

Human-Relevant Non-Animal Methodologies (NAMs)
Specialised Models Series: Metabolic, Digestive & Endocrine
Hepatic/Liver Models
Reference 5.1 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.

The liver is the central hub for xenobiotic metabolism, systemic homeostasis, and biochemical detoxification. Hepatic diseases—including Drug-Induced Liver Injury (DILI), Metabolic Dysfunction-Associated Steatohepatitis (MASH/NASH), cirrhosis, and biliary cholestasis—present exceptionally high hurdles in drug discovery and environmental safety profiling. Historically, preclinical toxicology has relied on in vivo animal testing. However, the mammalian hepatic microenvironment, enzymatic pathways, and immunological responses diverge fundamentally between species, rendering animal models highly non-predictive of human clinical outcomes.

At the physiological, molecular, and metabolic levels, human and rodent hepatic systems differ significantly:

- **Cytochrome P450 (CYP) Enzymatic Mismatch:** The metabolic clearance profile of a drug is dictated by Cytochrome P450 enzymes. Human and rodent CYP superfamilies exhibit profound disparities in gene expression, substrate specificity, and catalytic activity. For example, rodents express high levels of the CYP2C and CYP2D subfamilies, which process compounds through pathways non-existent in humans. Conversely, key human drug-processing enzymes, such as CYP3A4 and CYP2E1, have completely different induction kinetics and binding pocket geometries compared to their rodent orthologues, leading to routine false-negative predictions of drug safety and bioavailability.
- **Bile Acid and Transporter Discrepancies:** Rodents and humans utilise highly distinct bile acid pools and transporter networks. Rodents primarily synthesise hydrophilic bile acids (such as β -muricholic acid), which are significantly less toxic than the highly hydrophobic bile acids (such as chenodeoxycholic acid) dominant in humans. Furthermore, the expression, regulation, and drug-sensitivity of hepatic efflux transporters—such as the Bile Salt Export Pump (BSEP) and Multidrug Resistance-Associated Proteins (MRPs)—differ profoundly, meaning drug-induced cholestasis cannot be reliably predicted in rodent screens.
- **Non-Physiological MASH/NASH Induction:** Rodent models of progressive metabolic liver disease require either severe chemical poisoning—using toxic agents like carbon tetrachloride (CCl_4)—or highly unphysiological diets, such as the Methionine-Choline-Deficient (MCD) diet. While these methods force hepatic fat accumulation and fibrosis, they trigger severe systemic cachexia, muscle wasting, and profound immune suppression that do not mirror the progressive, obesity-linked, and inflammatory metabolic syndrome seen in human clinical MASH.
- **Acute Liver Failure and High Severity:** Regulatory acute systemic toxicity studies—such as high-dose paracetamol (APAP) challenges or acute CCl_4 exposures—inflict immense, high-severity suffering. These procedures cause rapid, widespread hepatic necrosis, excruciating abdominal pain, systemic hepatic encephalopathy, and high mortality rates, which act as massive, uncontrolled stress-induced confounding variables.

Transitioning to human-relevant Non-Animal Methodologies (NAMs)—such as multi-cellular microfluidic Liver-on-Chips, long-term functional 3D primary human hepatocyte (PHH) spheroids, and ex vivo human precision-cut liver slices (PCLS)—is a dual scientific and statutory mandate for UK academic research.

Landmark Regulatory & Scientific Momentum

This section details the transition from traditional animal-based hepatotoxicity assays to high-fidelity, human-relevant NAMs. By leveraging iPSC-derived organoids and liver-on-a-chip technologies, research institutions can replicate complex human hepatic responses to xenobiotics and metabolic stress with unprecedented accuracy.

- **3D Liver Microtissues:** Human iPSC-derived hepatocytes co-cultured with non-parenchymal cells to model human-specific hepatic response.
- **Liver-on-a-Chip:** Microfluidic platforms that simulate hepatic sinusoid shear stress and oxygen gradients, critical for replicating human-specific drug metabolism.
- **In Silico Predictive Toxicology:** Computational frameworks that integrate human hepatic molecular signatures to predict clinical hepatotoxicity.

