Strengths and Limitations of In Vitro Xenobiotic Metabolism Assays

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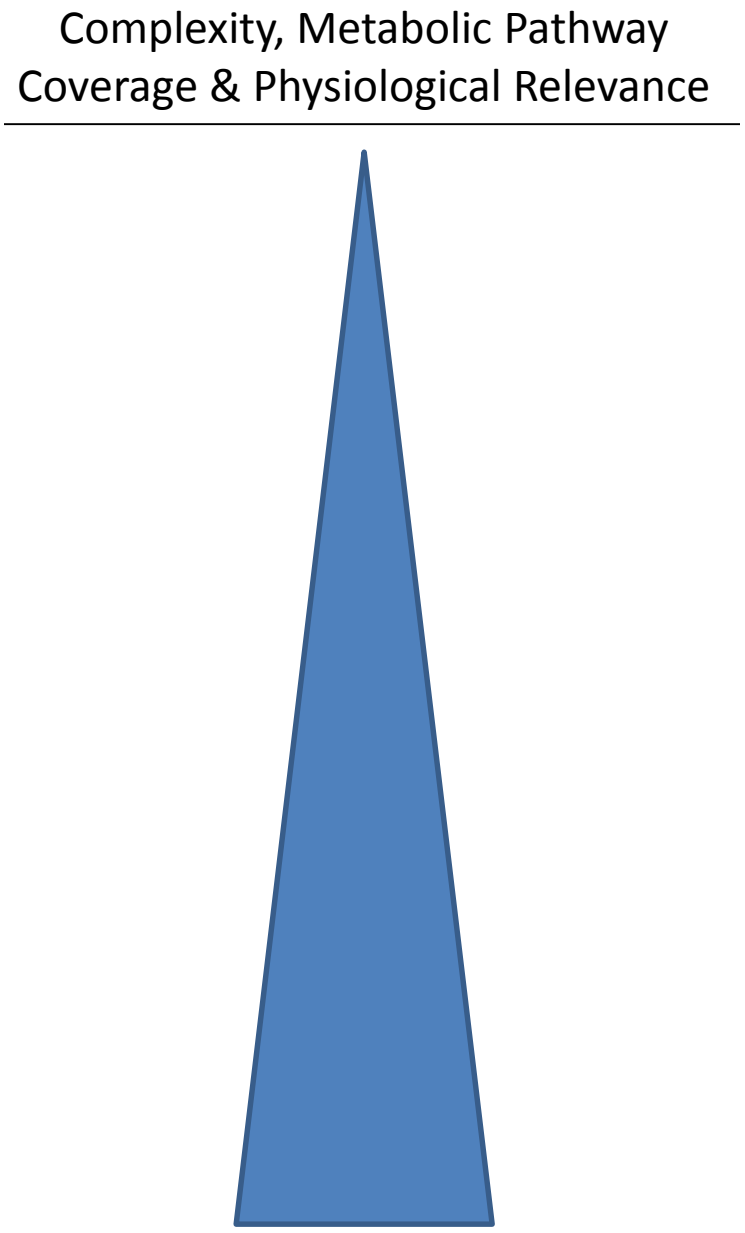
Disclaimer

The statements, opinions or conclusions contained therein do not necessarily represent the statements, opinions or conclusions of NIEHS, NIH or the United States government.

In Vitro Models to Study Drug Metabolism

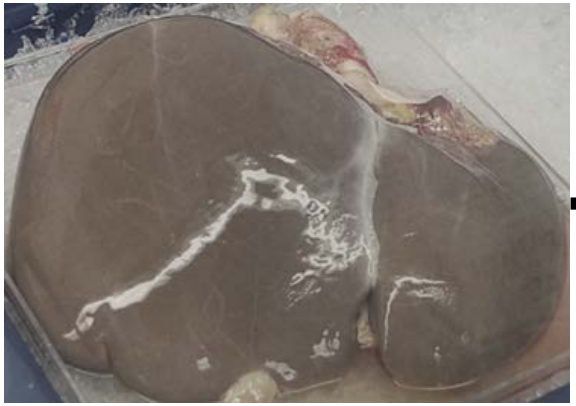
- Recombinant expressed enzymes
- Hepatic cell lines overexpressing metabolizing enzymes
- Subcellular fractions: microsomes/S9
- Differentiated hepatic cell lines
- Primary hepatocytes
  - Suspensions
  - Sandwich cultures
  - ‘NextGen’ culture models
- Liver Slices
- Isolated perfused organs

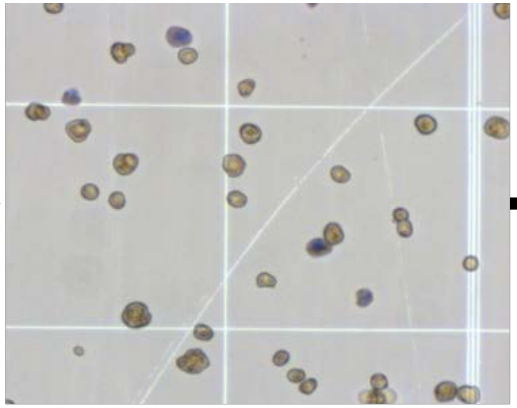


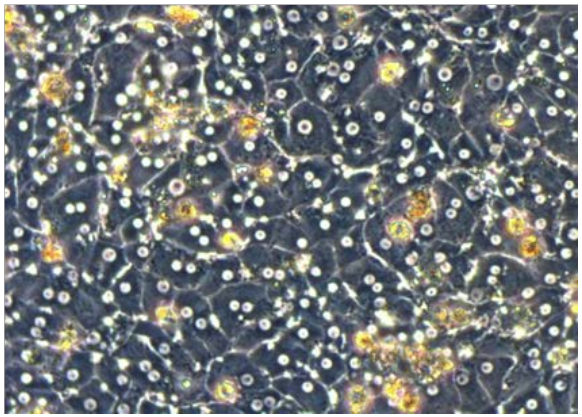
What is the 'Metabolism' Question?

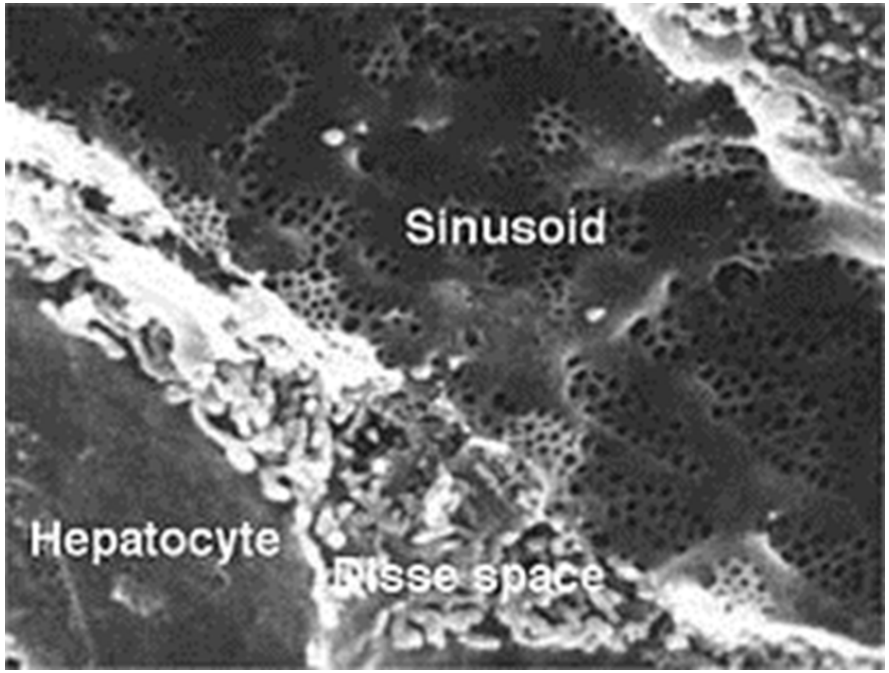
- Will a chemical be appreciably metabolized into alternate chemical structures? How much and how do we translate quantitatively to in vivo?
  - Primary hepatocytes, liver S9, liver microsomes, recombinant enzymes
  - IVIVE approaches to predict pharmacokinetics (i.e. C_{max}, C_{ss}, $T_{1/2}$, etc…)
- What metabolic pathways are likely involved in clearance (reaction phenotyping)?
  - Recombinant enzymes, liver microsomes/S9 with inhibitors or poor metabolizers
- What metabolite(s) will form and at what relative quantities/proportions?
  - Primary hepatocyte suspensions, liver microsomes/S9
- Is metabolic activation to toxic metabolites a potential issue for a chemical?
  - Liver S9 (e.g. Ames test for mutagenicity), primary hepatocytes (i.e. protein adducts, GSH conjugates), P450 over-expressing cell lines
- Will a chemical inhibit metabolism (i.e. TDI) that alters drug/chemical clearance?
  - Liver microsomes, primary hepatocyte suspensions
- Will chemical induce liver enzymes that alters drug/chemical clearance?
  - Primary hepatocyte sandwich cultures, HepaRG cultures

