CASE STUDY | DRUG INDUCED LIVER INJURY | LIVER

Revalia's human liver model detects troglitazone hepatotoxicity missed by conventional preclinical models

A platform for detecting drug induced liver toxicity

Study Snapshot

Revalia Bio is the technical lead for a contract worth up to $26.7 million awarded under the Advanced Research Projects Agency for Health (ARPA-H) Computational ADME-Tox and Physiology Analysis for Safer Therapeutics (CATALYST) program. The project, titled *An Integrated Human Data Approach to Unlock the Future of In Silico Learning Models*, supports Revalia's mission to redefine how drug pharmacokinetics and safety are predicted before clinical trials by generating human-relevant data from ex vivo perfused human kidneys and livers to support predictive machine learning (ML) and artificial intelligence (AI) models.

Troglitazone, a diabetes drug, was withdrawn from the market following reports of severe liver injury and acute liver failure and deaths in patients, despite extensive preclinical testing in nonhuman primates (NHPs) and clinical trials involving thousands of patients that failed to reliably predict severe hepatotoxicity [1–3]. In contrast, Revalia's Liver Toxicity Platform detected troglitazone-induced hepatotoxicity within just 72 hours at drug exposures comparable to those evaluated in NHP studies. By integrating pharmacokinetic, physiological, biochemical, and inflammatory measurements, the platform provided convergent biological evidence linking drug exposure to acute hepatotoxicity.

Early detection of human-specific toxicity using Revalia's Liver Toxicity Platform could have saved millions of dollars in clinical development, market withdrawal, and litigation costs and, more importantly, potentially saved lives.

Building on these findings, Revalia is evaluating additional compounds with distinct mechanisms of hepatotoxicity to establish the platform's ability to detect diverse forms of liver injury and support the development of ML- and AI-based models to predict hepatotoxicity in novel drug candidates.

THE ENGAGEMENT

Customer: Advanced Research Projects Agency for Health (ARPAH), a federal research program supporting research focused on Computational ADME-TOX and Physiology Analysis for Safer Therapeutics (CATALYST)

Program: Troglitazone-induced liver injury for hepatotoxicity disease modeling

Revalia offering: Liver Toxicity Study, intact perfused human donor livers

Perfusion window: Up to 72 hours per organ, with continuous physiologic monitoring

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The Challenge

Troglitazone, the first thiazolidinedione and PPARγ agonist approved to treat type 2 diabetes, entered the United States market in 1997 but was withdrawn in March 2000 following reports of severe hepatotoxicity and acute liver failure [4]. By withdrawal, the FDA had received 94 reports of drug-induced liver failure, and 63 deaths [1,2]. Despite extensive preclinical testing [3, 5], conventional animal models failed to reliably predict the severe liver injury observed in patients. This failure highlights a critical translational gap in preclinical drug development and establishes troglitazone as a relevant historical test compound for demonstrating the capabilities of Revalia's Liver Toxicity Platform.

Animal models can capture aspects of drug-induced liver injury, but species-specific differences in drug absorption, metabolism, susceptibility to toxicity, and physiological responses limit their ability to predict human hepatotoxicity. These limitations led Revalia to address two questions:

- Can an intact human ex vivo liver perfusion model detect early signals of troglitazone-induced hepatotoxicity that conventional animal studies failed to reliably predict?
- Can integrated measurements of drug exposure, metabolism, liver function, and injury establish a relationship between increasing drug exposure and progressive acute hepatotoxicity?

Revalia's ex vivo Human Liver Perfusion Platform addresses this critical translational gap by enabling the assessment of drug-induced liver injury in intact human livers while preserving native tissue architecture and integrated organ function. This approach enables the detection of early toxicity signals across multiple biological endpoints and provides a human-relevant platform for evaluating drug safety and investigating mechanisms of hepatotoxicity.

The Approach

Four human livers were perfused ex vivo for up to 72 hours and exposed to three escalating troglitazone infusions administered through the portal vein at 16.5-hour intervals. The initial dose was selected to achieve peak drug concentrations comparable to that observed in patients receiving therapeutic doses of troglitazone [6], while subsequent doses were designed to target higher drug exposure values extrapolated from troglitazone toxicity studies in small and large animals [3,5].

Troglitazone and its toxic metabolite, troglitazone sulfate, were quantified over time in venous perfusate using LC-MS/MS. Liver function, including bile production and lactate clearance, and circulating pro-inflammatory biomarkers were serially measured and compared with those of three control livers. This integrated approach links drug exposure and metabolism to complementary measures of liver function and injury.

