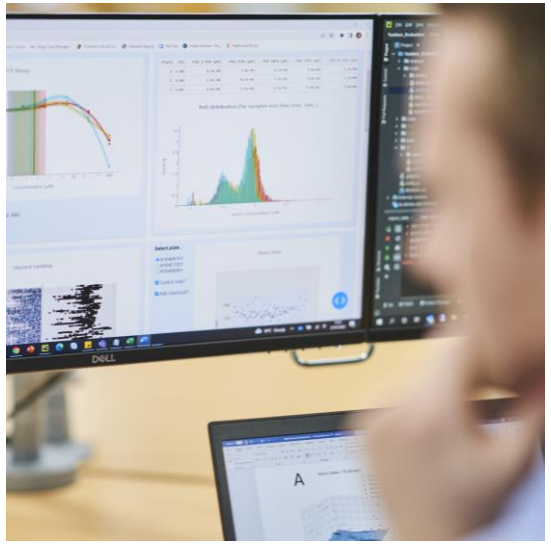
Use of complex hepatic models for a fit-for-purpose metabolism assessment of ingredients for use in Next Generation Risk Assessment

CHARLES RIVER-SOLVO Poster Blitz
10th May 2024



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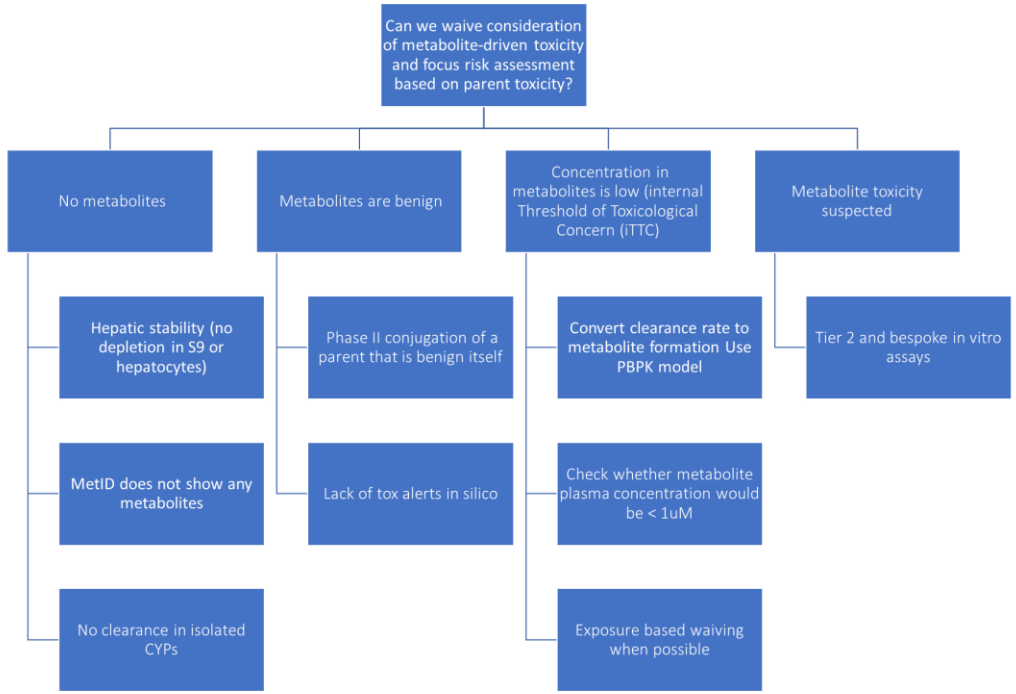
INTRODUCTION/BACKGROUND

- Next Generation Risk Assessments use a battery of in vitro tools and assays
- Metabolism framework/testing strategy developed
- Metabolism not always well assessed in in vitro cell assays (cell lines have poor metabolic capacity, exposure time not appropriate)
- We present two in vitro assays using of HepaRG cells



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Toxicology in Vitro

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Optimization of the HepaRG cell model for drug metabolism and toxicity studies

