Excerpt Analysis Report Acetaminophen

Functional Metabolic Safety Risk Assessment

Conclusion

- Acetaminophen experiments showed dose- and time-dependent effects. The longer the exposure and the higher the concentration, the greater the adverse impact on human liver cells. At a concentration of 1 mM, minor compromising effects were observed. At 3 mM, the effects were substantial, and at 10 mM, the cells were severely compromised.

Key Findings QSM™

- **Mitochondrial Health:** A dose-dependent reduction in membrane potential and proton leaking was observed across all concentrations, indicative of energetic compromise and reduced ATP production.
- **Oxidative Stress:** High oxidative stress was noted, resulting in massive depletion of NADPH and a breakdown of the pentose phosphate pathway to replenish NADPH at 10 mM.
- **Detoxification:** Detoxification was compromised, with reduced ammonia uptake and collapsing urea production starting at 1 mM acetaminophen.
- **Lipid Metabolisms:** Lipid biosynthesis was substantially compromised at concentrations higher than 0.3 mM.
- **Carbohydrate Metabolism**: The ability of liver cells to provide glucose was massively reduced at 3 mM & glycogen content dropped pointedly at 1 mM.

Data Analysis Tool QSM™

- QSM™ reveal metabolic cellular processes & fluxes, and assesses bioenergy metabolism & mitochondrial health.

Doppelganger Biosystem GmbH

Doppelganger

Case Study Acetaminophen

Background and Study Goal

Acetaminophen is an analgesic and antipyretic drug that works by inhibiting prostaglandin synthesis in the central nervous system and acting on the hypothalamus to lower fever. It is classified in the DILIrank severity class 5. Its effects have been well-described, but its broader impact on liver metabolism remains underexplored. We analyzed transcriptome signatures from human primary hepatocytes treated for 12, 24 or 72 hours with supplemented MCM media containing solvent control treated with acetaminophen (0.3, 1, 3 or 10 mM). We assessed metabolic and functional protein biomarkers to evaluate acetaminophen's pharmacodynamic effects on a molecular level.

Key Study Findings

Acetaminophen experiments showed a dose- and time-dependent effect. The longer the exposure and the higher the concentration, the greater the adverse impact on human liver cells. At a concentration of 1 mM, compromising effects were observed. At 3 mM, the effects were substantial, and at 10 mM, the cells were severely compromised.

A dose-dependent reduction in membrane potential and proton leaking was observed across all concentrations, leading to energy depletion and reduced ATP production. Furthermore, high oxidative stress was noted, resulting in massive depletion of NADPH and a breakdown of the pentose phosphate pathway to replenish NADPH at 10 mM.

Detoxification was also compromised, with reduced ammonia uptake and collapsing urea production starting at 1 mM acetaminophen. Lipid biosynthesis was substantially compromised at concentrations higher than 0.3 mM. The ability of liver cells to provide glucose was already massively reduced at 3 mM, and glycogen content dropped significantly starting at 1 mM.

The QSM™-driven dose-dependent analysis of functional cellular mechanisms allows for the optimization of dose finding for first-in-human experiments by minimizing safety risks and optimizing treatment effects.

Data Source and Analyses

- Data source: GSE248251, Comparison of transcriptomic profiles between HFPO-DA and positive controls for PPARa, PPARg or cytotoxicity in mouse, rat, and pooled human hepatocytes, 2044, Authors: Heintz MM, Klaren WD, East AW, Thompson CM, Haws LC
- BioAnalysis: bulk RNA-sequencing
- Analysis: Quantitative System Metabolism (QSM™) & Gene Set Enrichment Analysis (GSEA)

QSM™ Platform

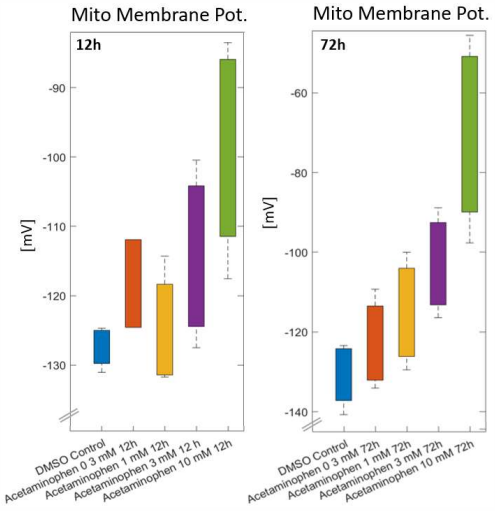
QSM™ Metabolic Phenotyping is the first analytical platform to quantify metabolic pathway activity biomarkers from single cells and tissues, including FFPE. QSM™ (Quantitative System Metabolism) equips researchers with the tools to assess the activity of 22 distinct metabolic pathways, offering actionable insights.