Revalia's Liver Toxicity Study

What was measured	How
Troglitazone and Troglitazone Sulfate	Serial perfusate sampling to longitudinally quantify troglitazone and its metabolite, troglitazone sulfate, to characterize drug pharmacokinetics, hepatic metabolism, and toxicity-associated changes in drug clearance.
Liver function	Serial perfusate and bile sampling to longitudinally monitor lactate clearance and bile output as indicators of hepatic metabolic and exocrine function, respectively
Circulating inflammatory biomarkers	Serial perfusate sampling to quantify markers of inflammation over time.

The Data

1. Troglitazone clearance aligned with pharmacokinetic predictions at therapeutic doses, with reduced clearances observed following dose escalation.

Using reference clinical pharmacokinetic data [6], a one-compartment model was developed to predict troglitazone exposure at a therapeutic dose and at escalating doses designed to achieve toxic exposures (left panel, black bars). At the therapeutic dose, observed troglitazone pharmacokinetics closely matched model predictions, with rapid formation of troglitazone sulfate. Together, these findings demonstrate that the ex vivo liver perfusion model preserves key aspects of hepatic drug metabolism and clearance.

At 6× the therapeutic dose, troglitazone clearance was slower than predicted, accompanied by increased troglitazone sulfate concentrations. At 12× the therapeutic dose, clearance slowed further, with additional accumulation of troglitazone sulfate. These progressive deviations from predicted pharmacokinetics indicate altered drug disposition at higher exposures, consistent with reduced effective clearance as toxicity developed. Notably, troglitazone sulfate accumulated with successive doses and remained elevated even after parent-drug concentrations declined. Previous experimental studies have implicated troglitazone sulfate in the disruption of hepatobiliary transport, providing a potential mechanistic link between metabolite accumulation and troglitazone-associated liver dysfunction. [7].