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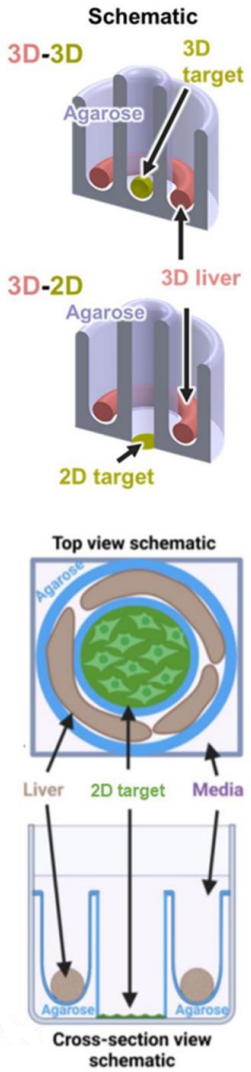
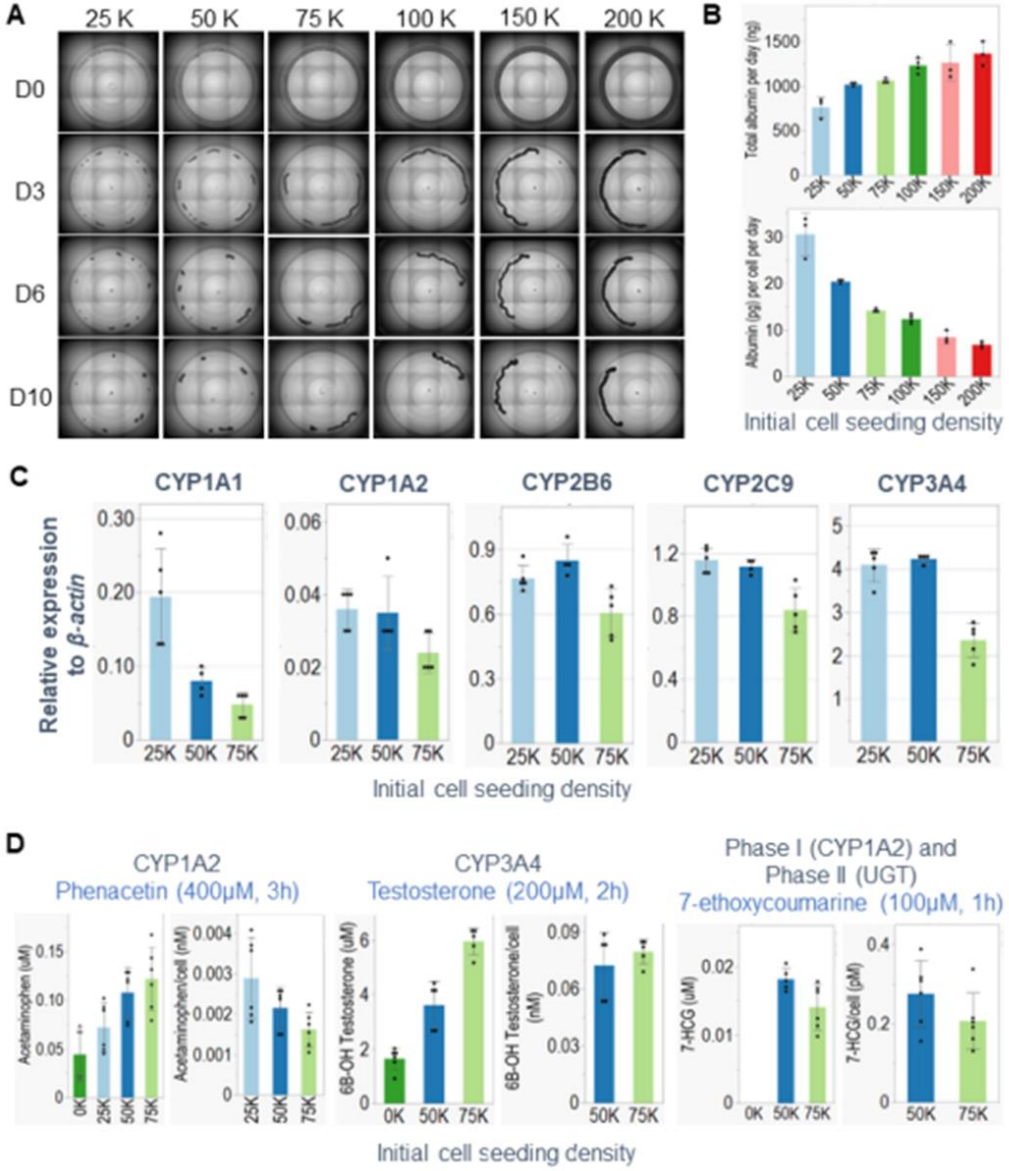
HEPARG TOROID IN CO-CULTURE SYSTEM

