

Human-Relevant Non-Animal Methodologies (NAMs)
Specialised Models Series: Metabolic, Digestive & Endocrine
NAFLD/NASH Models
Reference 5.7 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.

Non-Alcoholic Fatty Liver Disease (NAFLD) and its progressive form, Non-Alcoholic Steatohepatitis (NASH), represent a growing global health crisis. Current murine models fail to accurately replicate the complex human pathology of NASH. Key disparities include:

- **Metabolic Divergence:** Murine models often require exogenous toxic triggers (e.g., choline-deficient diets) that do not reflect the dietary/metabolic origins of human NAFLD.
- **Immune-Cell Profiling:** Human liver immune cell landscapes, specifically the activation patterns of Kupffer cells and infiltrating monocytes, show distinct molecular signalling compared to murine counterparts.
- **Severity:** Standard animal models involve prolonged, diet-induced chronic stress, leading to significant hepatic scarring and animal suffering, which are often non-predictive of human therapeutic outcomes.

Introduction & Regulatory Momentum

This section outlines the application of human-relevant liver-on-a-chip and organoid platforms designed to model the progressive stages of NASH, from simple steatosis to fibrosis and inflammation.

Integration Context: Roadmap Cohesion

NAFLD/NASH research (5.7) represents the clinical endpoint of the metabolic dysfunction explored in previous modules. It is intrinsically linked to the endocrine signalling (5.4), gut-microbiome crosstalk (5.5), and systemic metabolic dysfunction (5.6) described earlier. By utilising these integrated NAMs, researchers gain a comprehensive view of how systemic inputs drive liver pathology, allowing for a holistic Replacement strategy that tackles NASH at the organ and systems level.

Landmark Regulatory & Scientific Momentum

- **3D Liver Microtissues:** Human iPSC-derived hepatocytes and stellate cells co-cultured to model the transition from fatty liver to inflammation.
- **Liver-on-a-Chip:** Microfluidic platforms that simulate sinusoidal shear stress and oxygen gradients, critical for replicating NASH-induced fibrosis.
- **In Silico Disease Progression Models:** Computational frameworks that simulate the rate of fibrosis based on patient-specific lipid-metabolic profiles.