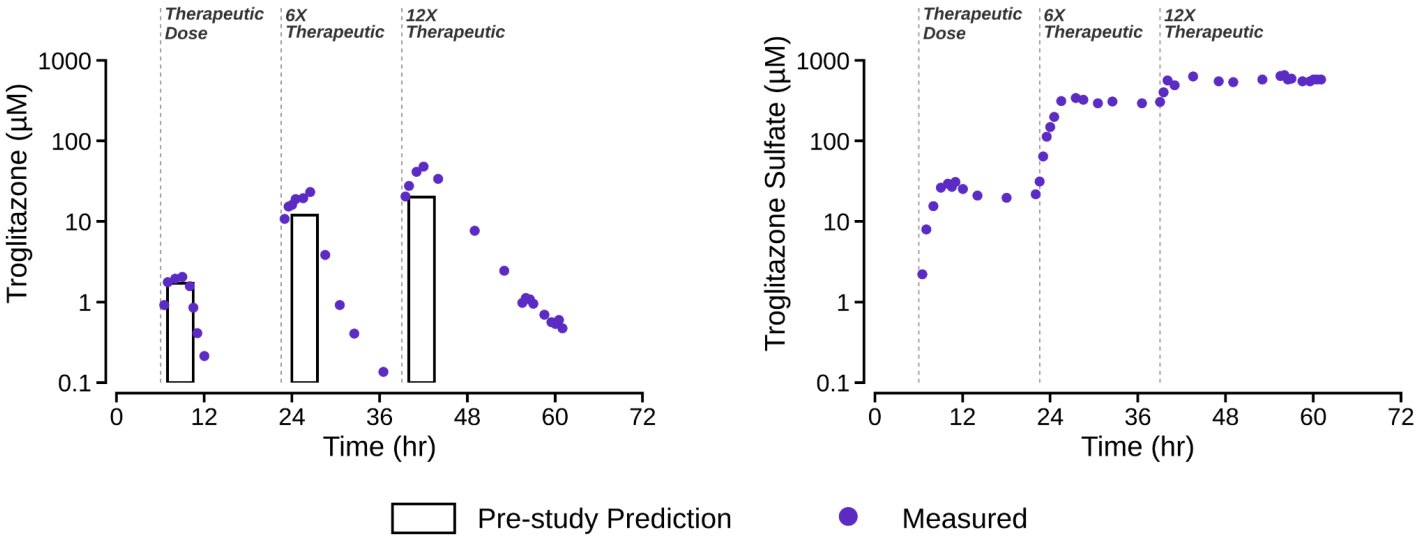


Figure 1. Ex vivo liver troglitazone pharmacokinetics align with clinical observations at therapeutic doses and reveal altered drug clearance at toxic exposures. ***Left panel*****:** Troglitazone concentrations were measured in serially collected venous perfusate during and following 4-hour portal vein infusions of 50 mg (therapeutic dose), 300 mg (6× therapeutic dose), and 600 mg (12× therapeutic dose). Black bars represent predicted troglitazone concentrations based on a one-compartment pharmacokinetic model, while purple dots represent measured concentrations from a representative liver.
Right panel: Troglitazone sulfate concentrations were measured in serially collected venous perfusate during and following 4-hour portal vein infusions of 50 mg (therapeutic dose), 300 mg (6× therapeutic dose), and 600 mg (12× therapeutic dose). Purple dots represent measured concentrations from a representative liver.

2. Escalating troglitazone exposure progressively impairs lactate clearance and reduces bile production.

Lactate clearance and bile production are clinically established indicators of hepatic metabolic and exocrine function, respectively, and are routinely assessed during ex vivo liver perfusion to evaluate organ viability for transplantation [8-10].

Prior to drug exposure, livers in both the reference control and troglitazone-treated groups cleared lactate to below the clinical viability threshold of 2.5 mM (left panel, red dashed line). Lactate clearance was maintained following the therapeutic dose of troglitazone (left panel, purple line). However, with escalating troglitazone doses, lactate progressively accumulated in the circulating perfusate, indicating impaired hepatic lactate clearance. In contrast, reference control livers-maintained lactate clearance throughout 72 hours of perfusion (left panel, black line).

Similarly, bile production was comparable between groups prior to drug exposure (right panel). Following the therapeutic dose, bile production decreased relative to reference controls, with further reductions observed following subsequent troglitazone doses (right panel).

Together, these findings demonstrate that escalating troglitazone exposure progressively impairs hepatic metabolic and exocrine functions, providing whole-organ physiological context for the observed alterations in drug disposition.

