LifeNet Health® LifeSciences

TruVivo® Application Brief:

Detecting Species-Relevant Thyroid Hormone Metabolism for Chemical Safety Evaluation

Background and Research Challenge

Liver-mediated clearance of thyroxine (T4) – particularly via T4 glucuronidation (T4G) – is a critical event in rodent liver–thyroid mode-of-action (MoA) pathways. *In vivo* induction of glucuronidation enzymes (UGTs) leads to reduced circulating T4, elevated TSH, and downstream thyroid histopathology.[1,3]

However, thyroid hormone metabolism and nuclear receptor activation differ quantitatively between rodents and humans, complicating extrapolation of rodent data to human risk. To improve quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) and reduce reliance on animal testing, researchers are adopting new approach methodologies (NAMs) to:

- Characterize species-specific liver-mediated T4 metabolism and glucuronidation
- Evaluate hepatic UGT induction and T4 clearance *in vitro*
- Support weight of evidence frameworks and QIVIVE for thyroid MoA evaluations
- Increase confidence in using data from human *in vitro* models for risk assessment

Researchers require an *in vitro* system that:

- Maintains rat and human hepatocyte phenotypes over the multi-day exposure periods used for nuclear receptor (NR) agonist studies
- Detects species-relevant patterns in chemical-induced changes in T4 metabolism
- Captures expected positive, negative, and species-specific reference compound responses
- Deliver quantitative data for integration into thyroid safety and risk evaluation workflows

The TruVivo® Solution

The TruVivo 2D+ hepatic system provides a physiologically relevant microenvironment enabling long-term culture of primary rat and human hepatocytes with stable morphology and species-specific sensitivity to nuclear receptor agonists and induced metabolic pathways.[1-6]

When used with the TruVivo Flex medium, the system provides adaptable supplementation optimized for non-human species, including physiologically relevant glucocorticoid levels necessary for maintaining rodent thyroid metabolism pathways and nuclear receptor response dynamics.[1-5]

Long-term stability of rat hepatocytes

- Rat hepatocytes maintain high attachment rates and stable morphology and functions for up to 14 days in both 24- and 96-well plate formats.[5]
- Albumin production remains stable from Days 7–14, confirming preserved function and metabolic capacity over repeated-dose assay windows.[5]

Fit-for-purpose for thyroid hormone metabolism assays

- Supports 7-day repeated exposure to NR agonists (PB, PCN, PCB153, RIF, CITCO, DEX) at non-cytotoxic levels[1-3, 5]
- Enables quantitative measurement of:
  - Baseline T4 glucuronidation (T4G)[3]
  - ΔT4G responses to NR agonists[1,3]
  - Species-specific NR-regulated CYP/UGT gene expression patterns[1,5]

Species-relevant performance

TruVivo preserves major rodent-human differences reported in NAM-based thyroid hormone metabolism assays, including:

- Higher baseline T4G in rat vs. human hepatocytes[3]
- Species-selective induction patterns for known CAR/PXR activators[3]
- Mechanistic gene expression consistent with compound-specific modes of action[5]

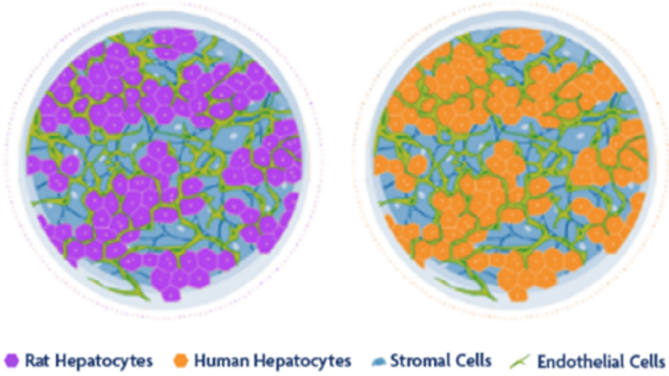


Figure 1. A schematic representation of TruVivo showing a top view of self-assembled rat (A) and human (B) hepatocyte colonies cultured with stromal and endothelial feeder cells.