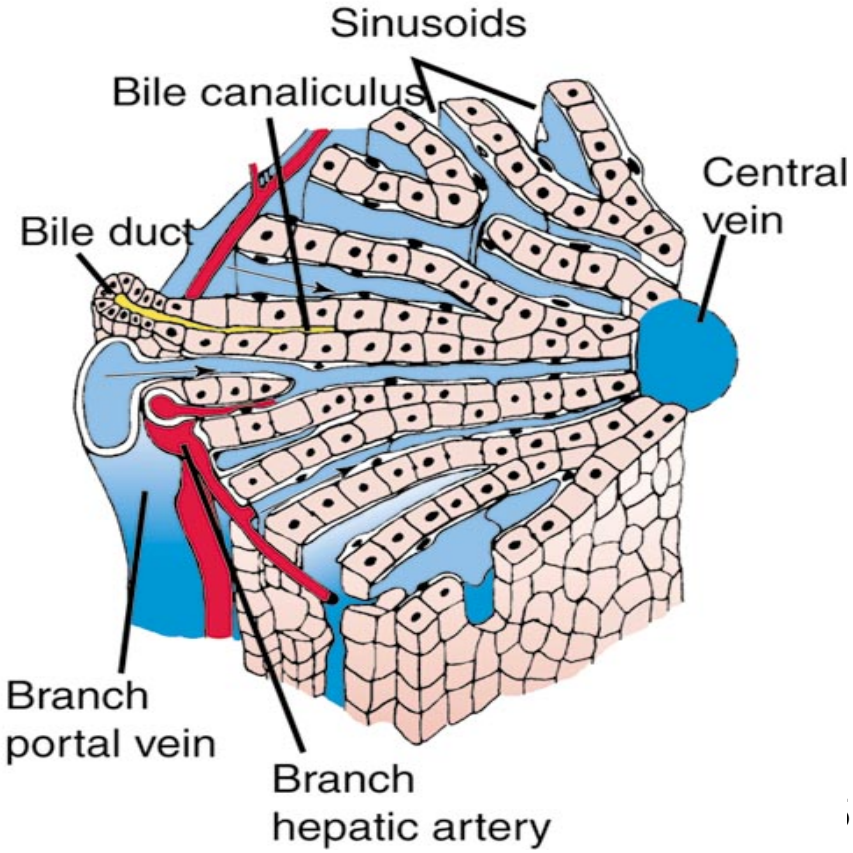
In Vitro Liver Models Derived from Human Liver Most Phys. Relevant



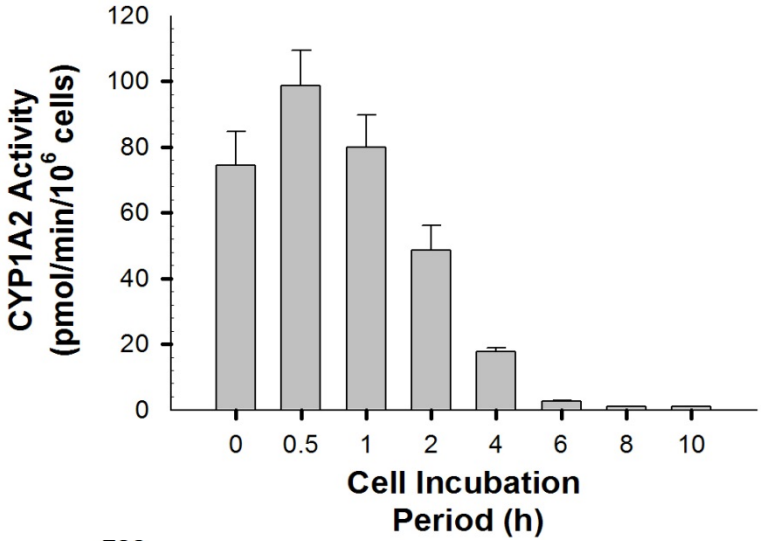


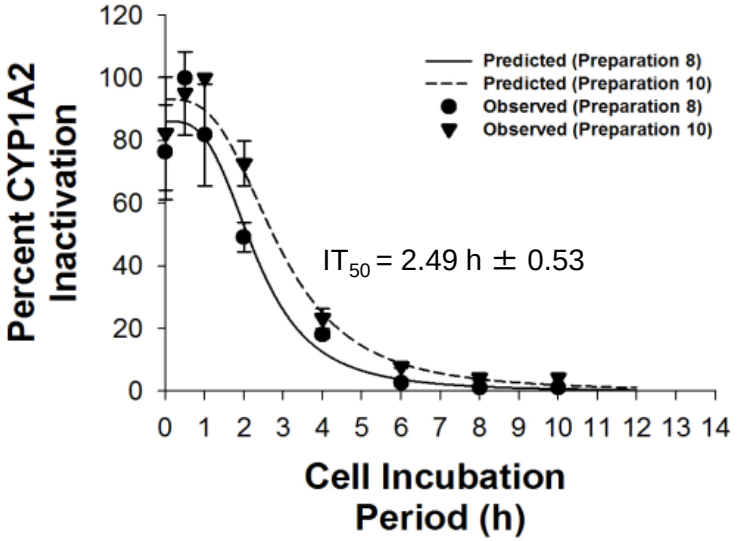


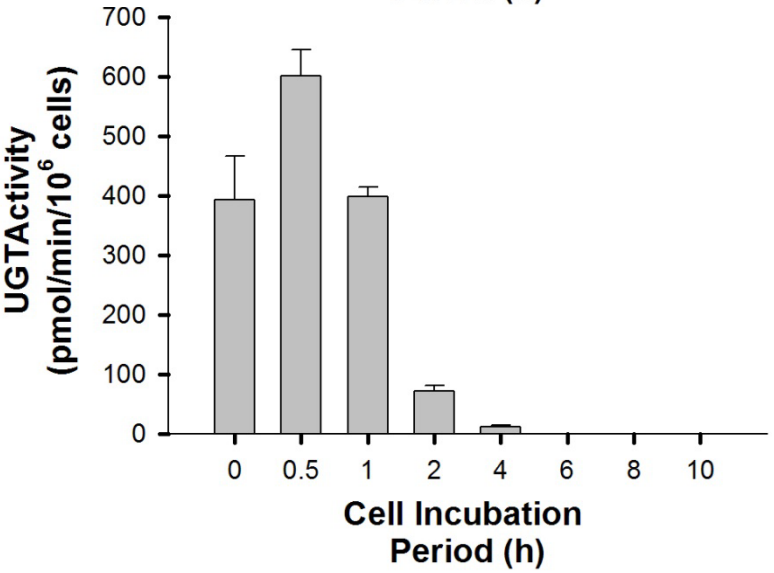


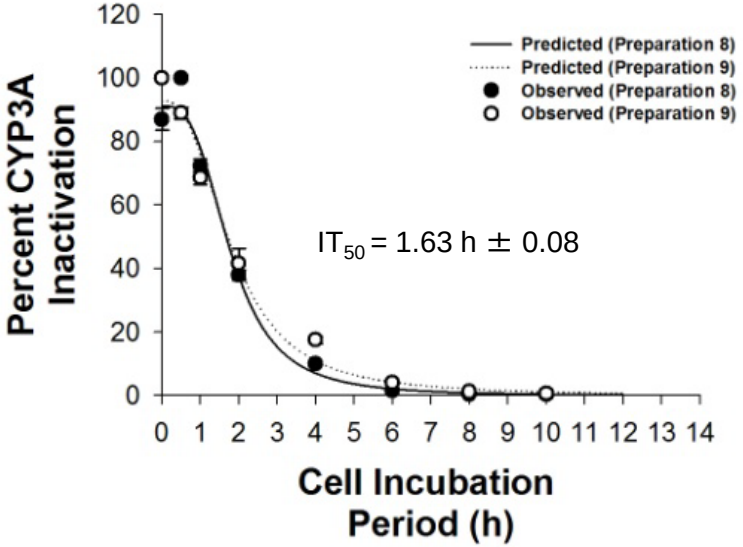


Loss of Xenobiotic Metabolism Competence with Isolated Primary Hepatocytes







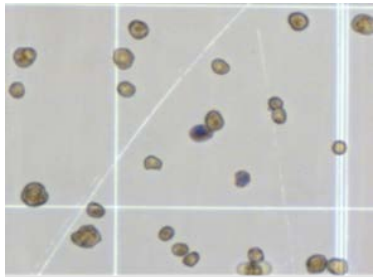


Smith et al. J. Pharm. Sci. 2012. v.101(10):3898.