Core Specialised Hepatic Platforms

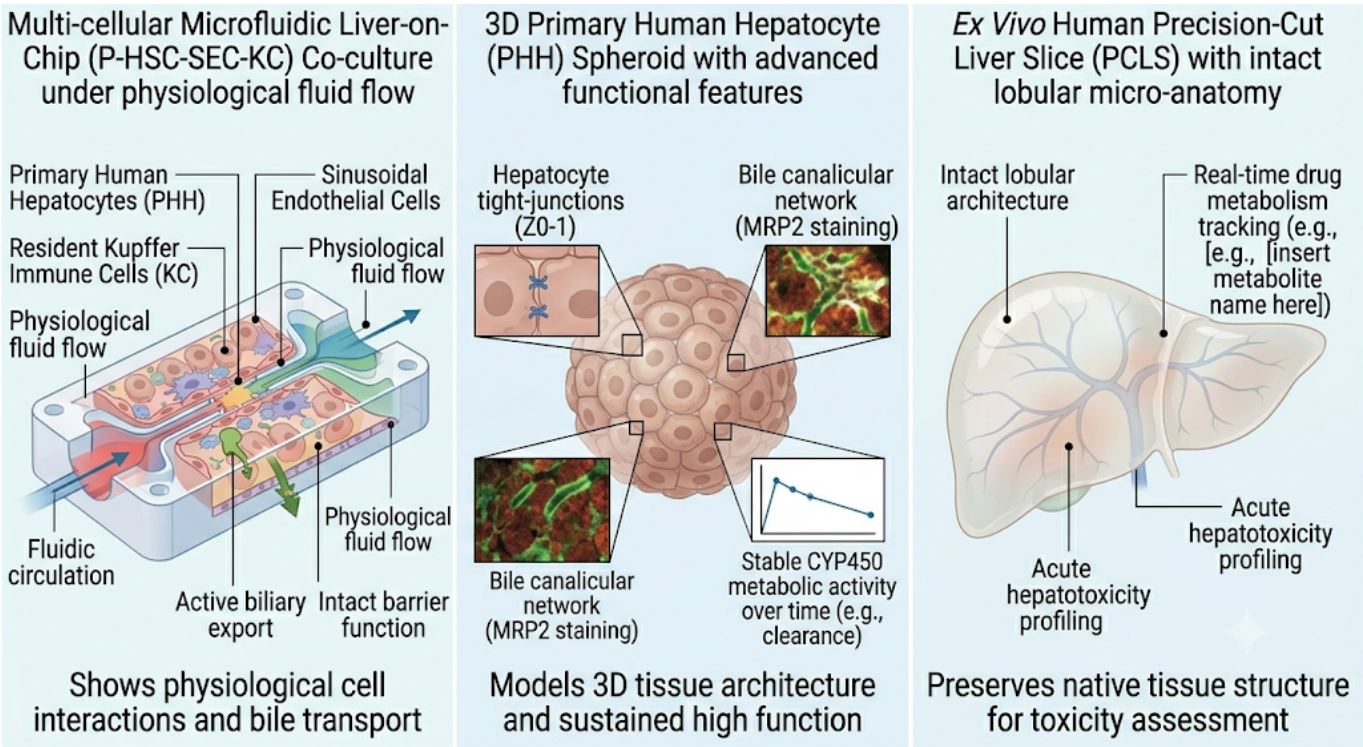


Figure 1: Core Specialised Hepatic Platforms — Multi-cellular microfluidic Liver-on-Chip showing primary human hepatocytes co-cultured with sinusoidal endothelial cells and resident Kupffer immune cells under physiological fluid flow, maintaining active biliary export (*left*); 3D primary human hepatocyte (PHH) spheroid showing functional hepatocyte tight-junctions, stable CYP450 metabolic activity, and bile canalicular networks (*centre*); and ex vivo human precision-cut liver slice (PCLS) showing intact lobular micro-anatomy used to track real-time drug metabolism and acute hepatotoxicity (*right*).