Doppelganger Biosystem GmbH (Germany) supports scientists in preclinical, clinical, and translational research by providing crucial insights into the function and activity of central cellular metabolic pathways.

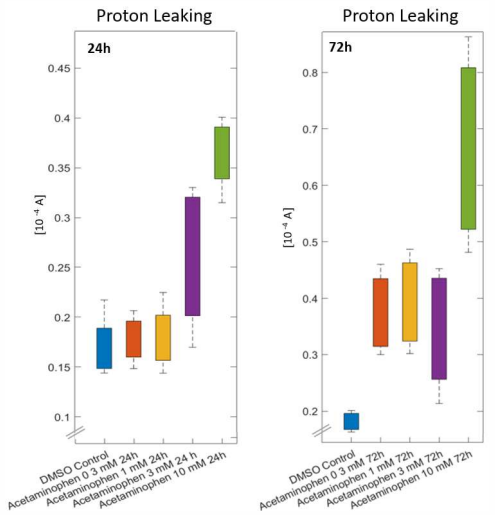
Key Results Acetaminophen

Mitochondrial Function Assessment

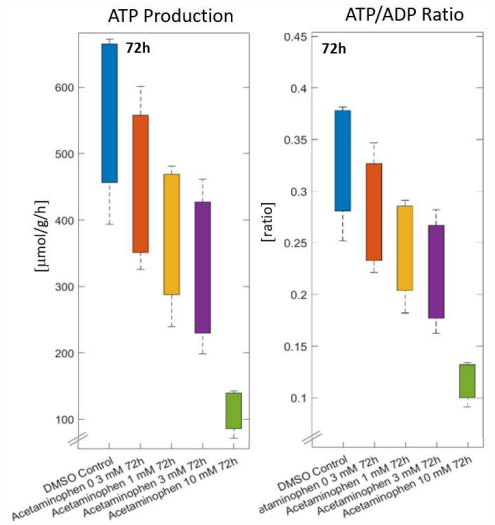
- A time- and dose-dependent effect can be observed with for acetaminophen causing increasing adverse effects on liver mitochondria. At 12h, a depolarization of the membrane potential can be observed, which is more pronounced at 72h. For 10 mM at 12h, the membrane potential reduction is approximately 25% to ~100 mV, and at 72h, a reduction of 60% to ~70 mV can be observed.



- Additionally substantial proton leaking can be observed. The effect at 24h shows a doubling, while at 72h a 4-fold increase is detected.

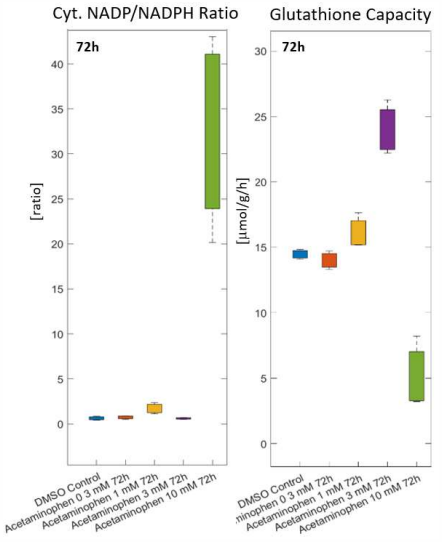


- In line with the reduced membrane potential and proton leaking observations, the ATP production capacity progressively worsens with higher concentrations (~6-fold decrease at 10 mM). This is also reflected in the ATP/ADP ratio, which significantly drops ~3 fold at 10 mM.



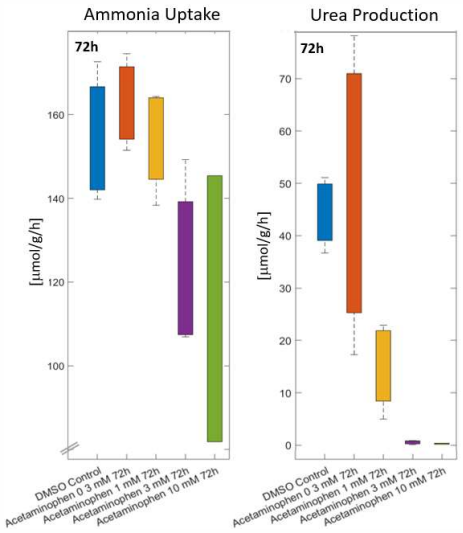
Oxidative Stress

- The NADP/NADPH ratio shows a significant increase at 10 mM, indicating massive depletion of NADPH and a limitation of the pentose phosphate pathway to replenish NADPH. Glutathione capacity is progressively increased at 1 mM and 3 mM before it drastically declines at 10 mM.