Core Specialised NAFLD/NASH Platforms

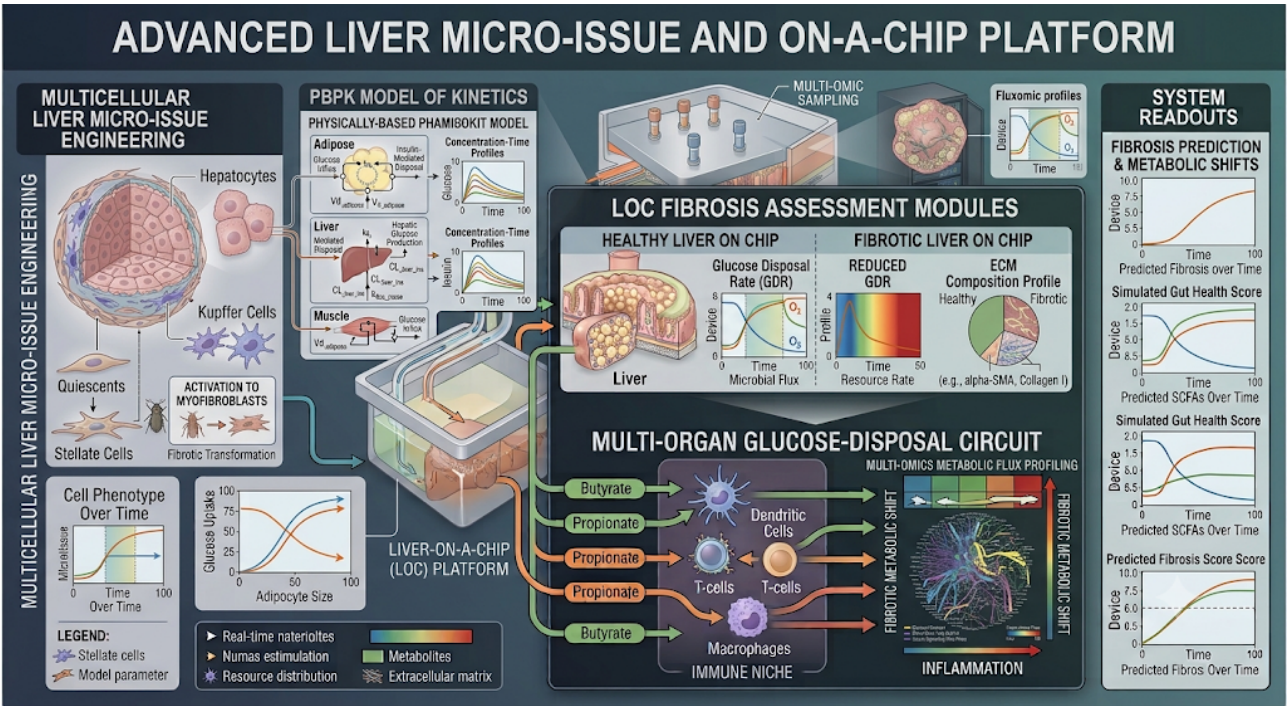


Figure 1: Multicellular liver microtissues (hepatocytes, Kupffer cells, and stellate cells); Liver-on-a-chip fibrosis assessment modules.

Table 1: Biological & Tissue-Engineered Platforms

Platform	Key Applications	Benefits for Universities & 3Rs
Multicellular Organoids	NASH progression (steatohepatitis)	Replaces chemical-injury rodent models
Liver-on-a-Chip	Fibrogenesis & shear stress modelling	Bypasses non-predictive toxic-diet protocols
Stellate-Cell Co-cultures	Liver fibrosis and scar tissue formation	Eliminates chronic fibrosis-inducing studies

Cross-Cutting Enabling Platforms

These cross-cutting enabling platforms provide the multi-scale predictive power required for NASH regulatory validation.

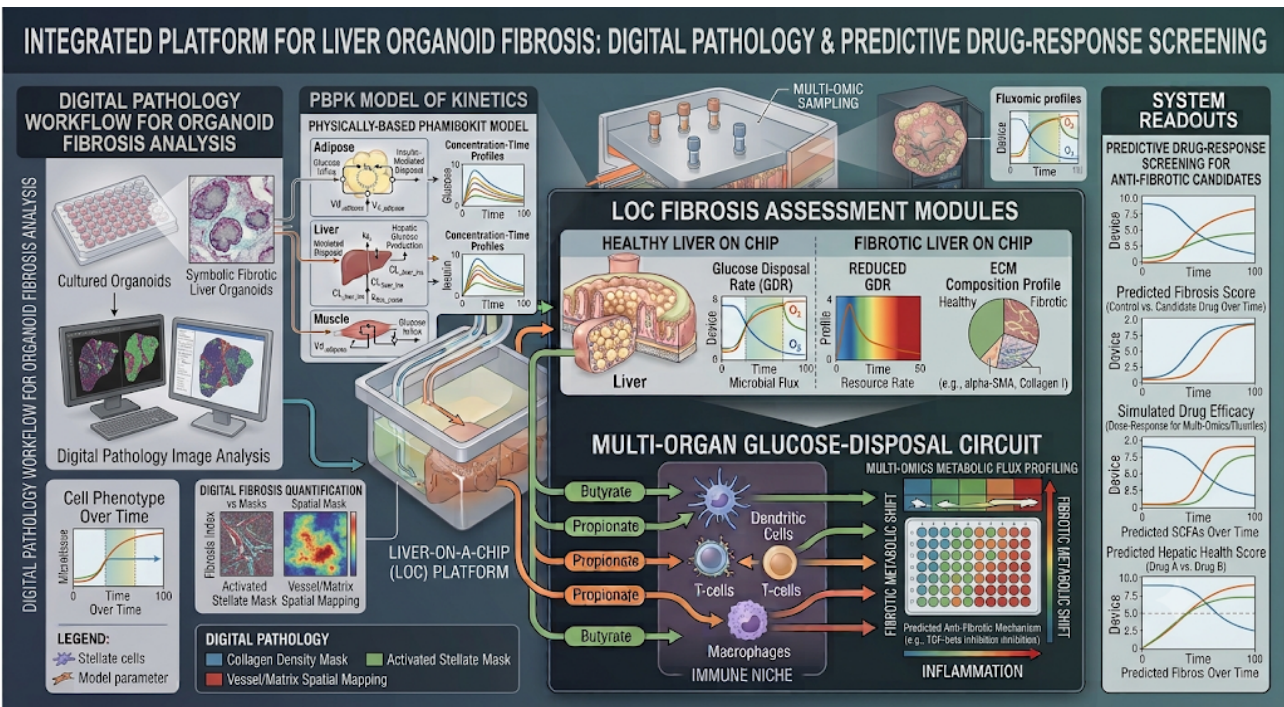


Figure 2: Digital Pathology for organoid fibrosis analysis; Predictive drug-response screening for anti-fibrotic candidates.

