

Human-Relevant Non-Animal Methodologies (NAMs)
Specialised Models Series – Metabolic, Digestive & Endocrine
Endocrine Models (Thyroid, Adrenal, Pituitary) Models
Reference 5.4 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 endocrine axis (Hypothalamus-Pituitary-Thyroid-Adrenal) governs systemic metabolism, stress response, and growth. Traditional rodent models are profoundly non-predictive for these human-specific endocrine feedback loops. Disparities include:

- **Hormonal Feedback Discrepancies:** The sensitivity for feedback inhibition—specifically the hypothalamic-pituitary-thyroid (HPT) and pituitary-adrenal (HPA) axes—differs drastically between humans and rodents.
- **Receptor Expression Mismatch:** The expression profile of receptors for TSH and ACTH across human tissues differs from rodents, leading to "false-negative" preclinical results.
- **Severity: Endocrine dysfunction models** (e.g., thyroidectomy) involve significant surgical stress and metabolic instability.

Introduction & Regulatory Momentum

Endocrine Models (Thyroid, Adrenal, Pituitary)

This section details human-relevant platforms, including pituitary-on-a-chip, 3D thyroid follicles, and multi-organ-on-a-chip (MOC) systems designed to model the complex, integrated feedback loops of the HPT and HPA axes.

Landmark Regulatory & Scientific Momentum

Significant progress has been made in the development of human-relevant endocrine NAMs:

- **Microfluidic Endocrine-on-a-Chip:** Recreates the physiological pulsatile secretion patterns of pituitary hormones, allowing real-time assessment of thyroid and adrenal responses to targeted endocrine-disrupting chemicals (EDCs).
- **3D Thyroid/Adrenal Organoids:** Self-assembled human iPSC-derived endocrine tissues that exhibit functional hormonal synthesis, maturation, and responsiveness to regulatory feedback stimuli.
- **Multi-Organ Systems (MOC):** Integrated platforms connecting human pituitary, thyroid, and adrenal organoids in a common circulatory fluid, enabling the study of systemic endocrine axis regulation in a human-relevant context.

