Comparison of different in vitro hepatocyte models for in vitro to in vivo extrapolation of liver-mediated thyroid hormone clearance in rats

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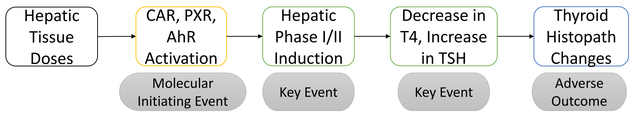
Background

Traditionally, rodent repeat dose studies have been used to describe liver-mediated thyroid effects and the key events for this mode of action (MoA) to support the human non-relevance of the adverse thyroid outcomes. The rodent liver-mediated thyroid hormone clearance has been recognized as not relevant to human health due to quantitative differences between species. Here we have utilized in vitro new approach methodologies (NAMs) to characterize rodent thyroid toxicity and establish human relevance without significant animal use. Given the quantitative differences between rat and human liver functions, it is crucial to illustrate the key event relationships underlying thyroid disruption and the liver-thyroid adverse outcomes in rodents. These alternative approaches can also help reduce animal testing by substituting some in vivo assays for thyroid endpoints. We have investigated two in vitro hepatocyte models for liver-induced thyroid toxicity and applied quantitative in vitro to in vivo extrapolation (QIVIVE) for the biological and mechanistic characterization of hepatic UGT induction and T4 clearance and metabolism in rats.

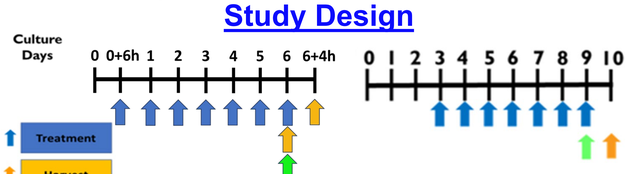


Liver mediated molecular initiating event and key event relationship leading to thyroid adverse outcomes.

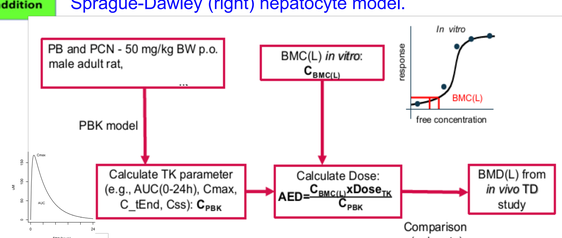
Objective

- Leverage two in vitro hepatocyte coculture models – a sandwich culture hepatocyte model with Wistar rat hepatocytes and a triculture model with Sprague Dawley rat hepatocytes.
- Characterize liver mediated thyroid metabolism and clearance – detect T4 clearance and T4 glucuronidation levels.
- Perform transcriptomics/qPCR to assess Cyp and Ugt expression changes in the liver.
- Extrapolate point of departure (POD) from in vitro data to in vivo scenarios using IVIVE to facilitate exposure driven next generation risk assessment (NGRA).

Study Design



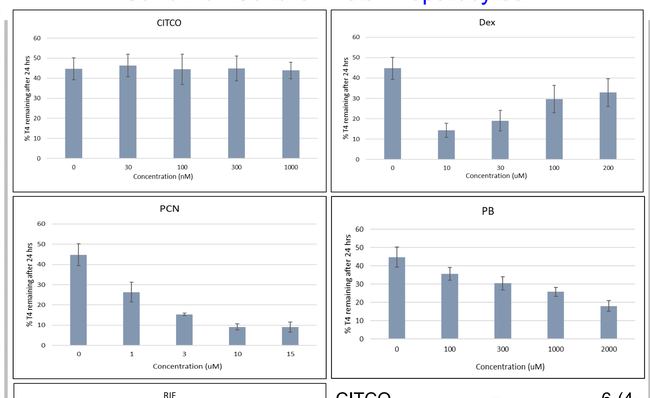
Schematics of exposure paradigm for Wistar (left) and Sprague-Dawley (right) hepatocyte model.



QIVIVE Approach for Wistar model – Reverse dosimetry to estimate administered equivalent dose (AED) for *in vivo* endpoints based on *in vitro* data.