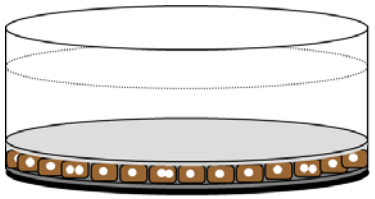
'Full' metabolic competence is NOT an intrinsic property of primary hepatocytes, but dependent on culture environment

Metabolic Competence

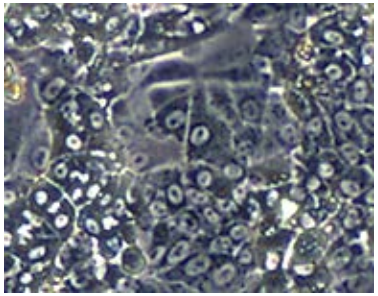
Suspension PHHs



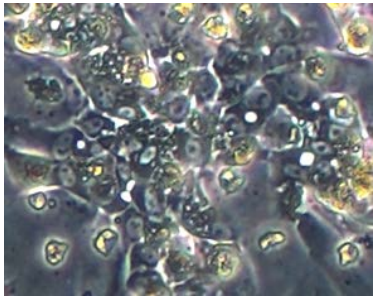
SC-PHHs



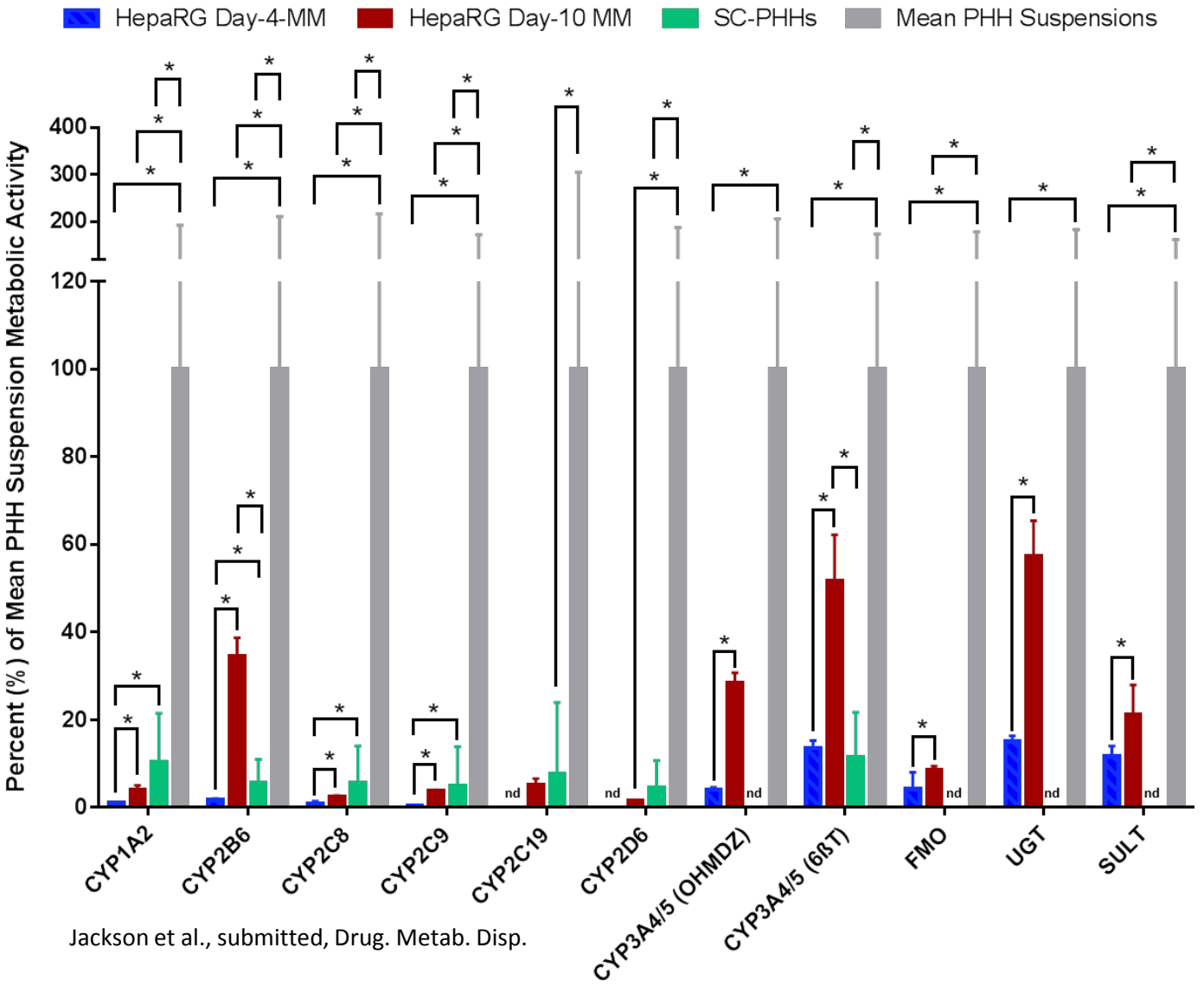
Day-10 HepaRG



Day-4 HepaRG



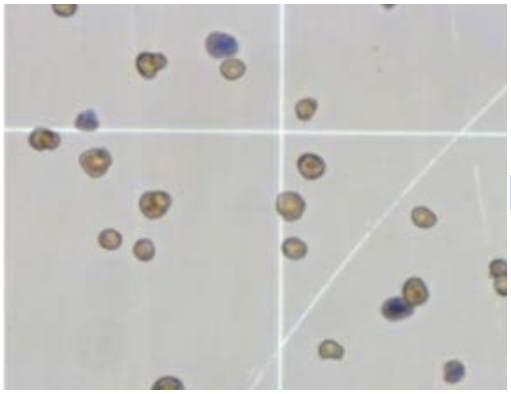
Comparison of Metabolic Competencies



Jackson et al., submitted, Drug. Metab. Disp.

- Metabolic competence/proportions not intrinsic, subject to environment
- 3D & flow models can greatly improve metabolic competence
- Vast majority of metabolic clearance assays in suspensions of PHHs

Metabolic Stability Assays (Substrate Depletion)



Primary Hepatocyte Suspensions

$1x10^6$ cells/well

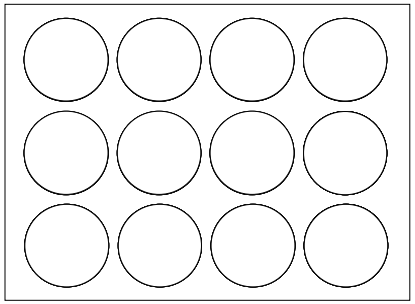
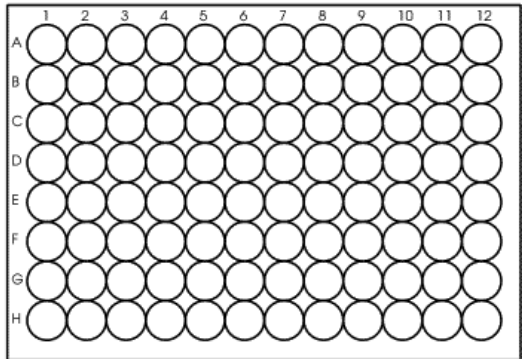


Plate containing media, 2X assay buffer & test compound (0.1, 1, 10µM)

37°C

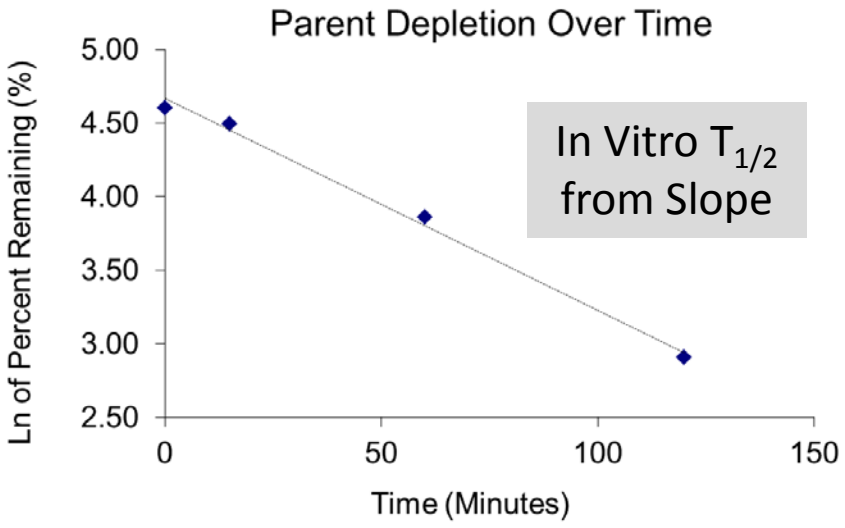
Remove aliquots over time



0 min
15 min
30 min
60 min
90 min
2 hr
3 hr
4 hr

- Assay conditions summary:
  - 1.2 mL initial reactions
  - WEM with ITS$^+$ & HEPES buffer
    - ITS+: insulin, transferrin, selenous acid, **BSA** (1.25 g/L), and linoleic acid
  - 120 rpm (orbital)
  - Assay suspension aliquots crashed in ACN (1:1)
  - Monitor viability (e.g. Trypan Blue)

~35-50 g/L albumin in human blood (35X in vitro levels used here)



In Vitro $T_{1/2}$ from Slope

Intrinsic Clearance CL_{int}

0022-3565/97/2831-0046$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 283, No. 1
Printed in U.S.A.