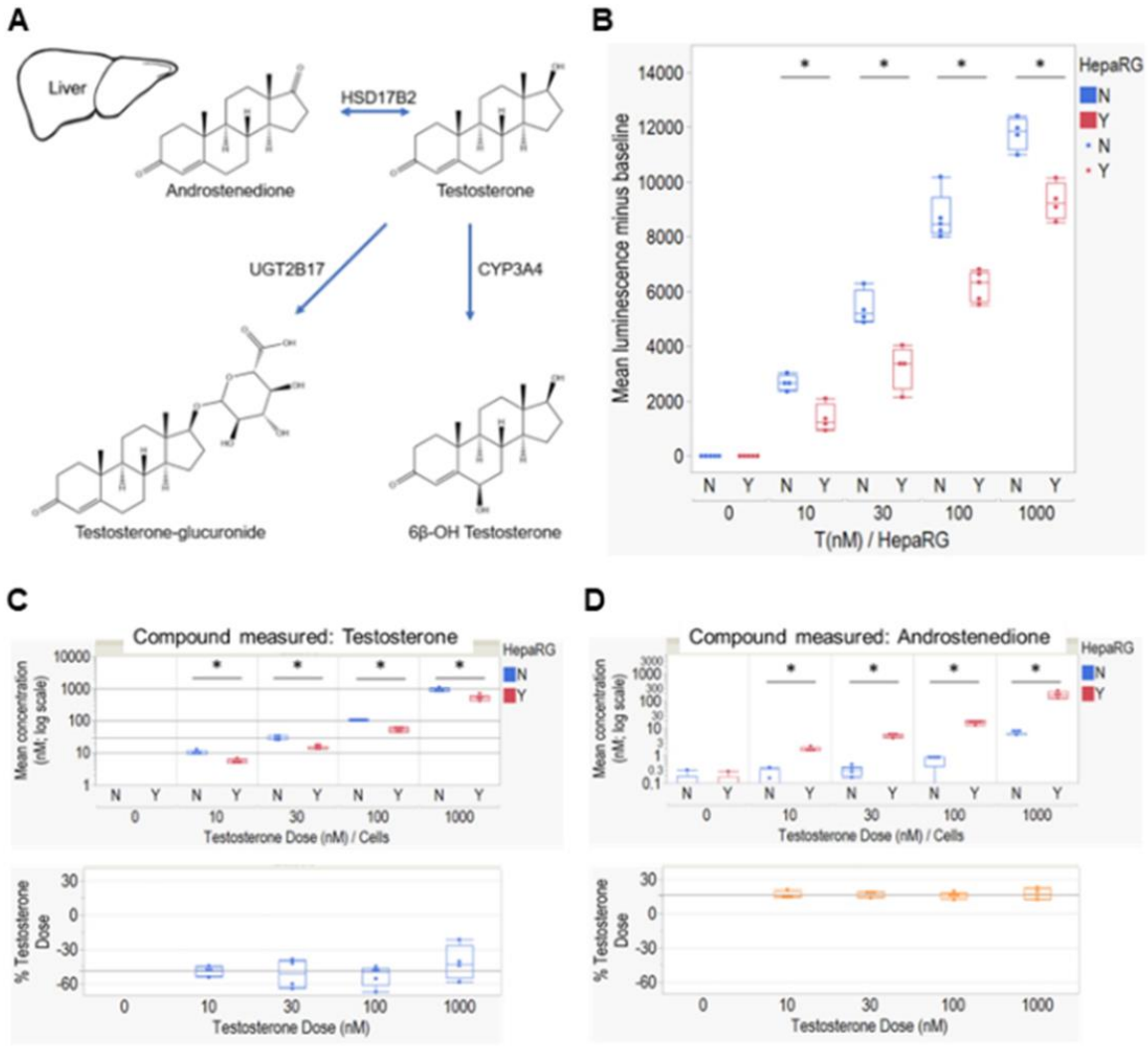


Design the HepaRG toroid:

- Optimisation of media
- Cell density with imaging to check sausage-like organoids formation
- mRNA expression of major CYPs
- Check agarose permeability for CYPS probes
- CYPs activity measured using probes (phenacetin, bupropion, testosterone, verapamil)
- LC-MS measurements of media and tissue lysates



HEPARG TOROID IN CO-CULTURE SYSTEM



Co-culture HepaRG Toroid and AR-Calux cells:

- Culture HepaRG toroid 10 days
- Add AR-Calux cells and wait 24h
- Treat with testosterone for 24h
- Effect of testosterone treatment is less pronounced in the AR-Calux cells when HepaRG toroid is present (detoxification)

› Toxicol Sci. 2024 Feb 9:kfae018. doi: 10.1093/toxsci/kfae018. Online ahead of print.