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

Platform / Model	Description	Key Applications	Benefits for Universities & 3Rs
Multi-Cellular Liver-on-Chip	Microfluidic channels lining human hepatocytes, endothelial, Kupffer, and stellate cells under continuous physiological perfusion.	Predicting chronic drug-induced liver injury (DILI), immune-mediated hepatotoxicity, and viral hepatitis (HBV/HCV) kinetics.	Directly replaces the highly distressing rodent chronic-dosing toxicity trials and regulatory repeat-dose testing.
3D Primary Hepatocyte Spheroids	Scaffold-free 3D micro-tissues of primary human hepatocytes maintaining high viability and metabolic function.	High-throughput screening of drug clearance, metabolite profiling, chronic low-dose toxicity, and enzyme induction/inhibition.	Replaces the routine use of animal-derived liver microsomes and fresh primary rodent hepatocyte isolation protocols.
MASH/NASH-on-Chip	Multi-cellular liver-on-chip exposed to human-relevant free fatty acids (oleic and palmitic acids) under continuous flow.	Modelling lipotoxicity, steatosis progression, inflammatory Kupffer cell activation, and stellate-mediated collagen deposition.	Eliminates the need for painful, non-physiological rodent diets (e.g., MCD diet) and severe chemical fibrosis protocols.
Ex Vivo Human Liver Slices (PCLS)	Thin, viable slices of non-transplantable human donor organs preserving intact lobular zoning and native tissue architecture.	Tracking localized metabolic clearance, drug-induced cholestasis, and testing anti-fibrotic compound efficacy in native human tissue.	Bypasses highly painful animal common bile duct ligation (BDL) surgeries used to induce cholestatic liver injury.

Cross-Cutting & Enabling Platforms for Hepatic Research

These platforms support multiple metabolic, pharmacokinetic, and toxicology research areas and are widely applicable across small-molecule target discovery, safety profiling, and clinical trial simulations. While physical liver-on-chips capture micro-structural niches, these cross-cutting digital and hybrid systems provide the quantitative, predictive pipelines required to validate high-maturity NAMs for regulatory-accepted, "fit-for-purpose" safety profiling.

As shown in Figure 2, combining machine learning with structural biology, in silico physiologically-based pharmacokinetics (PBPK), and quantitative Adverse Outcome Pathways (qAOPs) establishes a comprehensive preclinical pipeline. These enabling technologies are crucial for supporting qualified, human-predictive approaches under international standards like the FDA Modernization Act 2.0 and for strengthening legal replacement arguments under Section 2A of the *Animals (Scientific Procedures) Act 1986*.