JPET 283:46–58, 1997

The Prediction of Human Pharmacokinetic Parameters from Preclinical and *In Vitro* Metabolism Data

R. SCOTT OBACH, JAMES G. BAXTER, THEODORE E. LISTON, B. MICHAEL SILBER, BARRY C. JONES, FIONA MACINTYRE, DAVID J. RANCE and PHILIP WASTALL

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IN VIVO CLEARANCE OF ETHOXYCOUMARIN AND ITS PREDICTION FROM *IN VITRO* SYSTEMS

Use of Drug Depletion and Metabolite Formation Methods in Hepatic Microsomes and Isolated Hepatocytes

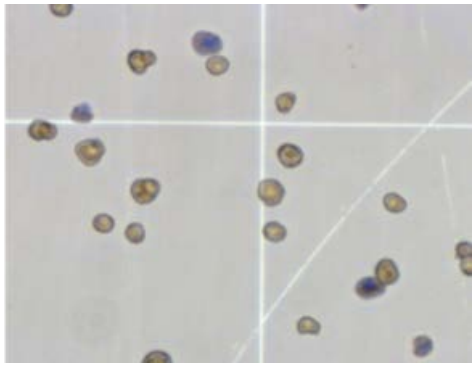
DAVID J. CARLILE, ALEX J. STEVENS, ELIZABETH I. L. ASHFORTH, DAXA WAGHELA, AND J. BRIAN HOUSTON

School of Pharmacy and Pharmaceutical Sciences, University of Manchester

$$CL_{int} = \frac{0.693}{t_{1/2}} \times \frac{\text{g liver wt}}{\text{kg body wt}} \times \frac{\text{mlincubation}}{\text{cells per incubation}} \times \frac{1.35 \times 10^6 \text{ cells}}{\text{g liver wt}}$$

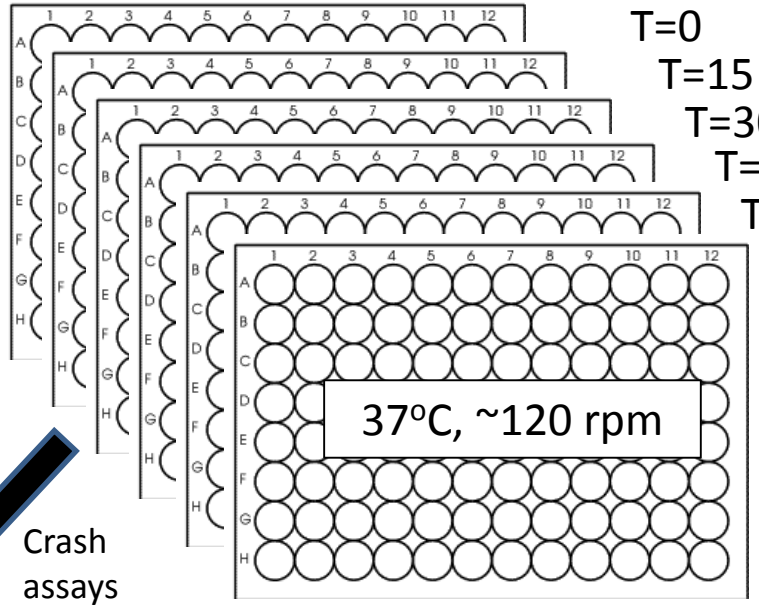
For a first order reaction with [S] << Km, the $t_{1/2}$ values should be constant

Metabolic Stability with ToxCast (Phase I & Phase II) Chemicals



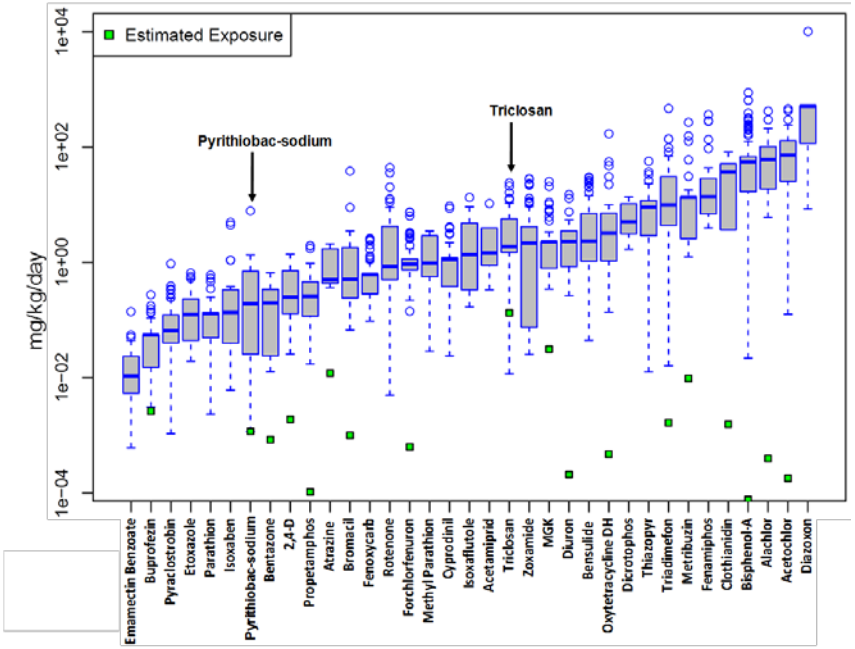
Pooled Suspensions of Primary Hepatocytes from >10 donor preps

50k cells/well

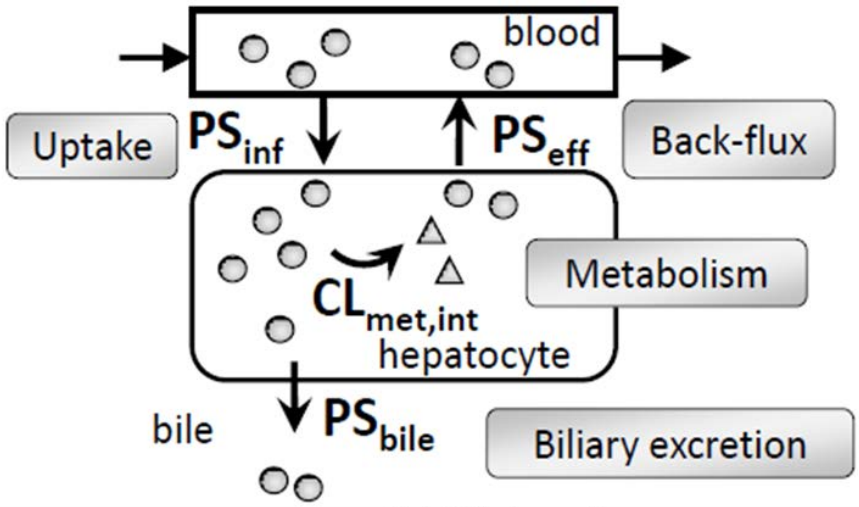


Crash assays with ACN

Collaboration between The Hamner, EPA, and Life Tech



- Assay conditions summary:
  - 100 µL assays
  - 1 & 10 µM concentrations
  - WEM with ITS$^+$ & HEPES buffer
    - ITS+: insulin, transferrin, selenous acid, **BSA** (1.25 g/L), and linoleic acid
  - 300 rpm (orbital)
  - Assay suspension aliquots crashed in ACN (1:1)
  - Monitored viability (e.g. Trypan Blue)
  - Heat-treated & no-cell controls included to assess non-specific binding/recovery



*Yuichi Sugiyama figure

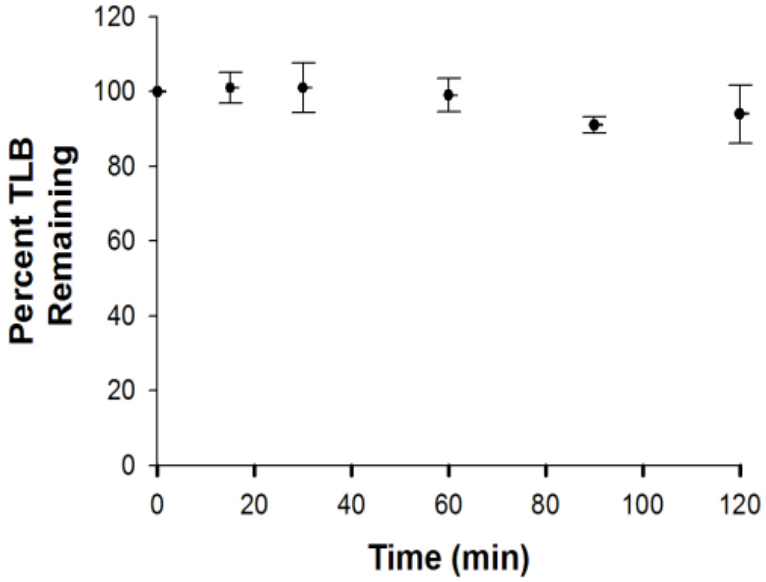
Strengths of Suspension Hepatocyte CL_{int} Approach:

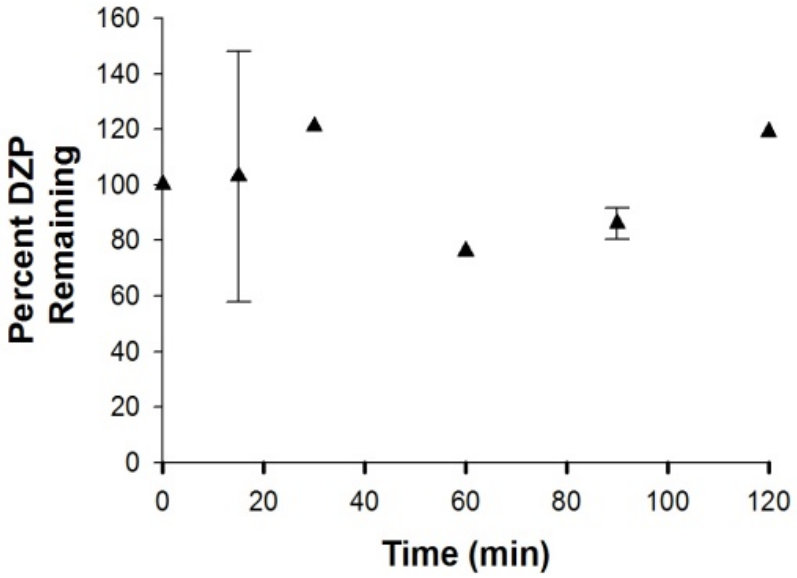
- Suspension hepatocytes historically proven to be a useful model system , superior to microsomes/S9 due to Phase II metabolism capacity, de novo cofactor synthesis
- Suspension hepatocytes support a broad complement of transformation types and active uptake transport
- Suspension hepatocytes represent in vivo-like levels/proportions of metabolic competence
- Monitoring loss of parent captures broad complement of metabolism pathways
- Crashing cells & supernatants to maximize recovery of parent chemical can improve mass balance
- Useful CL_{int} predictions obtained, largely in agreement with in vivo metabolic clearance (within ~2-3 fold)

Limitations of the Suspension Hepatocyte CL_{int} Approach Used to date:

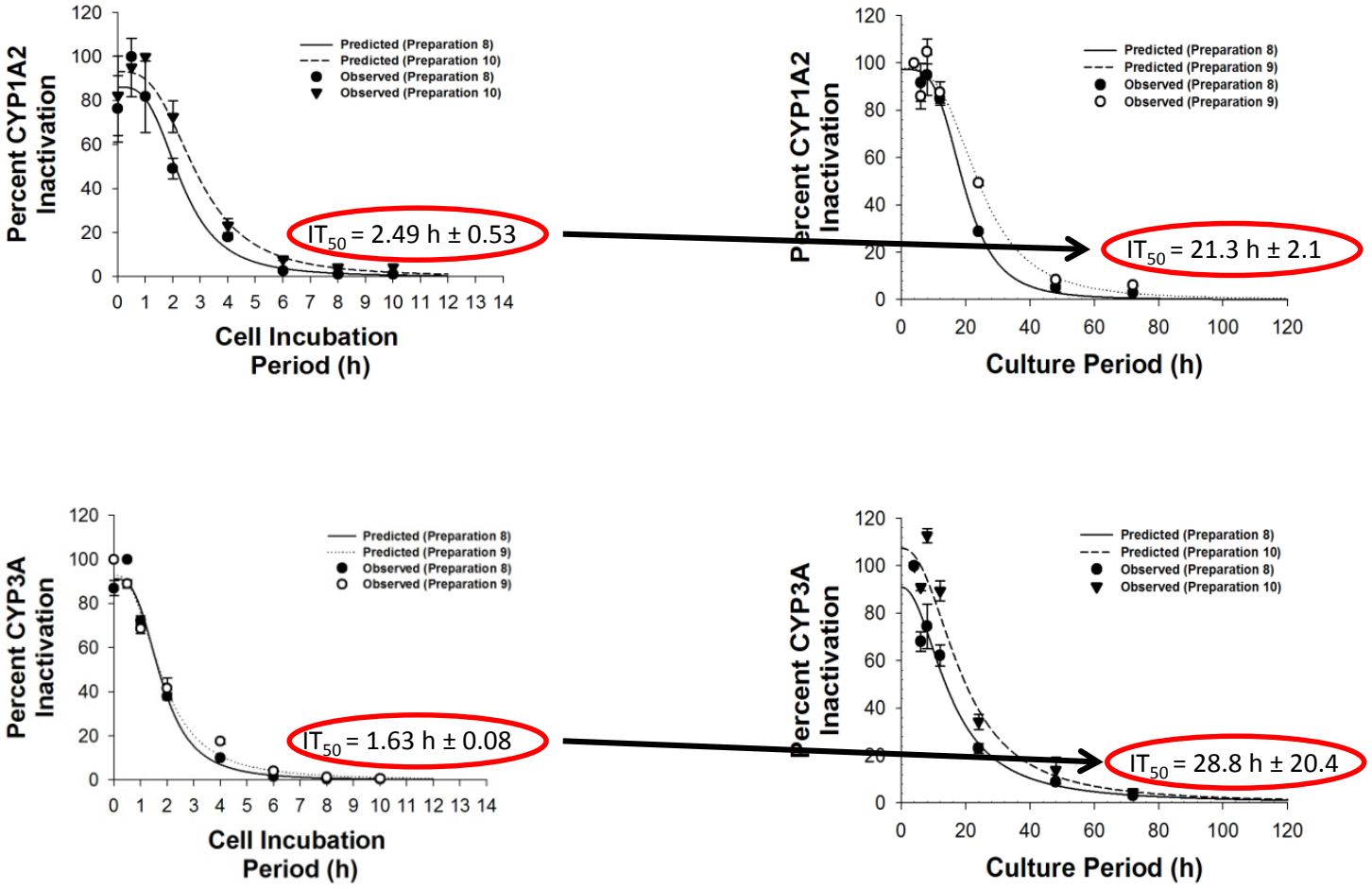
- Suspensions of hepatocytes are short lived (~2-4 hrs)
- Suspensions hepatocyte metabolic competence half-lives ~1-2 hrs limiting effectiveness with lower turnover compounds that could also alter metabolite profile outcomes
- Physiological levels of serum proteins (i.e. albumin) were not used during incubations (~30-fold lower)
- Crashing cells and supernatants together limits ability to understand partitioning kinetics and the impact of uptake transport
- Suspension hepatocytes thought to be devoid of canalicular efflux transport and limited in basolateral efflux that may alter metabolism outcomes

Suspension PHHs Often Ineffective with Lower Turnover Compounds?



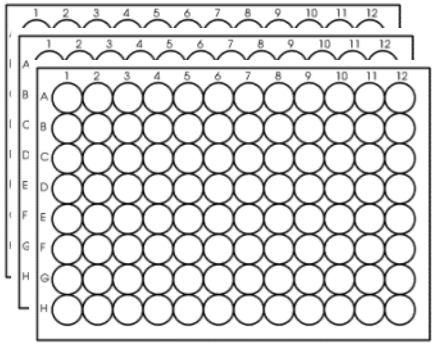


Temporal Effects on CYP1A2 & CYP3A Activities in Suspension vs. Plated Cultures



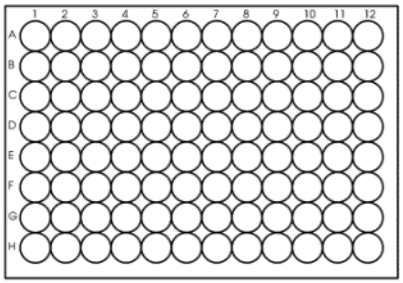
Smith et al., J. Pharm. Sci. 2012, Oct, v.101(10), 3989-4002