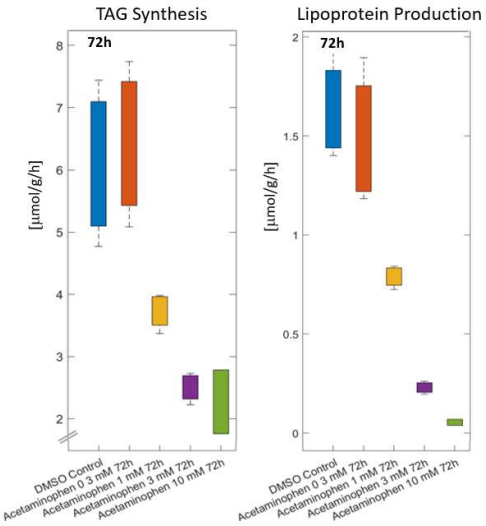
Detoxification

- Ammonia uptake by the liver is reduced by approximately 25% at 3 mM and 10 mM. Urea production drops to almost zero at 3 mM and 10 mM, and by ~66% at 1 mM, indicating that detoxification of ammonium is highly compromised.



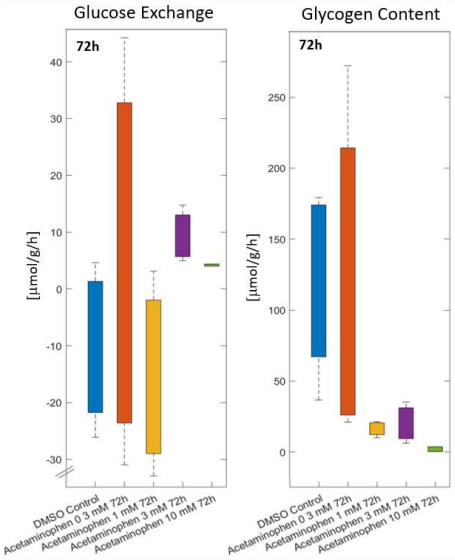
Lipid Metabolism

- Lipid biosynthesis for fatty acids (not shown), TAG and lipoprotein is progressively reduced, further indicating a debilitating impact of higher acetaminophen concentrations.



Carbohydrate Metabolism

- The liver produces glucose to supply the body. Here, a negative "Glucose Exchange" value means net efflux or hepatic glucose production, while a positive value means net influx of glucose or hepatic glucose consumption.
- High concentrations of acetaminophen lead to a halt in hepatic glucose production.
- Furthermore, the glycogen content is massively reduced to almost zero at 10 mM, indicating high energetic stress for cells exposed to acetaminophen concentrations higher than 0.3 mM.



Principal component analysis for all proteins

- The PCA analysis for 72h showed a clear concentration-dependent separation of the respective concentration groups from control.

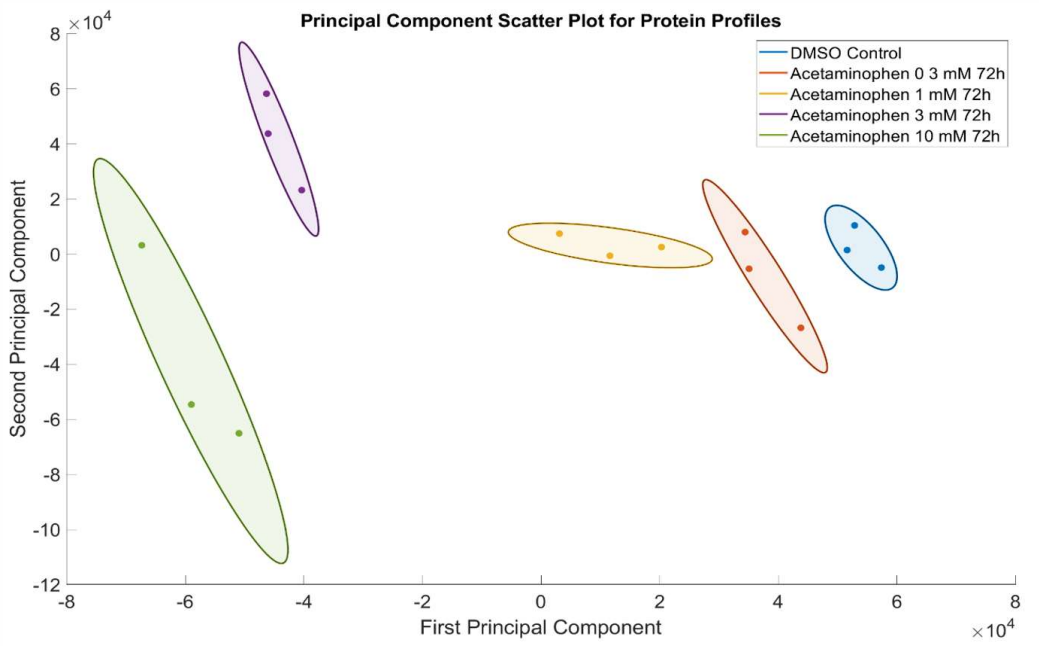


Table of Contents

Please request the full report (case study #52) at frankjunker@doppelganger-bio.com