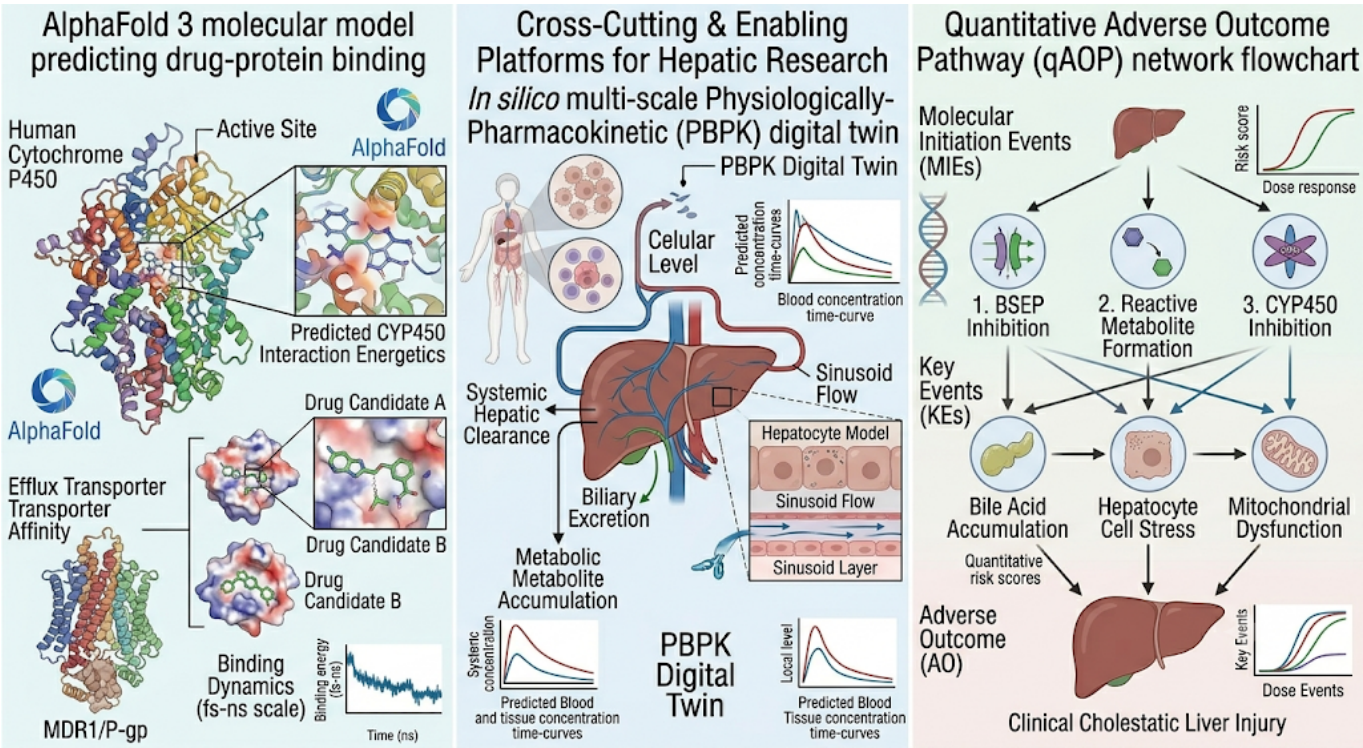


Figure 2: Cross-Cutting & Enabling Platforms for Hepatic Research — AlphaFold 3 molecular model predicting the binding dynamics of drug candidates interacting with human Cytochrome P450 active sites and efflux transporters (*left*); in silico multi-scale Physiologically-Based Pharmacokinetic (PBPK) digital twin simulating systemic hepatic clearance, biliary excretion, and metabolic metabolite accumulation (*centre*); and a quantitative Adverse Outcome Pathway (qAOP) network flowchart linking molecular initiation events (such as BSEP inhibition) to clinical cholestatic liver injury (*right*).

Table 2: Genetic, Computational & Framework-Driven Platforms

Platform / Model	Description	Key Applications	Benefits for Universities & 3Rs
In silico PBPK Clearance Simulators	Multi-scale mathematical models simulating human hepatic blood flow, enzyme kinetics, and systemic metabolite concentration over time.	Predicting human-specific drug clearance, calculating localized tissue exposures, and optimizing clinical dose regimens.	Bypasses highly invasive rodent and canine pharmacokinetic (PK) blood-sampling and mass-balance studies.
Molecular Dynamics & CYP Docking	AI-driven structural predictions mapping the interactions of small molecules with human hepatic enzymes (CYP3A4, CYP2C9, CYP2D6).	Screening compound libraries for CYP inhibition, predicting drug-drug interaction (DDI) risks, and optimizing metabolic stability.	Replaces animal-tissue-derived cell membrane preparation protocols and radioactive ligand-binding assays.
Patient-Derived iPSC Hepatic Models	Reprogrammed patient-specific iPSCs differentiated into hepatocytes and assembled into diseased, multi-cellular liver organoids.	Precision modelling of genetic metabolic disorders (e.g., lysosomal storage diseases, Wilson's disease) and screening gene therapies.	Bypasses the need for breeding and maintaining specialised transgenic rodent strains with human-restricted metabolic mutations.
qAOP-Integrated Hepatic Screeners	Quantitative Adverse Outcome Pathways linking molecular initiation events (e.g., mitochondrial stress) to clinical steatohepatitis.	Regulatory chemical safety assessment, environmental pollutant screening, and high-throughput hepatotoxicity target prioritisation.	Integrates multiple in vitro data points into a legally defensible weight-of-evidence Replacement package for Project Licence applications.

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

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 Hepatology & Microfluidics:** Training programmes should bridge the gap between bench-top hepatic biology, microfluidic circuit engineering, and complex pharmacokinetic data analysis. Researchers require dual proficiency in maintaining long-term primary cell co-cultures and leveraging the computational fluid dynamics software necessary to interpret flow-mediated metabolic profiles.
- **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 hepatic differentiation, hepatic spheroid maintenance, and AI-driven metabolic docking are increasingly critical for modern metabolic 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.

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.