CL_{int} in 'Early' Cultures of PHHs



Collagen-I coated, plated pre-wetted with 60 µL plating media

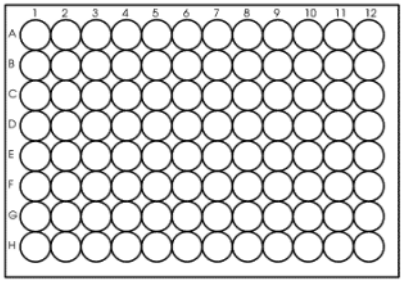
Plate Cryo Heps @ 50,000 cells/well



Allow attachment for ~4 hrs

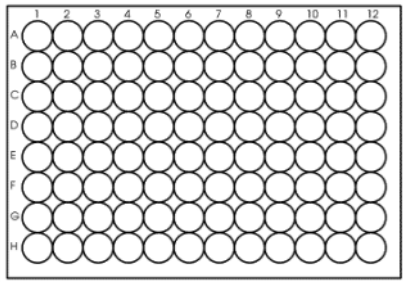
Remove spent culture media &

Add cmpds 1 µM final in WEM with HEPES



Incubate for T=0, 0.25, 1, 2, 6, 18, 24, and 48 hrs

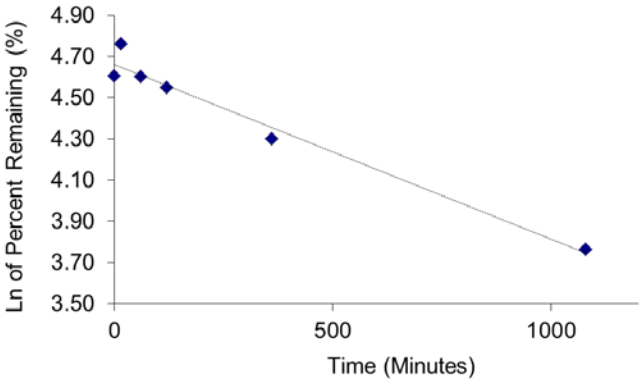
300 rpm shaking

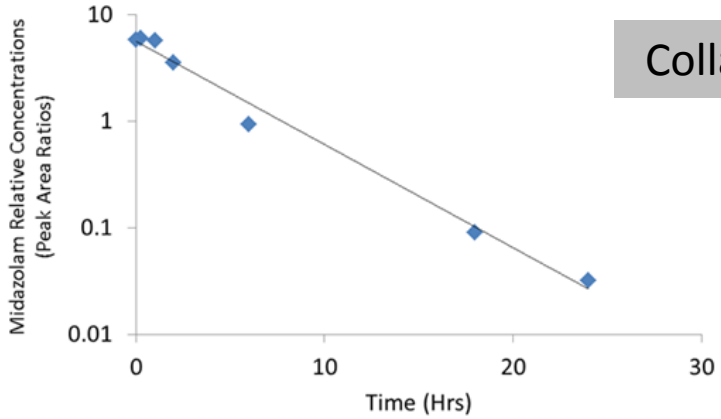


Collect supernatants, flash freeze sups & plates for analytical

ACN crash & analytical on sups & cell fractions

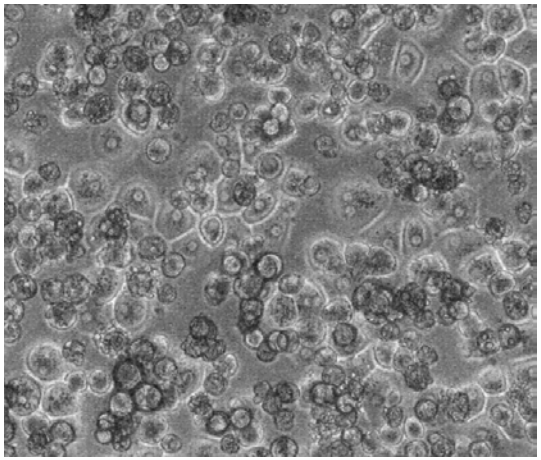
Tolbutamide depletion over time



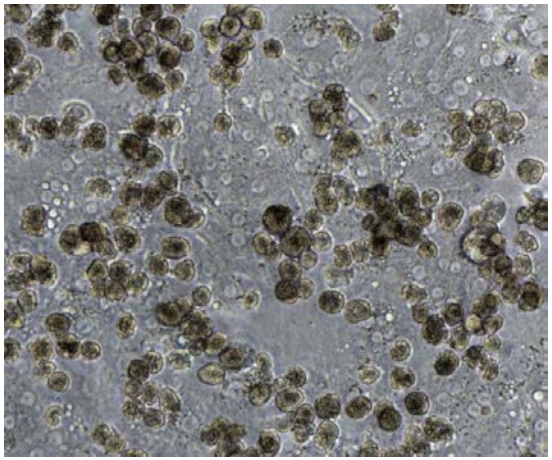


Collaboration-BI

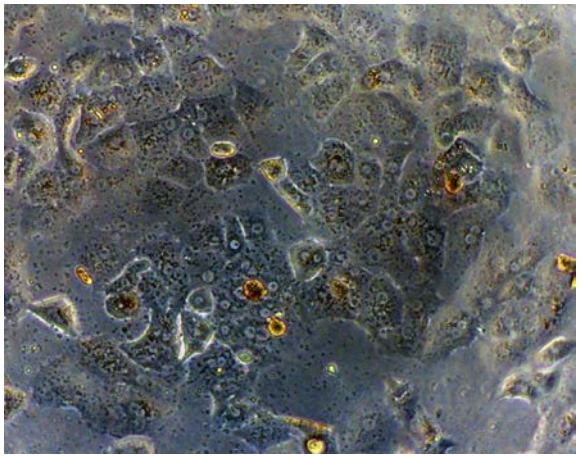
Assay Optimization of CL_{int} in 'Early' Cultures of PHHs



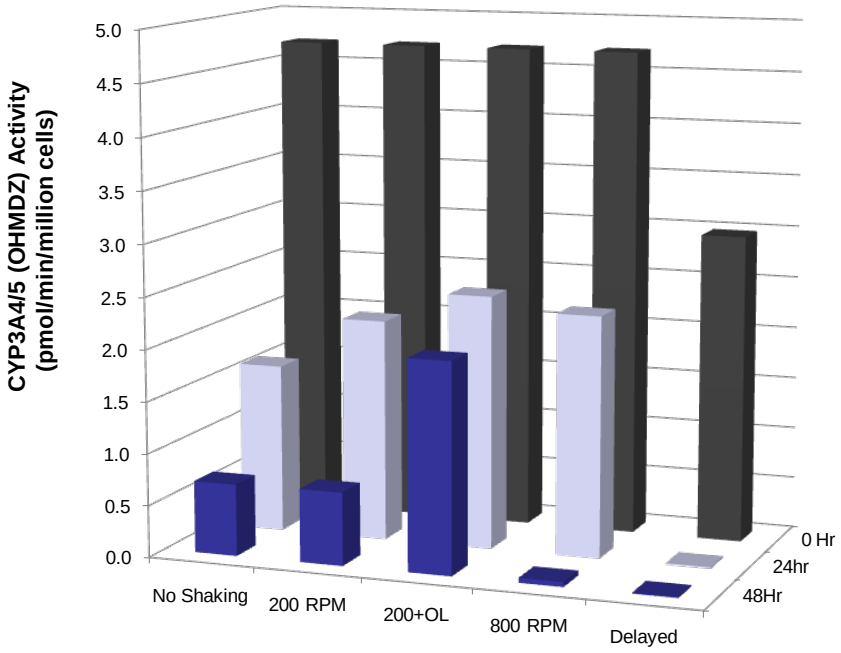
Initial Attachment

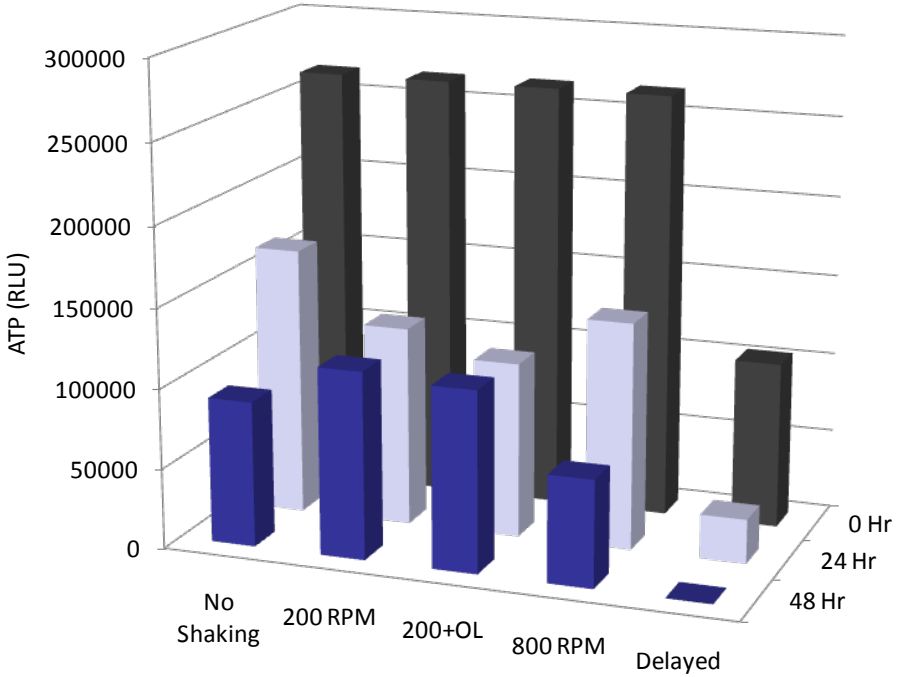


48 hrs, 200 RPM



48 hrs, 800 RPM





Results with 'Early' PHH Cl_{int} Model

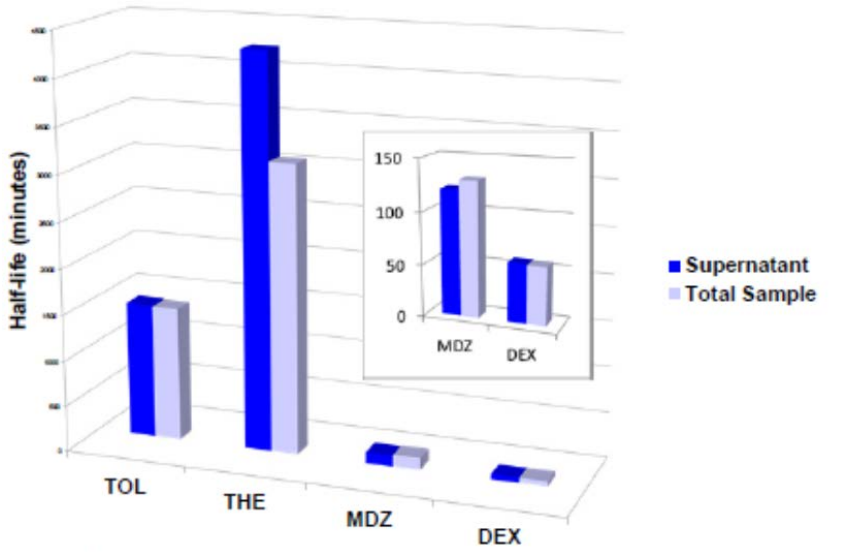
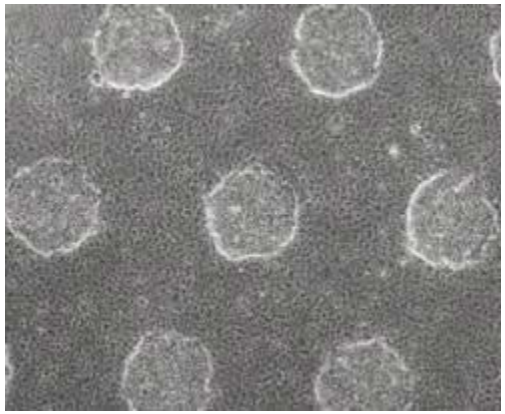


Figure 1. Effect of supernatant and total sample analysis on the observed half-life of dextromethorphan, midazolam, tolbutamide, and theophylline at a seeding density of 50k cells/well.