Table 2: Genomic & Computational Frameworks

Platform	Key Applications	Benefits for Universities & 3Rs
Digital Pathology AI	Automated fibrosis scoring	Replaces manual animal-tissue histology
Metabolic Pathway Sim	Predicting anti-fibrotic drug efficacy	Bypasses murine metabolic screening
Systems Biology Map	Human NASH gene signature mapping	Regulatory WoE for Replacement

Strategy • Skills & Capacity

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

To successfully transition from traditional murine protocols to human-relevant NAMs in NAFLD/NASH research, institutional capacity must be expanded. This requires a dual focus on advanced technical expertise in organoid handling and the development of robust bioinformatics capabilities to manage complex, multi-omic datasets derived from human tissue models. Furthermore, professional development must address the regulatory landscape of ASPA 1986, ensuring that researchers can build rigorous, evidence-based cases for Replacement that will satisfy AWERB and ASRU review boards. By institutionalising this training, universities reduce reliance on legacy models while simultaneously accelerating clinical translation.

Table 3: Skills •Training, and Capacity Building

Capability Domain	Focus Area	Impact on 3Rs/Replacement
Technical	Multicellular Liver Organoid Culture	Direct Replacement of animal models
Bioinformatics	Fibrotic Gene-Signature Analysis	Bypasses murine fibrosis histology
Regulatory	ASPA 1986 Compliance & AWERB	Facilitates faster ASRU approvals
Computational	Anti-Fibrotic Drug Efficacy Prediction	Eliminates invasive animal screening

Clinical Translation Case Studies

Case Study 1: Liver-on-a-Chip vs. Murine "Toxic-Diet" Models

A therapeutic candidate for NASH demonstrated anti-fibrotic effects in murine "choline-deficient" models but failed in human trials. Liver-on-a-chip platforms, utilizing patient-derived stellate cells, correctly predicted the lack of anti-fibrotic activity in humans. This failure underscores the non-predictive nature of rodent models; the Organ-on-a-Chip platform serves as the "fit-for-purpose" Replacement, satisfying the ASPA 1986 mandate.

Case Study 2: Replacement of High-Severity NASH Protocols

Replacement of high-severity murine models—which induce liver damage through long-term high-fat diets and toxic stimulants—with a human-relevant Liver-Organoid-on-a-Chip. This NAM allows researchers to study fibrogenesis in a human context, creating a robust, ethically compliant Replacement that avoids the systemic suffering inherent in animal models, directly satisfying the ASPA 1986 requirement.

Executive Summary & Compliance Dashboard

Executive Summary

The reliance on murine models for NASH research is a leading cause of translational failure, as these models often fail to replicate human liver pathology and fibrogenesis. This roadmap provides a strategic transition to human-relevant NAMs, including multi-cellular liver organoids and microfluidic chips. By prioritizing these models, institutions improve predictive accuracy and meet the statutory ASPA 1986 mandate to replace regulated animal procedures with scientifically superior alternatives.

Compliance Dashboard

Focus Area	Primary Animal Model	NAM Replacement Technology	ASPA 1986 Status
NAFLD/NASH	Murine Diet-Induced Fibrosis	Multi-cellular Liver Organoids	High

Maturity Matrix

- Phase 1 (Exploratory):** Audit animal use in NASH studies.
- Phase 2 (Pilot):** Integrate liver organoid NAMs into research.
- Phase 3 (Integration):** Use MOC circuits for systemic NASH modelling.
- Phase 4 (Standardisation):** Replace 100% of high-severity protocols.

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 fundamental hepatic and gastrointestinal models to complex metabolic syndrome and NAFLD/NASH models (5.7)—demonstrate that replacement is not a siloed scientific exercise but an integrated institutional strategy. By mapping these specialised NAMs into a unified framework, the institution moves 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.