Development of a human liver microphysiological co-culture system for higher throughput chemical safety assessment

Blanche C Ip [1 2], Samantha J Madnick [1 2], Sophia Zheng [1], Tessa C A van Tongeren [3], Susan J Hall [1], Hui Li [1], Suzanne Martin [4], Sandrine Spriggs [4], Paul Carmichael [4], Wei Chen [5], David Ames [5], Lori A Breitweiser [5], Heather E Pence [5], Andrew J Bowling [5], Kamin J Johnson [5], Richard Cubberley [4], Jeffrey R Morgan [1 2], Kim Boekelheide [1 2]

Affiliations + expand

PMID: 38335931 DOI: 10.1093/toxsci/kfae018

HEPARG AS A TOOL TO RISK ASSESS REACTIVE METABOLITES

Cell Stress Panel is a high throughput imaging assay developed to assess up to 36 biomarkers.

Reactive metabolites formed in situ can influence levels of:

- GSH, ROS MMP, LDH and ATP
- DNA (damage)
- PLD and Steatosis

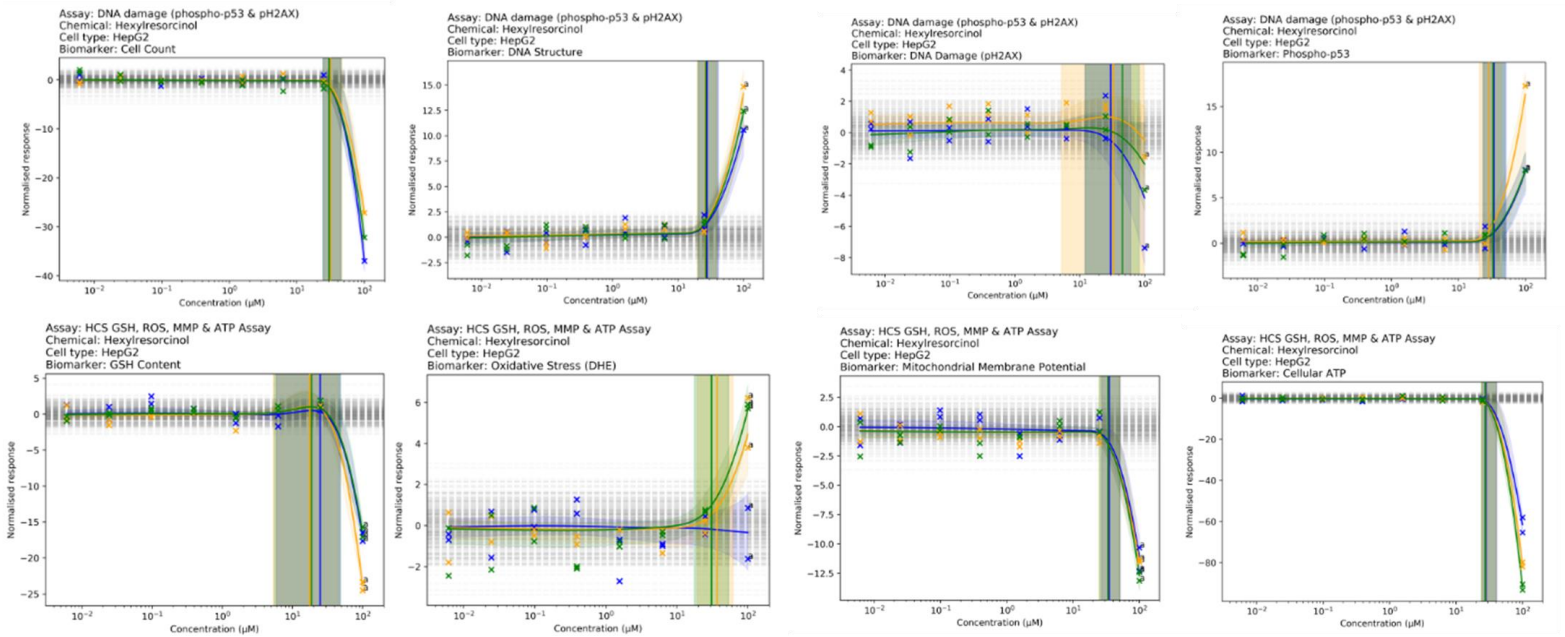
All these biomarkers have shown a good prediction for DILI but can be useful for more generic toxicities in cells.