- Extended to a broader set of ~30 chemicals
- Began evaluation of pooled plateable approach
- Overlay had little effect on CL_{int} within 48hrs evaluated

Compound	Class	In vivo $Cl_{non\text{-}renal,\ obs}$	% Q_h	In vitro CL_h (mL/min/kg)
S-Warfarin	Acidic	0.1	0.5	0.7
Tolbutamide	Acidic	0.4	2	0.8
S-Mephenytoin	Basic	0.5	2	1.5
Alprazolam	Neutral	0.8	4	0.4
Theophylline	Neutral	1.1	5	2.1
Clozapine	Basic	2.9	14	3.4
Triazolam	Neutral	4.7	23	1.7
Prednisolone	Neutral	4.9	24	3.6
Zolpidem	Neutral	5.7	28	2.1
Accuracy	% within 2-fold			56
	% within 3-fold			89

‘NextGen’ In Vitro Liver Models to Predict Metabolic Clearance



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Drug Metab Dispos 41:2024–2032, December 2013

Special Section on Prediction of Human Pharmacokinetic Parameters from In Vitro Systems

Meeting the Challenge of Predicting Hepatic Clearance of Compounds Slowly Metabolized by Cytochrome P450 Using a Novel Hepatocyte Model, HepatoPac

Tom S. Chan, Hongbin Yu, Amanda Moore, Salman R. Khetani, and Donald Tweedie

Drug Metabolism and Pharmacokinetics, Boehringer Ingelheim Pharmaceuticals, Ridgefield, Connecticut (T.S.C., H.Y., D.T.); Hepregen Corporation, Medford, Massachusetts (A.M.); and Mechanical and Biomedical Engineering, Colorado State University, Fort Collins, Colorado (S.R.K.)

Drug Metabolism and Disposition
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Drug Metab Dispos 42:394–406, March 2014

Bridging In Vitro and In Vivo Metabolism and Transport of Faldaprevir in Human Using a Novel Cocultured Human Hepatocyte System, HepatoPac

Diane Ramsden, Donald J. Tweedie, Tom S. Chan, Mitchell E. Taub, and Yongmei Li

Drug Metabolism & Pharmacokinetics, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Connecticut

TABLE 7

Hepatic clearance of faldaprevir estimated from HepatoPac data and comparison with in vivo CL/F

Method	Hepatic Clearance of Faldaprevir at:	
	120 mg QD	240 mg QD
	ml/min	
Well stirred	9.37	8.08
Well stirred + uptake	31.1	22.9
Poulin method	49.4	42.7
CL/F observed	67.4	19.7
In vivo CL (F = 0.5–1)	33.7–67.4	9.85–19.7

- Vastly improved longevity over suspensions of PHHs
- Maintain levels of metabolic competence exceeding 4-day cultures of PHHs (< suspension PHHs)
- Appear useful for multiple questions including:
  - CL_{int}
  - Metabolite formation
  - Metabolite profiling
  - Chemical accumulation

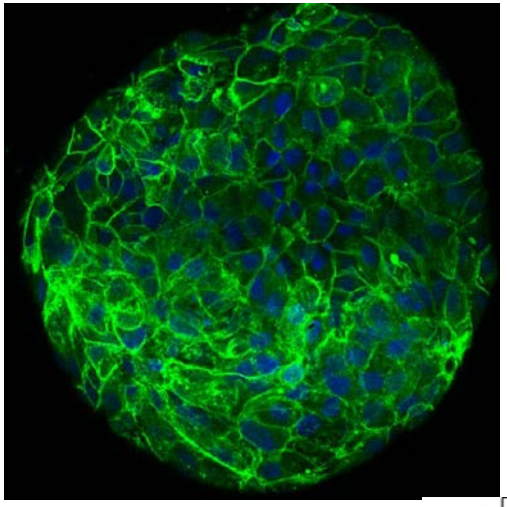
Xenobiotic Metabolism Competence Mapping

- Develop xenobiotic metabolism assay suite
  - Liver enzyme specific activity panel (& mRNA)
  - Metabolite profiles over time (with targeted ID)
  - Metabolic clearance (Cl_{INT}) capacities
- Evaluate xenobiotic metabolism 'competence' in various in vitro models representing adult liver, pediatric liver, Tox21 assays at various states of cellular differentiation
- Contextualize xenobiotic metabolism competence, grounded in metabolite profiles over time, that collectively define metabolic competence states
- Deploy physiologically-relevant (i.e. levels/ proportions) xenobiotic metabolism to in vitro toxicology research
  - direct, conditioned chemicals, co-cultures, flow systems
- Link parent and metabolite chemical structures to phenotypic (high content imaging) and high resolution 'omics responses (i.e. transcriptomics)

TABLE 5
Separation of Database into Low, Intermediate, and High Clearance Chemicals

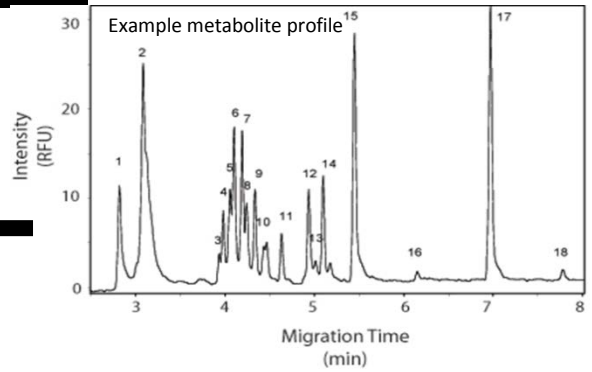
Age group	Ratio of child to adult $t_{1/2}$: Low clearance chemicals[a]	High clearance chemicals[b]	High + intermediate clearance[c]
Premature neonates	not available	3.34 ± 1.27	4.18 ± 1.26
Full-term neonates	3.40 ± 2.18[d]	1.99 ± 0.67	2.38 ± 0.59
1 week–2 months	4.34 ± 0.62[e]	1.85 ± 0.38	1.96 ± 0.41
2–6 months	1.25 ± 0.31	0.90 ± 0.26	0.94 ± 0.28
6 months–2 years	0.57 ± 0.16	0.26 ± 0.12	0.52 ± 0.14
2–12 years	0.60 ± 0.11	0.72 ± 0.24	0.72 ± 0.10

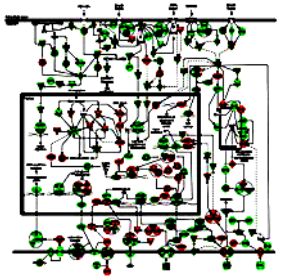
Ginsberg G. et al., (2002) *Tox. Sci.*, v. 66, pp. 185-200



- Suspension PHHs
- Hepatopac
- Tox21 cell lines
- Stem cells (i.e. iPSCs)

http://depts.washington.edu/sfields/metabolites/





'omics/ pathways

Cell biology phenotypes (i.e. proliferation)

In Silico Models for Reverse TK IVIVE When No In Vitro Data Are Available

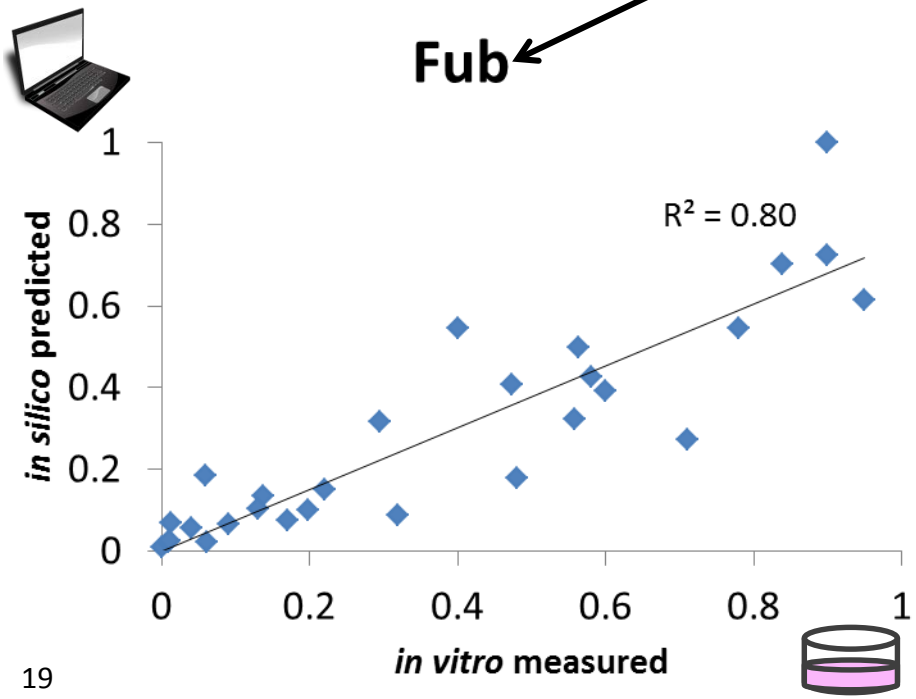
Nisha Sipes Compiled 28 Tox21 cmpds

- *in silico* parameters (Fub, CLint)
- *in vitro* parameters (Fub, CLint)
- *in vivo* measured C_{max} values (human, Drug Matrix)