Table 3: Skills •Training, and Capacity Building

Capability Domain	Focus Area	Impact on 3Rs/Replacement
Technical	iPSC-derived hepatocyte 3D culture	Direct Replacement of animal models
Bioinformatics	Hepatic transcriptome data analysis	Bypasses murine liver-injury histology
Regulatory	ASPA 1986 Compliance & AWERB	Facilitates faster ASRU approvals
Computational	PBPK liver-clearance simulations	Eliminates invasive animal PK studies

Clinical Translation Case Studies

Case Study 1: Human Liver-on-Chip Correcting Rodent Drug-Induced Liver Injury (DILI) False Negatives

- **Background:** A novel clinical drug candidate designed for a metabolic indication demonstrated an exceptional safety profile, showing zero hepatic toxicity or enzyme elevation across extensive preclinical testing in both rats and dogs. However, during early Phase I clinical trials, human volunteers experienced severe, acute hepatotoxicity and elevated liver enzymes, forcing the immediate termination of the program.
- **The Paradigm Shift:** An academic pharmacology department re-evaluated the failed compound using a human-relevant Liver-on-Chip platform. The human-relevant system correctly identified the risk, demonstrating that rodent and canine Cytochrome P450 active sites and metabolic pathways do not mirror human-specific reactive metabolite generation.
- **The Methodology:** Researchers co-cultured primary human hepatocytes with human endothelial and Kupffer cells inside a microfluidic device under continuous flow. Upon introducing the drug candidate, the chip recorded a rapid, dose-dependent accumulation of a highly reactive hepatotoxic metabolite—specifically generated by human CYP3A4 but absent in rodents—leading to acute mitochondrial dysfunction, oxidative stress, and subsequent hepatocyte death.
- **University & Legal Takeaway:** This study highlights how human-relevant Liver-on-Chip platforms can successfully replace animal drug-induced toxicity screening, proving to the Home Office under ASPA 1986 that the animal preclinical safety data had been actively misleading.

Case Study 2: Long-Term 3D Hepatocyte Spheroids Replacing High-Severity Rodent MCD Diet Models of MASH/NASH

- **Background:** Preclinical evaluation of therapeutics for metabolic dysfunction-associated steatohepatitis (MASH) historically relies on feeding mice a Methionine-Choline-Deficient (MCD) diet. This high-severity protocol induces rapid hepatic steatosis and inflammatory cell infiltration, but also inflicts immense systemic distress on the animals, including dramatic weight loss, chronic muscle wasting, systemic cachexia, and high mortality rates before study completion.
- **The Paradigm Shift:** A university research department implemented multi-cellular 3D human liver spheroids, completely replacing the need for high-severity rodent metabolic dietary protocols for anti-fibrotic target validation.
- **The Methodology:** The team generated 3D hepatic spheroids containing primary human hepatocytes, hepatic stellate cells, and resident Kupffer cells. By exposing the spheroids to human-relevant concentrations of free fatty acids (oleic and palmitic acids) and glucose, they successfully recapitulated the clinical features of human MASH—including lipid droplet accumulation, inflammatory cytokine release (TNF- α , IL-6), and active collagen deposition by stellate cells. They measured anti-steatotic drug efficacy in real time via non-invasive imaging, testing compounds in a highly human-relevant micro-environment without live animals.
- **University & Legal Takeaway:** This study showcases a highly predictive human alternative that completely replaces high-severity animal dietary protocols, aligning perfectly with Home Office and ASRU expectations for the application of high-maturity Replacement models.

Executive Summary & Compliance Dashboard

Executive Summary

The reliance on murine models for hepatotoxicity and liver disease research is a significant barrier to translational success, often failing to mirror human-specific hepatic metabolic pathways. This roadmap provides a strategic transition to human-relevant NAMs, including iPSC-derived hepatic organoids and advanced microfluidic liver platforms. By prioritising these models, institutions improve predictive toxicology and satisfy 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
Hepatic Toxicity	Murine Hepatotoxicity Protocols	iPSC-derived Liver Organoids	High

Maturity Matrix

- Phase 1 (Exploratory):** Audit animal use in liver toxicity studies.
- Phase 2 (Pilot):** Integrate 3D liver organoids into research workflows.
- Phase 3 (Integration):** Implement multi-organ microfluidic liver circuits.
- Phase 4 (Standardisation):** Replace 100% of high-severity liver injury 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 Cohesion

As the foundational pillar of the metabolic series, Hepatic/Liver models (5.1) provide the core infrastructure for subsequent modules. The liver-specific metabolic flux and clearance mechanisms established here are the primary determinants of the systemic responses studied in Endocrine Signalling (5.4), Microbiome Crosstalk (5.5), and the complex pathologies of Metabolic Syndrome (5.6) and NAFLD/NASH (5.7). By centralising liver NAMs, universities create an interconnected regulatory framework where Replacement strategies in hepatic physiology directly support and validate the systemic metabolic research that follows.