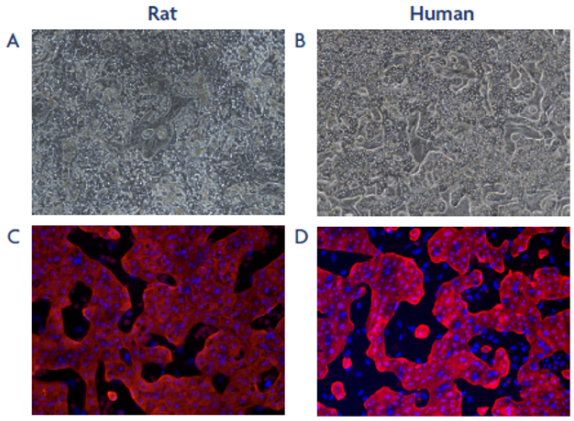
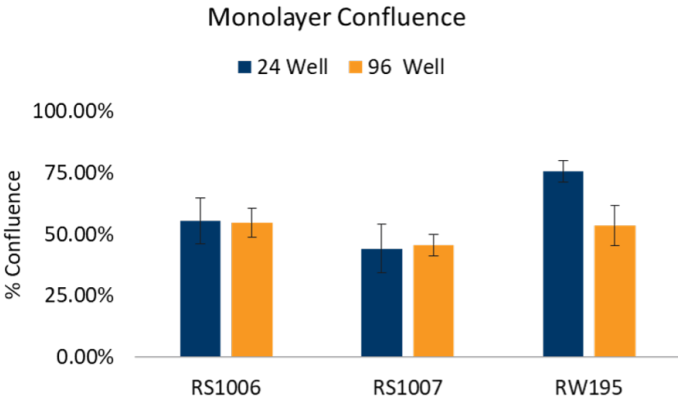


Figure 2. Representative brightfield images of rat (A) and human (B) cultures on day 10 and confocal laser microscopy images of rat (C) and human (D) cultures (blue: nuclei [DAPI], red: hepatocytes [Cytokeratin-18]) on day 14 in TruVivo.[4]

Key Findings and Validation

Finding	Evidence
TruVivo supports long-term stability needed for thyroid MoA studies	• Rat hepatocytes maintained stable morphology and function for 14 days in 24- and 96-well formats across multiple donors[5] • Albumin production remains stable (days 7–14), confirming sustained hepatocyte function required for multi-day exposures[5]
TruVivo detects quantitative species differences in baseline T4 glucuronidation	• T4G formation is substantially higher in rat vs. human hepatocytes, fully consistent with known rodent-human differences[2,3]
Species-specific ΔT4G induction patterns are preserved	**Rat (Sprague Dawley):** • PCB153 and PCN strongly increase T4G[2,3] • PB increases T4G at higher concentrations[2,3] • CITCO and RIF show no T4G response, as expected for negative controls[2,3] **Human:** • PCB153 causes significant increases in T4G[2,3] • RIF increases T3 (not T4), consistent with PXR biology[2,3] • PCN, PB, CITCO show minimal or no T4G induction[2,3]
Mechanistic CYP/UGT responses are maintained	• Rat hepatocytes show expected CYP2B1 and CYP3A induction, with PCN > PB for CYP3A-related activity.[3] • Human hepatocytes show CYP2B6/CYP3A4 and UGT1A1 induction with RIF and PCB153.[3] • UGT2B1 upregulation in rat following PCN exposure reflects rodent-specific UGT regulation.[2]
TruVivo reproduces expected positive and negative reference controls	• Control profiles match *in vivo* expectations and published NR-agonist behavior • Positive rat controls: PCB153, PCN, PB[1,3] • Negative rat controls: CITCO, RIF[1,3] • Positive human control: PCB153[2,3] • Negative human control: PCN[2,3] • Human-selective responses: RIF (CYP/UGT induction; ↑T3)[2,3]



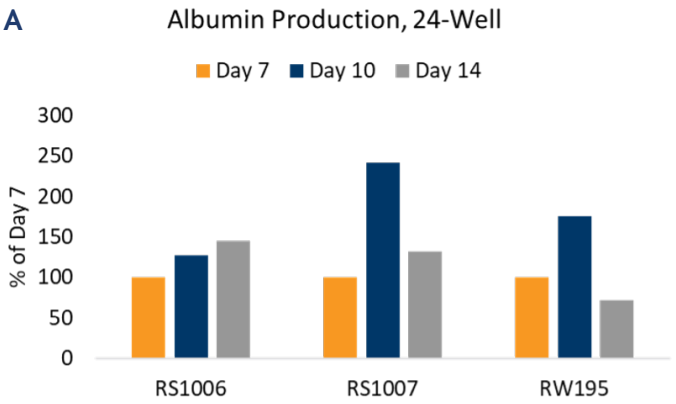


Figure 3 (Above). TruVivo supports attachment and sustained confluence of rat hepatocytes over 14 days in culture.[5] Monolayer confluence of primary rat hepatocytes from three lots was assessed on day 14 of culture in the TruVivo system in both 24- and 96-well formats. All lots maintained ≥50 % confluence, meeting the predefined performance specification. Data demonstrates that TruVivo supports long-term hepatocyte monolayer integrity across multiple donors and plate formats.[5]