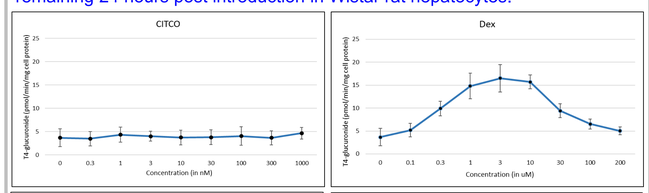
Results

Sandwich Culture Wistar Hepatocytes

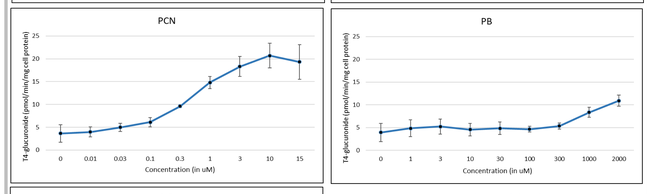


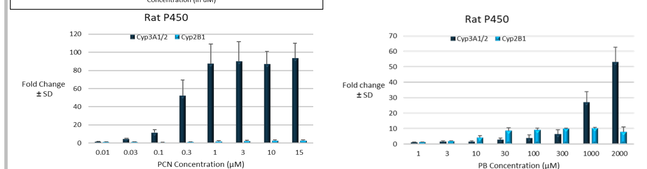
CITCO – 6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime
Dex - Dexamethasone
PCN - Pregnenolone 16α-carbonitrile
PB - Phenobarbital
RIF - Rifampicin

T4 clearance in hepatocytes presented as the percentage of T4 hormone remaining 24 hours post introduction in Wistar rat hepatocytes.

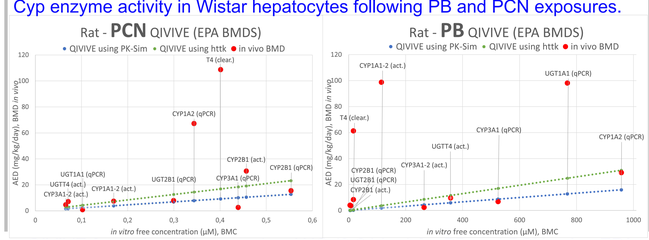


T4 glucuronide (T4-G) formation in Wistar hepatocytes 24 hours post T4 administration, as a measure of T4 metabolism in liver. PCN and PB show increased glucuronide formation in the rat liver cells, whereas RIF has no change in glucuronide formation.



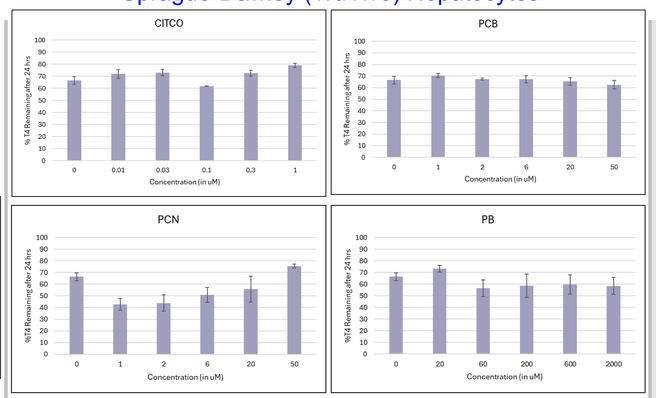


Cyp enzyme activity in Wistar hepatocytes following PB and PCN exposures.



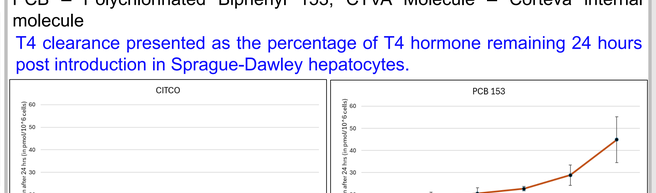
QIVIVE for male adult rats using AUC (0-24 hrs) for PB and PCN using EPA BMDS model.