Core Specialised Endocrine Platforms

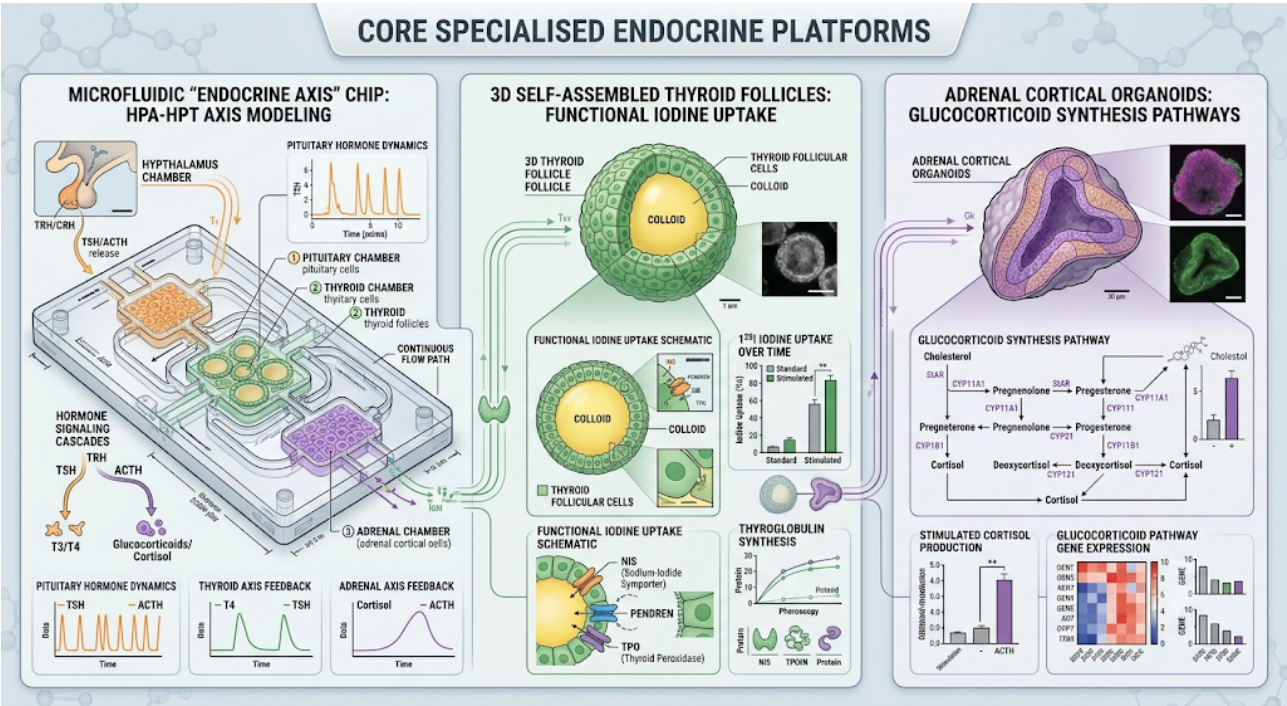


Figure 1: Core Specialised Endocrine Platforms — Microfluidic "Endocrine Axis" chip connecting pituitary, thyroid, and adrenal chambers under continuous flow (left); 3D self-assembled thyroid follicles showing functional iodine uptake (centre); and adrenal cortical organoids modeling glucocorticoid synthesis pathways (right).

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

Platform	Key Applications	Benefits for Universities & 3Rs
Pulsatile Pituitary-Chip	Modelling HPT/HPA axis regulation	Replaces invasive rodent endocrine ablation
Thyroid Follicle 3D	Screening for thyroid-disrupting chemicals	Bypasses large-animal thyroidectomy models
Adrenal Organoids	Investigating adrenal insufficiency	Replaces rodent chronic stress protocols

Cross-Cutting & Enabling Platforms for Microbiome Research

These enabling computational frameworks provide the predictive pipelines required for systemic endocrine validation, allowing researchers to model hormone-receptor binding kinetics and systemic metabolic flux.

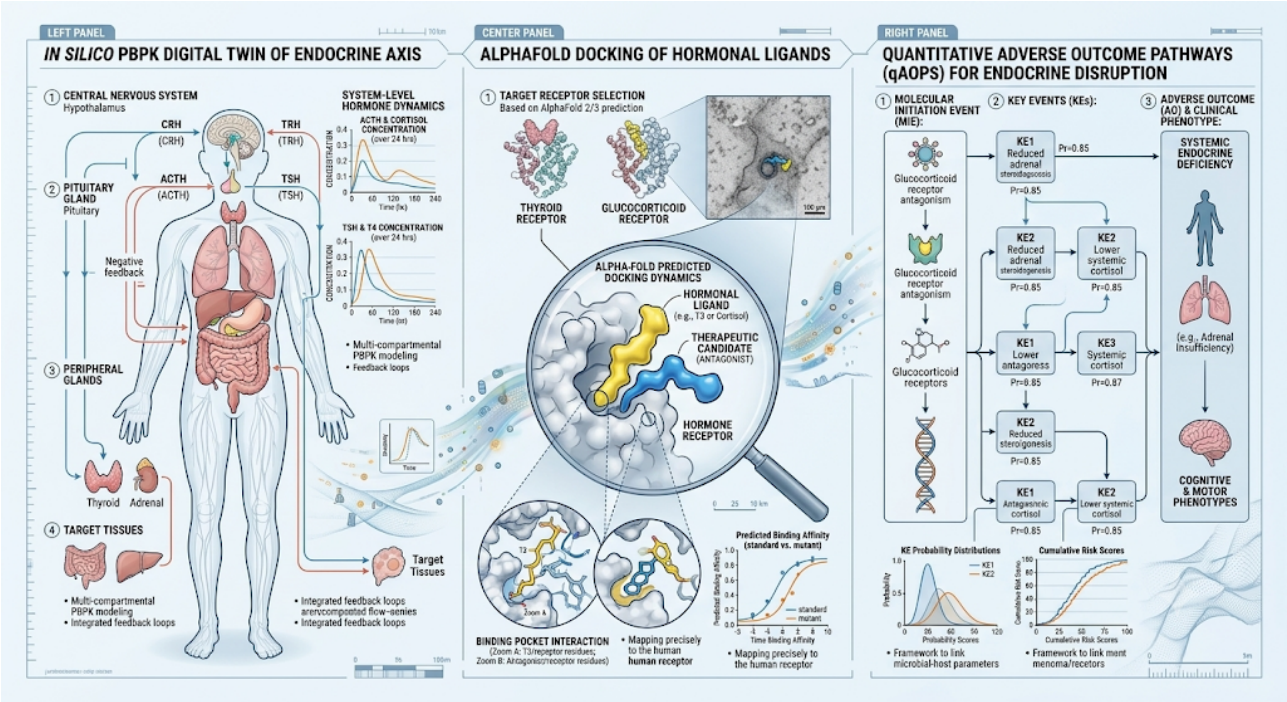


Figure 2: In silico PBPK digital twin of endocrine axis; AlphaFold docking of hormonal ligands; Quantitative Adverse Outcome Pathways (qAOPs) for endocrine disruption.