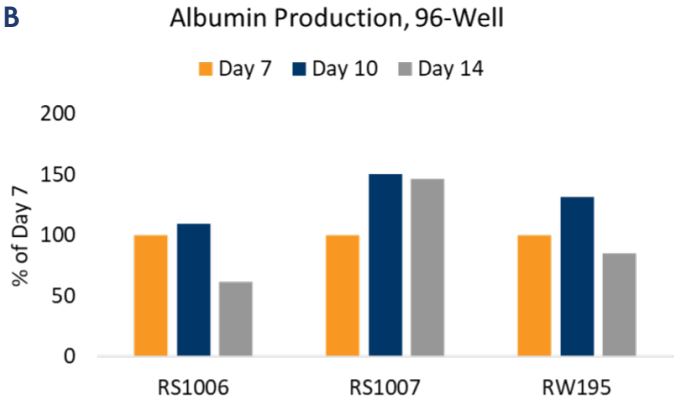
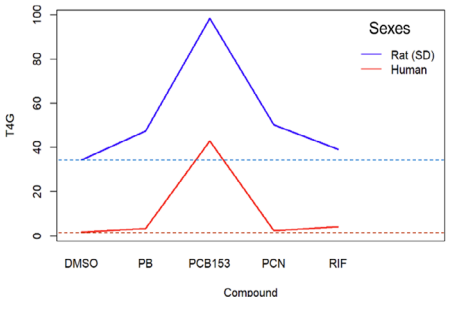
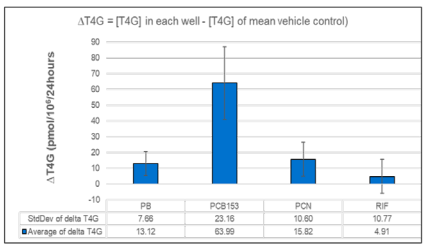


Figure 4 (Right). TruVivo maintains albumin production in rat hepatocytes for up to 14 days in culture.[5] Albumin secretion was measured in three lots of primary rat hepatocytes cultured in the TruVivo system using 24-well (A) and 96-well (B) formats. Albumin levels were assessed on days 7, 10, and 14, and data are presented as percent change relative to day 7 values. Hepatocytes maintained stable albumin production over time in both formats, supporting the functional integrity of the cultures over extended culture periods.[5]

A) Interaction Plot: Compound and Species



B) ΔT4G induction in Rat (SD) after Reference compound exposure (Mean of three runs)



C) ΔT4G induction in Human after Reference compound exposure (Mean of three runs)

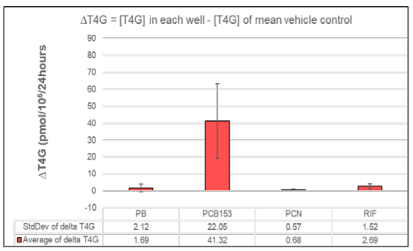


Figure 5 (Above). TruVivo captured clear, quantitative species differences in T4 glucuronidation: rats showed ~10-fold higher baseline T4G than humans, and this species separation was maintained across all exposure groups. PCB153 produced strong induction in both species, PB and PCN responses were rat-specific, and RIF produced modest induction only in human hepatocytes. Data generated by Corteva Agrisciences.[3]