Toxicol Sci. 2020 Jul; 176(1): 11–33. PMCID: PMC7357173
Published online 2020 May 6. doi: 10.1093/toxsci/kfaa054 PMID: 32374857

Journal Article

Identifying and Characterizing Stress Pathways of Concern for Consumer Safety in Next-Generation Risk Assessment

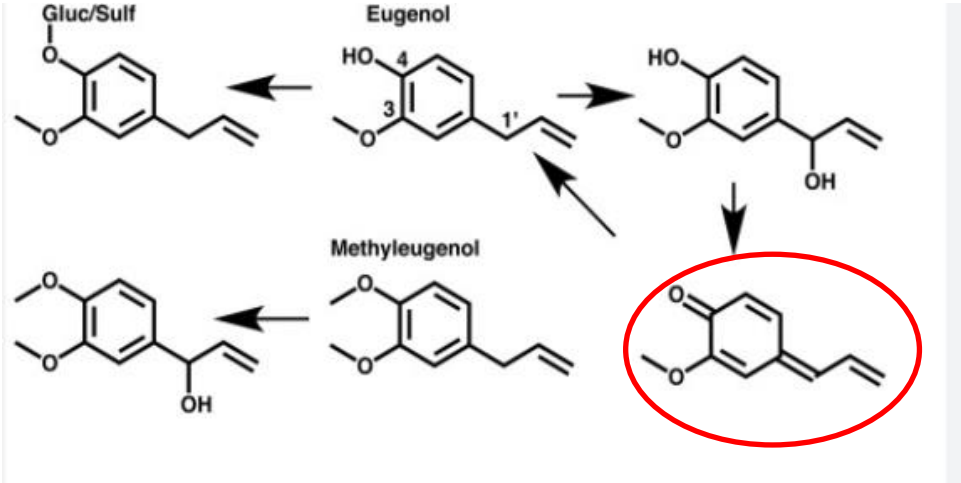
Sarah Hatherell,[k1] Maria T Baltazar,[k1] Joe Reynolds,[k1] Paul L Carmichael,[k1] Matthew Dent,[k1] Hequn Li,[k1] Stephanie Ryder,[k2] Andrew White,[k1] Paul Walker,[k2] and Alistair M Middleton[k1]



HEPARG AS A TOOL TO RISK ASSESS REACTIVE METABOLITES

Chemical ID	CAS number	MW (g/mol)	Reactive metabolite of interest (a)	Cytotoxicity top dose in assay (µM) (b)
Diclofenac [sodium salt]	15307-79-6	318.13	Quinoneimine (after CYP formation of 5-hydroxydiclofenac)	500
Acetaminophen	103-90-2	151.16	NAPQI (quinoneimine)	2000
Sunitinib [malate]	341031-54-7	532.56	Quinoneimine (formed after CYP induced oxidative defluorination)	200
Fialuridine	69123-98-4	372.09	Metabolites generated via CYPs	500
Troglitazone	97322-87-7	441.54	Quinone and o-quinone methide	200
Ketoconazole	65277-42-1	531.43	Reactive metabolite via CYP3A4	500
Cyclophosphamide	6055-19-2	279.1	Phosphoramide mustard	2000
Eugenol	97-53-0	164.2	Quinone type	500
Methyl eugenol	93-15-2	178.23	Non-reactive metabolite	500
Hydroquinone	123-31-9	110.11	Quinone	200
Retrorsine	480-54-6	351.39	Dehydroretrorsine via CYP3A4	500
4-Hexylresorcinol	136-77-6	194.27	Quinone type	500

Example:



- Plate design allow for the analysis of 12 compounds
- Chemicals selected have a high potential for reactive metabolite formation (often quinone types)
- Tested in plated HepG2 (up to 72h), plated HepaRG (up to 72h) and HepaRG spheroids (up to 14 days)
- Derive PoDs using internal BIFROST model to assess the impact of metabolism on bioactivity.

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CONCLUSION/NEXT STEPS

- HepaRG cells are a useful in vitro tool
- Demonstrated good Metabolic activity when cultured in 3D (here in toroid)
- Demonstrated stable metabolite formation in amounts sufficient to have an effect on target cells
- Further work to demonstrate effect of reactive metabolites on the HepaRG cells themselves currently ongoing

Thank You

Unilever SEAC
Sandrine Spriggs
Richard Cubberley
Sharon Scott
Maria Baltazar

Brown University
Kim Boekelheide
Blanche Ip

Cyprotex UK
Caroline Bauch
Paul Walker

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