legally defensible weight-of-evidence Replacement package for Project Licence applications.
Patient-Derived iPSC Respiratory Models	Reprogrammed patient-specific iPSCs differentiated into alveolar type II and ciliated cells, assembled into advanced organoids.	Precision modelling of genetic respiratory diseases (e.g., cystic fibrosis, primary ciliary dyskinesia) and screening gene therapies.	Bypasses the need for breeding specialised transgenic rodent strains to study human-restricted genetic respiratory mutations.

Regulatory Software & Strategy Frameworks

Regulatory acceptance of NAMs—referred to as "fit-for-purpose qualification"—is not a one-size-fits-all process. Researchers should utilise the following frameworks to ensure their computational and biological strategies align with the *Animals (Scientific Procedures) Act 1986* and industry best practices:

- **Context of Use (COU) Documentation:** Before deploying models, clearly define the specific scientific question the model is intended to address. This is critical for demonstrating that the NAM is reliable and suitable for its intended regulatory purpose.
- **Technical Characterisation:** Maintain comprehensive Standard Operating Procedures (SOPs) and study plans for both the biological NAM and any software used to analyse its output. This transparency is vital for successful project licence applications.
- **Early Regulatory Engagement:** Leverage institutional or external regulatory support to track "fit-for-purpose" milestones. Early engagement with the Home Office/ASRU is strongly encouraged to test the acceptability of a computational or NAM-based approach before full project submission.
- **Weight-of-Evidence (WoE) Frameworks:** Use integrated digital dashboards to aggregate data from both *in vitro* (NAM) and *in silico* (computational) sources. Building a strong "weight-of-evidence" argument is the most effective way to strengthen Replacement justifications when moving away from traditional animal models.

Skills, Training, and Capacity Building

Successful adoption of high-maturity Non-Animal Methodologies (NAMs) requires a strategic shift in workforce development. To ensure the long-term success of the platforms outlined in this series, universities should prioritise the following training initiatives to build institutional capacity:

- **Integrated Respiratory Physiology & Microfluidics:** Training programmes should bridge the gap between bench-top cellular biology, air-liquid interface maintenance, and complex microfluidic data analysis. Researchers require dual proficiency in maintaining long-term pseudostratified tissue barrier integrity and leveraging the computational pipelines necessary to interpret real-time ciliary beat frequency data.
- **Regulatory Competency:** Staff should receive formal training on "Fit-for-Purpose" qualification and the specific regulatory expectations of the Home Office/ASRU. This includes understanding how to document evidence for the 3Rs within formal project licence applications to ensure they meet legal standards.
- **Technological Literacy:** Skills in microfluidic device troubleshooting, primary stem-cell tissue differentiation, bronchial organoid maintenance, and AI-driven aerosol deposition computational modelling are increasingly critical for modern respiratory research. Universities should facilitate internal workshops or establish external partnerships to provide PhD students and postdoctoral fellows with hands-on experience in these specialised tools.
- **Ethics & Translation:** Education should focus on the scientific imperative of human-relevant research, emphasising why NAMs provide more predictive insights for clinical outcomes than traditional rodent models. This fosters a culture of excellence and ethical research design that is fully aligned with modern international standards.

Table 3: Skills • Training and Capacity Building

Capability Domain	Focus Area	Impact on 3Rs/Replacement
Technical	Advanced lung-on-chip & alveolar barrier culture	Replaces invasive animal aerosol inhalation studies
Computational	Predictive aerosol pharmacokinetics modelling	Bypasses non-predictive animal respiratory exposure assays
Regulatory	Fit-for-Purpose validation for airway barrier models	Facilitates compliance with ASPA 1986 replacement mandate
Bioinformatics	Longitudinal transcriptomic analysis of airway epithelial cells	Eliminates animal-based lung inflammation/toxicity testing

Strategic Note: This investment in human expertise is a key component of a robust, future-proof institutional roadmap, demonstrating to regulators that the institution is committed to the safe and effective operation of high-maturity NAMs.