Sprague-Dawley (TruVivo) Hepatocytes

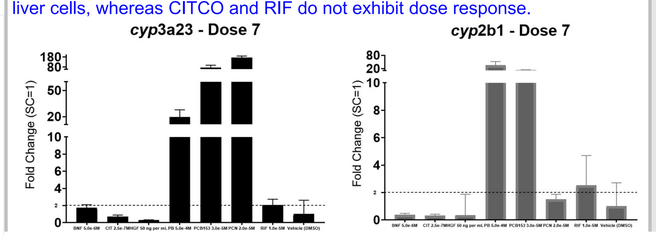


PCB – Polychlorinated Biphenyl 153, CTVA Molecule – Corteva internal molecule

T4 clearance presented as the percentage of T4 hormone remaining 24 hours post introduction in Sprague-Dawley hepatocytes.



T4-G formation in TruVivo hepatocytes 24 hours post T4 administration. PCB153 and PB show significantly increased glucuronide formation in the rat liver cells, whereas CITCO and RIF do not exhibit dose response.



qPCR results showing gene expression changes in Cyp3a and Cyp2b genes.

Discussion

- Phenobarbital and PCN show a high rate of T4 clearance and glucuronide formation in both Wistar and Sprague-Dawley Hepatocyte models.
- PCB 153 shows a strong response in T4 clearance and metabolism in the Sprague-Dawley TruVivo rat model. This compound is therefore used as a positive reference inducer in the TruVivo rat hepatocytes.
- Both CITCO and RIF did not show any response in T4 clearance or glucuronidation in both the rodent models as expected. Therefore, these two compounds are negative in the rodent liver T4 clearance assay. Dex showed an increased response initially, that eventually declined at the higher concentrations.
- Both PB and PCN exposures induced P450 enzyme activity as expected. However, Cyp3a related enzyme activity showed a more prominent increase in activity compared to Cyp2b1. Further, this increased activity were more pronounced for PCN exposures compared to PB.
- Extrapolating BMC(L) from mRNA and activity of hepatic enzymes to the *in vivo* data shows better accuracy compared to extrapolation of BMC(L) for T4 clearance *in vitro* data for both PB and PCN.
- The BMD(L) of T4 levels in vivo was larger than the predicted AED in all cases, which can be attributed to the thyroid feedback regulatory mechanism in vivo enhancing the resilience of thyroid hormone homeostasis.

Future Directions

- ✓ Transcriptomic analysis to generate POD (point of departure) for IVIVE. Also, assess changes in biomarkers for liver enzymatic activity in thyroid metabolism.
- ✓ IVIVE to compare predicted in vivo AEDs from in vitro results with experimentally derived AEDs from in vivo studies.
- ✓ Advocate for replacement of some in vivo assays with in vitros following in depth analysis of qIVIVE data to determine the robustness of these models.

References

1. Noyes PD *et. al.* Evaluating Chemicals for Thyroid Disruption: Opportunities and Challenges with in Vitro Testing and Adverse Outcome Pathway Approaches. Environ Health Perspect. 2019 Sep;127(9):95001. PMID: 31487205.
2. Phillips JR *et. al.* BMDExpress 2: enhanced transcriptomic dose-response analysis workflow. Bioinformatics. 2019 May 15; PMID: 30329029.
3. Interuniversity Institute for Biostatistics and statistical Bioinformatics. "EFSA Platform for Bayesian Benchmark Dose Analysis." EFSA Supporting Publications 19.12 (2022): 7740E.
4. Fabian E *et. al.* In vitro-to-in vivo extrapolation (IVIVE) by PBTK modeling for animal-free risk assessment approaches of potential endocrine-disrupting compounds. Arch Toxicol. 2019 Feb;93(2):401-416. PMID: 30552464.
5. Breen, Miyuki, et al. "High-throughput PBTK models for in vitro to in vivo extrapolation." Expert opinion on drug metabolism & toxicology 17.8(2021): 903-921.
6. Eissing T *et. al.* A computational systems biology software platform for multiscale modeling and simulation: integrating whole-body physiology, disease biology, and molecular reaction networks. Front Physiol. 2011 Feb 24;2:4. PMID: 21483730.

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