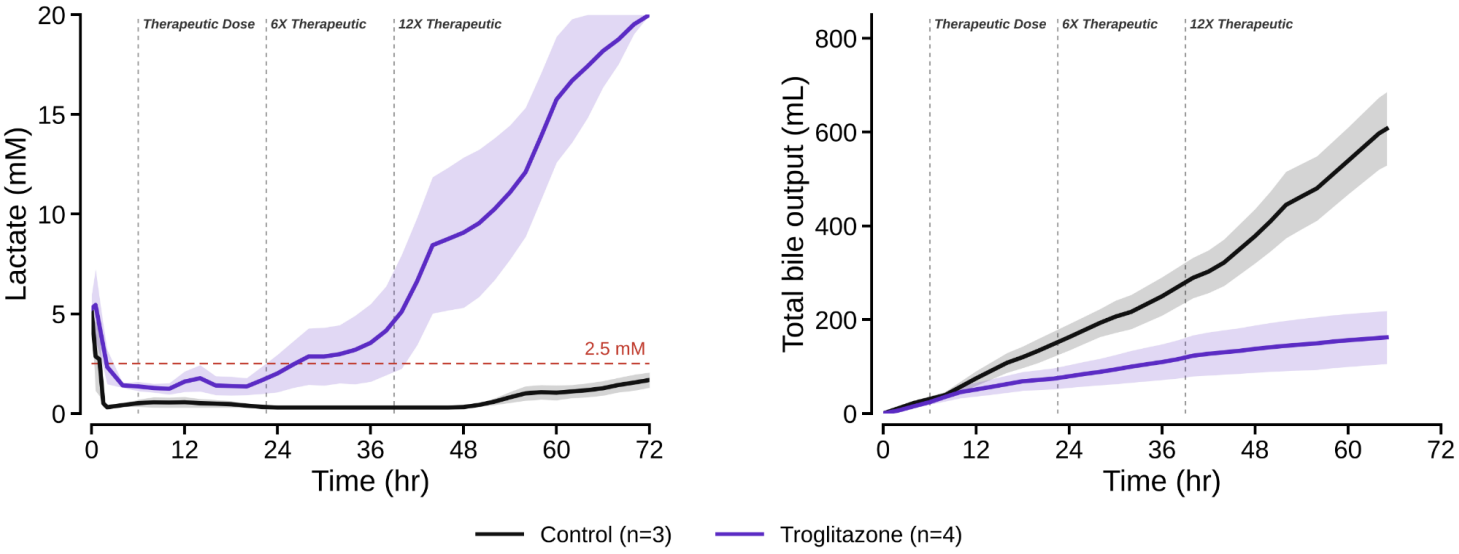


Figure 2. Escalating troglitazone exposure progressively impairs lactate clearance and reduces bile production, two critical indicators of whole-organ hepatic function. ***Left panel:*** Circulating lactate concentrations were measured in serial arterial perfusate samples collected during extended perfusion of four troglitazone-treated livers (purple line) and three reference control livers (black line). ***Right panel:*** Bile production was measured serially during extended perfusion to determine total bile output in four troglitazone-treated livers (purple line) and three reference control livers (black line). In both panels, solid lines represent group means, and shaded regions indicate the standard error of the mean (SEM).

3. Escalating troglitazone exposure leads to progressive inflammatory signaling.

Preclinical studies have reported that increased pro-inflammatory signaling occurs downstream of troglitazone-induced hepatocellular damage and may further exacerbate hepatic injury [11,12].

MIP-1α is a pro-inflammatory chemokine involved in the recruitment and activation of immune cells. Circulating MIP-1α levels remained relatively stable in control livers throughout 72 hours of perfusion (upper panel, black line). In contrast, MIP-1α levels increased sharply following the third troglitazone

dose (upper panel, purple line), with overall levels significantly elevated compared with controls (upper panel, bar graph).

MCP-1 is a pro-inflammatory chemokine involved in monocyte recruitment and inflammatory responses to tissue injury. Circulating MCP-1 levels increased after 24 hours of perfusion in control livers (lower panel, black line). However, this increase was more pronounced in troglitazone-exposed livers (lower panel, purple line), with overall levels significantly elevated compared with controls (lower panel, bar graph).

Together, these findings demonstrate an amplified inflammatory response accompanying the progression of troglitazone-induced liver injury, providing additional biological evidence of drug-induced hepatotoxicity.