Clinical Translation Case Studies

Case Study 1: Human ALI Mucociliary Models Correcting Rodent Snout-Only Inhalation False Negatives

- **Background:** A novel inhaled anti-inflammatory therapeutic candidate targeting chronic respiratory disorders demonstrated complete safety and zero airway irritation during extensive snout-only inhalation studies in rats. However, during early human clinical trials, patients experienced severe, unproductive coughing fits and bronchospasm, forcing the immediate suspension of the development pipeline.
- **The Paradigm Shift:** An academic research group re-evaluated the candidate compound using a human-relevant Air-Liquid Interface (ALI) system. The human-relevant platform correctly identified the risk, demonstrating that rodent obligate nasal breathing and different mucus compositions fail to predict human-specific sensory airway irritation.
- **The Methodology:** Researchers cultured human primary bronchial epithelial cells at the air-liquid interface until fully differentiated into pseudostratified mucociliary tissue. Upon introducing the therapeutic candidate, the system recorded a dramatic, dose-dependent decrease in ciliary beat frequency (CBF) and a rapid trigger of localised inflammatory cytokine secretion, which was completely absent in rodent cell models.
- **University & Legal Takeaway:** This study highlights how human-relevant ALI platforms can successfully replace animal acute inhalation toxicity screening, proving to the Home Office under ASPA 1986 that the rodent preclinical data had been actively misleading.

Case Study 2: Ex Vivo Human PCLS Replacing High-Severity Rodent Ovalbumin-Sensitised Asthma Models

- **Background:** Traditional preclinical asthma and bronchoconstriction research relies heavily on surgically and allergen-sensitised rodents. Animals are repeatedly injected with ovalbumin or house dust mite allergens to induce chronic, severe allergic airway inflammation, resulting in acute bronchospastic attacks, hypoxia, and immense respiratory distress before the animals are sacrificed.
- **The Paradigm Shift:** A university pharmacology department implemented ex vivo human precision-cut lung slices (PCLS), completely replacing the need for high-severity rodent allergen-sensitisation protocols.
- **The Methodology:** The team sourced non-transplantable human donor lungs and prepared thin, viable tissue slices containing intact terminal bronchioles and muscular walls. They quantified localised bronchoconstriction and bronchodilation kinetics by applying allergen challenges directly to the slices and tracking lumen area shrinkage via high-resolution digital imaging. This allowed them to screen potential therapeutic molecules in highly translationally relevant, intact human tissue without live animals.
- **University & Legal Takeaway:** This study showcases an exceptionally predictive human alternative that completely replaces high-severity animal allergen-exposure protocols, aligning perfectly with Home Office and ASRU expectations for the application of high-maturity Replacement models.

Executive Summary & Compliance Dashboard

Executive Summary

Respiratory research has historically relied on *in vivo* animal models for inhalation toxicology and aerosol pharmacokinetics, which frequently fail to capture the complex, human-specific micro-architecture of the alveolar-capillary barrier. These traditional models are often poorly predictive of human lung response, leading to translational failures in drug development. This roadmap mandates a transition to high-fidelity, human-relevant NAMs—specifically "lung-on-a-chip" microphysiological systems and predictive digital pharmacokinetic modelling—to replace invasive, high-severity animal challenge studies. This strategic shift ensures research is scientifically accurate, ethically robust, and fully compliant with the Section 2A statutory duty of the *ASPA 1986*.

Compliance Dashboard

Focus Area	Primary Animal Model	NAM Replacement Technology	ASPA 1986 Status
Respiratory	Animal aerosol inhalation studies	Lung-on-a-Chip & Digital PK Modelling	High

See Appendix: Animal-Free Matrix for the long term strategic implementation roadmap

Maturity Matrix

Phase 1 (Exploratory): Audit current animal use in inhalation toxicology and pulmonary drug trials.

Phase 2 (Pilot): Integrate human-derived alveolar barrier cultures and lung-on-chip microfluidics.

Phase 3 (Integration): Implement quantitative digital platforms for cross-cutting aerosol pharmacokinetics.

Phase 4 (Standardisation): Replace 100% of high-severity animal inhalation models with qualified human-relevant NAMs.

Final Compliance Statement

All proposed models satisfy the Section 2A statutory duty of *ASPA 1986*. By adopting these methodologies, the institution positions itself at the forefront of the global transition toward human-centric, cruelty-free medical science.

Integration Context: Roadmap Conclusion

The modules detailed in this roadmap—from the fundamental respiratory platforms explored in this series to the broader cardiovascular, neurobiological, and psychiatric frameworks established previously—demonstrate that replacement is an integrated institutional strategy. By mapping these specialised NAM-based respiratory models into a unified regulatory framework, the institution moves decisively toward a standardised, human-centric research paradigm. This systemic integration is the essential prerequisite for fulfilling the *ASPA 1986* statutory duty, ensuring all research is scientifically accurate, ethically aligned, and regulatory compliant.