Detecting Indirect Thyroid Disrupting Chemicals *In Vitro* Through Induction for T4 Metabolism

Need

Chemicals that disrupt thyroid function can act indirectly by affecting how thyroid hormones (TH), such as thyroxine (T4) and triiodothyronine (T3), are metabolized in the liver. Rats are at greater risk than humans for the indirect thyroid disruption, due to species differences in TH conjugation rates, nuclear receptor sensitivities to xenobiotics, and their higher serum concentrations of thyroxine binding globulin (TBG) in humans compared to rats.

Solution

The species differences in the changes in T4 serum concentrations is important as it suggests an adverse event in the rat may or may not have relevance to human safety. To properly test chemicals for their potential to disrupt thyroid function indirectly through hepatic metabolism of T4, it is important to compare rat and human liver models that closely mimic the *in vivo* compound-induced perturbations in TH clearance mechanisms and enable cross-species translation.

The LifeNet Health TruVivo 2D+ hepatic system provides both rat and human species options in order for this comparison to be performed.

Accurate & reliable data

Fast turnaround times

Unsurpassed expertise

Collaborative approach

Pilot Study

ASSAY PARAMETERS	PROTOCOL
Cell Model	TruVivo (with rat and human hepatocytes)
Plate Format	96-well
No. Reference Compounds (CAR/PXR activators)	2
No. of Concentrations	6
Replicates	2
TA exposure time	24 hours
End Points	LDH cell viability
End Points Gene expression CAR/PXR activation	CYP2B6/CYP2B1, CYP3A4/CYP3A1/3A23, and HPRT/Hprt.
Time to complete	3-4 weeks

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Talk with one of our experts for help with general inquiries, protocol details, or becoming a new client.

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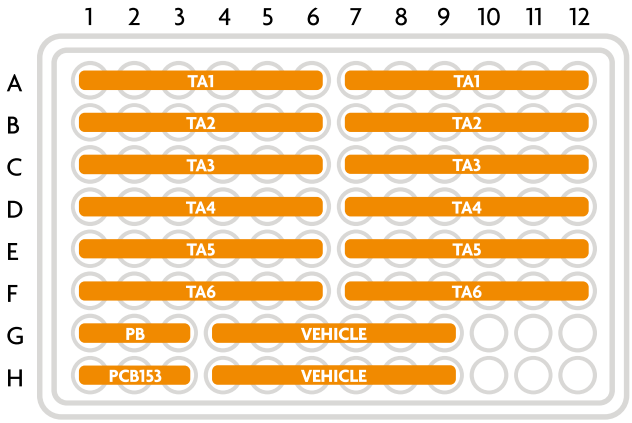
Pilot Plate Layout (Rat and Human)

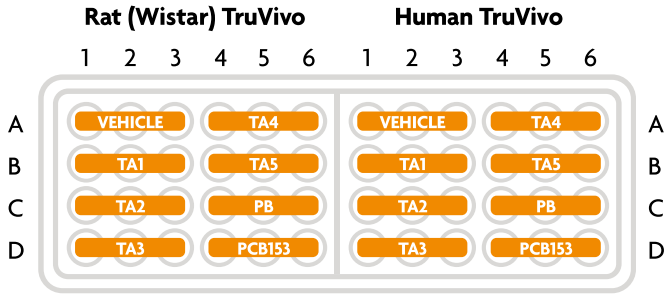


Experimental Procedure

ASSAY PARAMETERS	PROTOCOL
Cell model T4-G Qualified	TruVivo (Rat (Wistar) and Human Primary Hepatocytes or Sprague-Dawley Rat)
Plate Format	24-well
Replicates	3 per treatment
No. TA concentrations	1
Reference Compounds (2) (CAR activators)	PCB153 (30 µM) and Phenobarbital (2000 µM)
Treatment time with Test Articles and reference compounds	5-7 days
Addition of Thyroxine (T4)	On day 9
Thyroxine exposure time	24 hr (Day 10 harvest)
Thyroxine (T4)	T4 (0.1 µM) with 13C-T4 as internal standard
Endpoints for cell health	Urea and LDH leakage (days 7)
Endpoints for Thyroid metabolism	T4-glucuronide (T4-G)
End points for CAR activation	CYP2B6/CYP2B1, CYP3A4/CYP3A1/3A23, and HPRT/Hprt.
Bioanalytical method	LC/MS/MS
Time to completion	3-4 weeks
Regulatory	Non-GLP or GLP compliant
Deliverables	Full report including: graphs, tables, statistical analysis, relative T4 metabolism rates between rat and human. Discussion on PoD or EC50 relative to exposure risk based on known in vivo data

If the maximum test article (TA) concentration is not known a pilot study can be performed to determine CAR activation and cytotoxicity utilizing 6 TA concentrations. From this experiment a single concentration will be selected for the T4 study.

Example Plate Layout



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