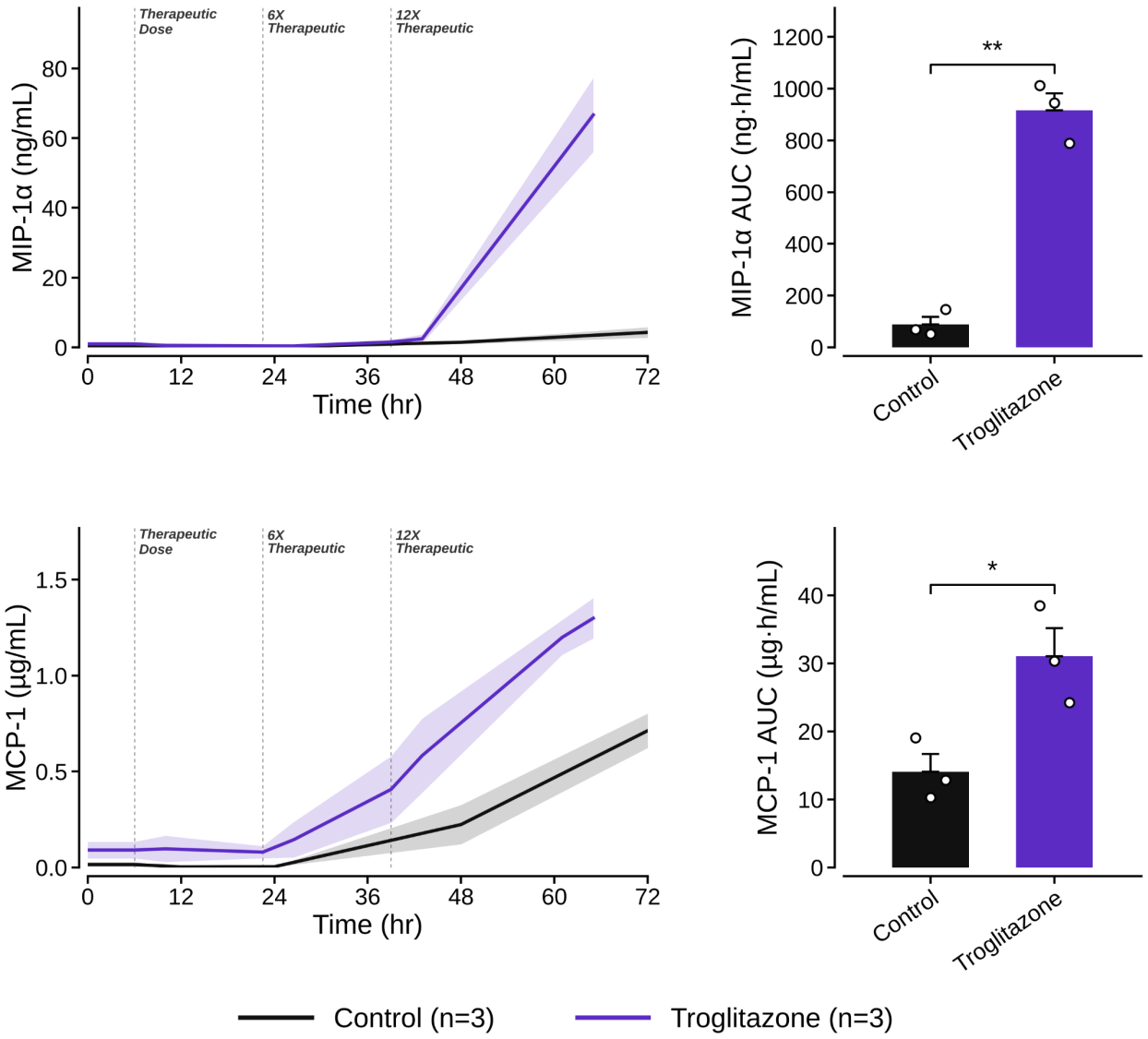


Figure 3. Escalating troglitazone exposure elevates circulating levels of pro-inflammatory chemokines involved in leukocyte recruitment. A multiplex immunoassay (MSD) was used to quantify MIP-1α and MCP-1in serial arterial perfusate samples collected during extended perfusion of four troglitazone-treated livers (purple line) and three reference control livers (black line). Data are presented as absolute chemokine concentrations over time (line graphs) and area under the curve (AUC, bar graphs). For line graphs, solid lines represent group means, and shaded regions indicate the standard error of the

mean (SEM). For bar graphs, group means are shown with SEM, and individual livers are represented as black outlined circles. Statistical differences in AUC were assessed using unpaired, two-tailed Welch's t-tests (*$p < 0.05$; **$p < 0.01$).

The Result

The Revalia Liver Toxicity Platform demonstrated the ability to detect troglitazone-induced hepatotoxicity across multiple biological endpoints, linking drug exposure to altered pharmacokinetics, impaired hepatic function, and increased inflammatory signaling in intact human livers. These findings highlight the platform's ability to detect human-relevant hepatotoxicity that prolonged conventional animal studies failed to reliably predict.

These findings, together with data generated using additional hepatotoxic compounds under the ARPA-H CATALYST award, are being used to characterize distinct patterns and mechanisms of liver injury and support the development of machine learning and artificial intelligence models aimed at predicting drug-induced hepatotoxicity of novel compounds.

Furthermore, the ability of the ex vivo perfused human liver system to reproduce expected clinical pharmacokinetic behavior at therapeutic exposures demonstrates the platform's ability to evaluate drug disposition and hepatic metabolism of novel compounds.

What Else This Applies To

The study design and experimental framework extend beyond troglitazone and can be applied to a broad range of compounds, therapeutic modalities, and research objectives using intact human liver. These applications include:

- **Additional hepatotoxic compounds:** Evaluation of known hepatotoxic agents, including acetaminophen, diclofenac, tolcapone, and amiodarone, all of which are actively being studied at Revalia.
- **Diverse therapeutic modalities:** Safety and toxicity assessment of small molecules, biologics, viral vector-based gene therapies, oligonucleotides, siRNA, and mRNA-lipid nanoparticles (LNPs).
- **Multiple administration routes:** Evaluation of clinically relevant delivery approaches, including intra-arterial bolus or infusion, portal vein bolus or infusions, direct parenchymal injection, and other translatable delivery techniques.
- **Expanded research applications:** Beyond toxicity assessment, the platform supports studies of therapeutic delivery and biodistribution and early efficacy.

WORKING WITH REVALIA

Revalia Bio maintains intact, perfused human donor organs for pharmaceutical research. Sponsors dose their own molecules in functioning human organs and get spatial, functional, and molecular data that animal models and in vitro systems cannot produce.

For more details, visit us @ www.revaliabio.com.

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