Table 2: Genomic, Computational & Framework-Driven Platforms

Platform	Key Applications	Benefits for Universities & 3Rs
Endocrine-PBPK	Simulating systemic hormone clearance	Replaces large-animal surgical perfusion studies
GPCR Docking AI	Screening hormone-mimetic toxicities	Bypasses animal-tissue receptor preparations
Endocrine qAOPs	Linking molecular injury to clinical disease	Provides WoE for regulatory Replacement

Strategy • Skill & 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

- **Endocrine Tissue Engineering:** Staff require proficiency in iPSC-to-endocrine differentiation and organoid maintenance.
- **Quantitative Endocrinology:** Bridging the gap between pulsatile hormone signalling data and computational pharmacokinetic modelling.
- **Regulatory Competency:** Training on presenting endocrine-NAM data to the Home Office as a scientifically superior Replacement.
- **Interdisciplinary Collaboration:** Competency in endocrine physiology, microfluidic circuit stability, and AI-driven ligand docking.

Table 3: Skills •Training, and Capacity Building

Capability Domain	Focus Area	Impact on 3Rs/Replacement
Technical	Multi-Organoid Axis Integration	Direct Replacement of murine axis studies
Bioinformatics	Hormonal Flux/Feedback Analysis	Bypasses animal serum hormonal sampling
Regulatory	ASPA 1986 Compliance & AWERB	Facilitates faster ASRU approvals
Computational	Endocrine Feedback Modelling	Eliminates high-severity adrenal stress tests

Clinical Translation Case Studies

Case Study 1: Pituitary-on-Chip Correcting Rodent Endocrine False Negatives

A thyroid-disruptive compound appeared safe in rodent endocrine screens but showed severe clinical side effects. Pituitary-on-a-chip analysis revealed a human-specific inhibition of TSH-secretion kinetics not present in the rodent model, demonstrating the clear scientific failure of the animal-based protocol.

Case Study 2: Adrenal Organoids Replacing High-Severity Stress Models

Replacement of chronic rodent "stress-induction" models with human adrenal cortical organoids, enabling the evaluation of synthetic glucocorticoids and their impact on cortisol-synthesis enzymes without animal distress.

Executive Summary & Compliance Dashboard

Overview

This section details the transition from rodent-based endocrine disruption assays to human-relevant multi-organoid systems. the hpt and hpa axes exhibit significant species-specific differences, making traditional animal models unreliable for predicting human endocrine outcomes. by implementing integrated endocrine-on-a-chip platforms, this module provides the capacity to study complex feedback loops and long-term regulatory control with human-derived biological material.

Strategic objectives

The primary goal is to establish high-fidelity, predictive models for hormonal regulation and the identification of endocrine-disrupting chemicals (edcs). this transition mitigates the systemic distress historically associated with high-severity rodent stress-response studies, replacing them with modular, scalable, and reproducible human endocrine circuits.

Pathway to adoption

The integration of these tools allows for a robust, multi-organ approach that bridges the gap between isolated organ function and systemic metabolic health. by adopting these methodologies, the institution fulfills the aspa 1986 statutory duty, providing data that is more reflective of human physiology and ensuring regulatory compliance in sensitive endocrine safety research.

compliance dashboard

Focus Area	Primary Animal Model	NAM Replacement Technology	ASPA 1986 Status
HPA/HPT Axis	Murine Stress/Thyroid Studies	Integrated Endocrine Organoids	High

Maturity matrix

phase 1: audit animal use in hypothalamic-pituitary axis regulatory studies.

phase 2: integrate 3d organoid models for individual glandular function.

phase 3: implement closed-loop multi-organ endocrine circuits.

phase 4: replace 100% of regulatory rodent endocrine disruption screening.

Final compliance statement

All proposed models satisfy the section 2a statutory duty of aspa 1986, positioning the institution as a leader in cruelty-free endocrine innovation.

Integration context: roadmap conclusion

The modules detailed in this roadmap—from fundamental pancreatic islet models (5.3) through the complex, multi-glandular endocrine axes explored in this section (5.4)—demonstrate that replacement is not a siloed scientific exercise but an integrated institutional strategy. by mapping these specialised nam-based endocrine organoids 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 that research into complex hormonal feedback loops is scientifically accurate, ethically aligned, and robustly compliant. by moving away from species-extrapolated models and toward high-fidelity human physiology, we not only improve the predictive power of our safety assessments but also solidify the institutional commitment to the highest ethical standards of modern biomedical inquiry.