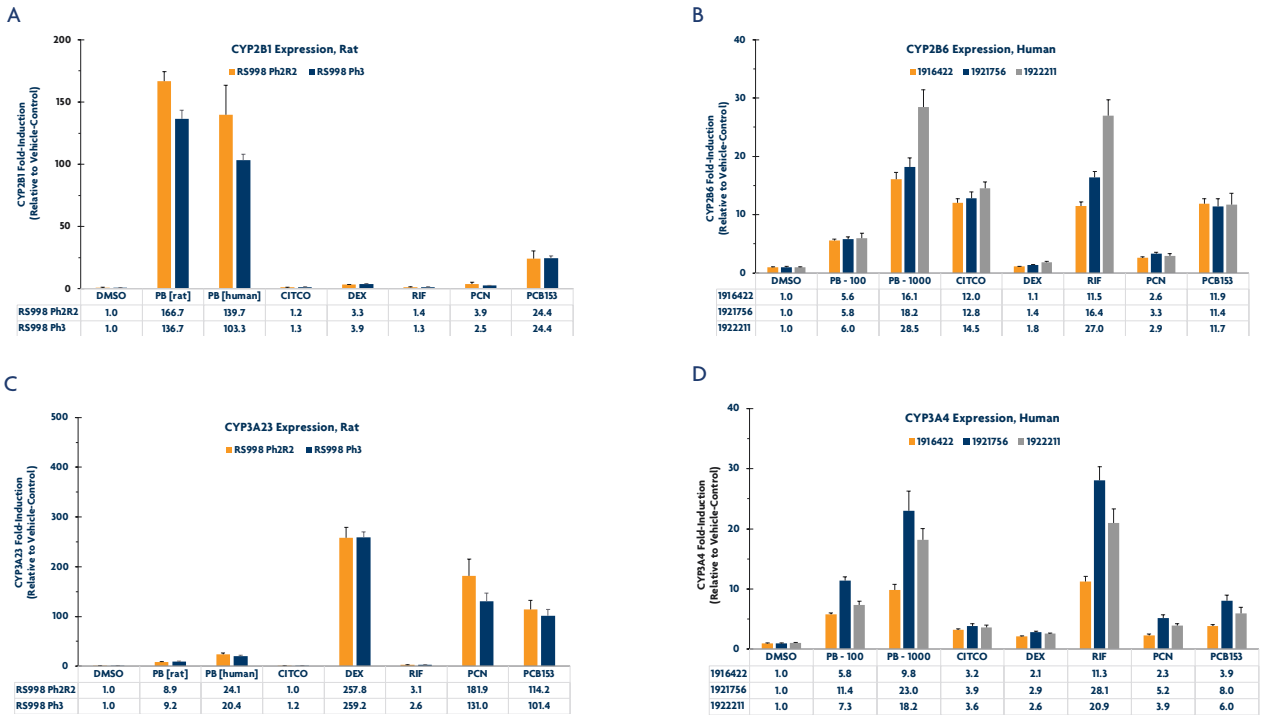


Figure 6 (Above). Species-specific nuclear receptor-mediated gene expression in TruVivo Flex. CYP2B1 (A), CYP2B6 (B), CYP3A23 (C), and CYP3A4 (D) expression in rat or human hepatocytes after nuclear receptor agonist treatment, showing distinct species-specific responses. TruVivo enabled detection of distinct species differences in nuclear receptor-mediated gene regulation, supporting its application for assessing compound dependent changes in T4 clearance when Flex media was used.[5]

Why Researchers Choose TruVivo

Researchers Need	TruVivo Advantage
Long-term rat hepatocyte model for indirect thyroid MoA studies	14-day stability in confluence and albumin secretion enables extended exposures and repeated dosing.
Capture of species-relevant T4 metabolism	~10× higher baseline T4G in rat vs human hepatocytes, respectively, with preserved species-selective responses.
Reliable reference controls	PCB153 (both species), PB/PCN (rat), RIF (human), CITCO (negative).
Mechanistic confidence (NR signaling intact)	Preserved CAR/PXR-driven CYP/UGT responses verify mechanistic integrity.

References

1. **Bhattacharya *et al.* (2025),** *Comparison of In Vitro Hepatocyte Models for IVIVE of Thyroid Hormone Clearance in Rats*. SOT 2025.
2. **Raza *et al.* (2023),** *In Vitro Triculture Model to Evaluate Human Relevance of Chemical-Induced Thyroid Toxicity.* SOT 2023, Abstract 4532/P408.
3. **Raza *et al.* (2025),** *Comparative T4-glucuronidation Assay in Primary Human and Rat Hepatocytes in TruVivo.* SOT 2025, Abstract 3938/H421.
4. **Odanga *et al.* (2025),** *Morphology and Functionality of Primary Rodent Hepatocytes in TruVivo® Flex.* ISSX 2025, Abstract P41.
5. **LifeNet Health Internal Data.,** Data on file at LifeNet Health. Study #LNH-P-111-004.
6. **Weaver *et al.*,** *The morphology, functionality, and longevity of a novel all human hepatic cell-based tri-culture system.* Toxicology *In Vitro*, volume 86, 2023. https://doi.org/10.1016/j.tiv.2022.105504