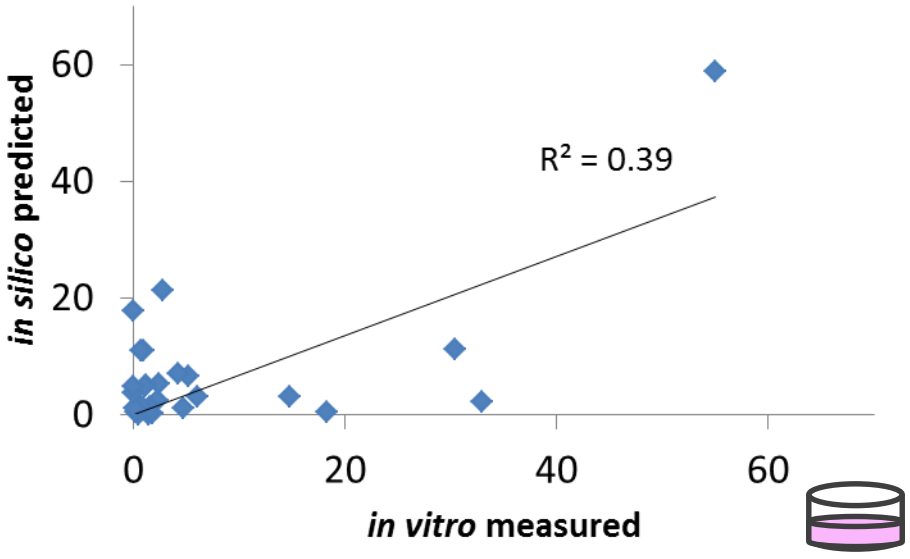
In Silico CL_{int} Models
CYP1A2
CYP2C9
CYP2C19
CYP2D6
CYP3A4

Weighted sum by relative expression levels in human liver

Fub



CLint



In Silico Models for Reverse TK IVIVE When No In Vitro Data Are Available

Nisha Sipes

- *in silico* p
- *in vitro* p
- *in vivo* m (human,

Models
2
9
19
6
4

Accepted Manuscript

Title: QSARs for estimating intrinsic hepatic clearance of organic chemicals in humans

Author: Alessandra Pirovano Stefan Brandmaier Mark A.J. Huijbregts Ad M.J. Ragas Karin Veltman A. Jan Hendriks

PII: S1382-6689(16)30017-5
DOI: http://dx.doi.org/doi:10.1016/j.etap.2016.01.017
Reference: ENVTOX 2442

To appear in: *Environmental Toxicology and Pharmacology*

Received date: 8-9-2015
Revised date: 19-1-2016
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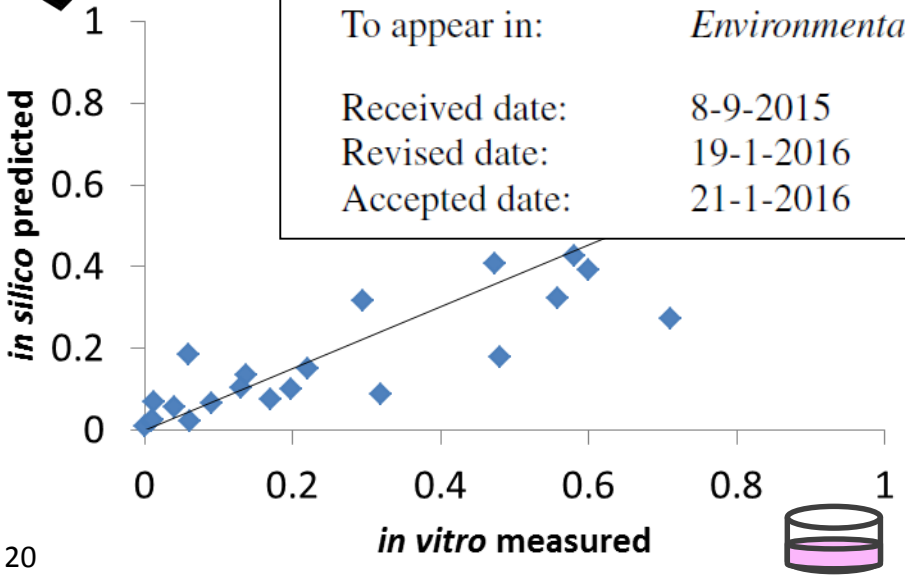
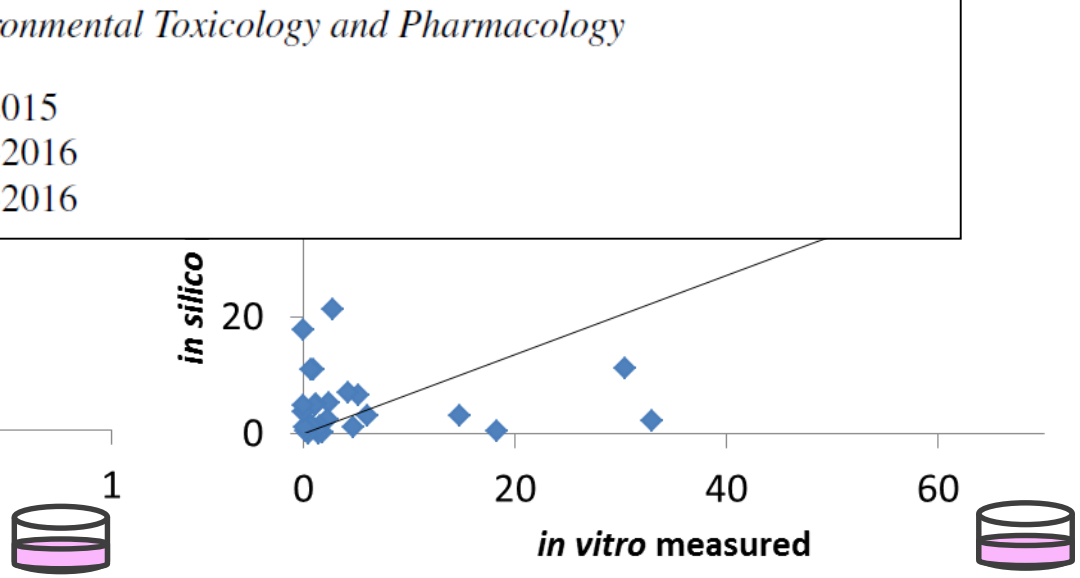
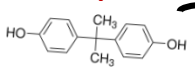


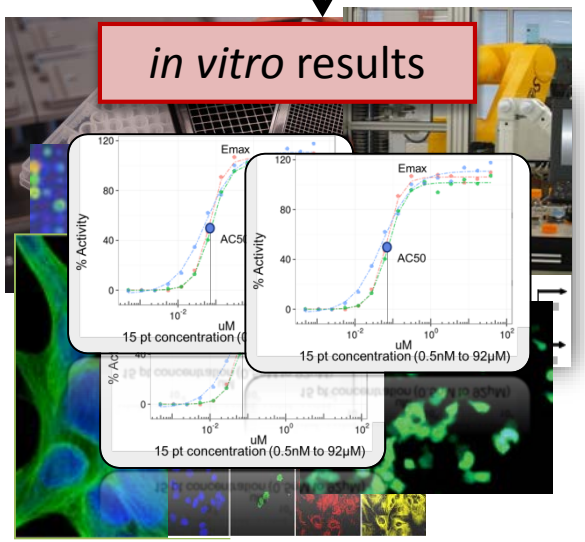


How Do We Apply These In Vitro to In Vivo Translation Approaches to Tox21 Where Metabolism Data is Not Available?

Compound



in vitro results



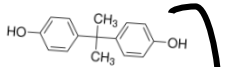
IVIVE

Chemical Assay
AC_{50} E_{max}

?

Chemical Biomarker
C_{max}
Efficacy

Compound



Nisha Sipes

Please visit Nisha's Poster!

IVIVE with:

In Vivo Data
In Vitro CL_{int}, fub predictions
In Silico CL_{int}, fub predictions

Computational Models to Correlate *In Vitro* to *In Vivo* Activity

Sipes[1] NS, Wambaugh[2] JF, Pearce[2] R, Wetmore[3] BA, DeVito[1] MJ, Auerbach[1] SS, Ferguson[1] SS
[1]NTP/NIEHS/NIH, [2]NCCT/ORD/EPA, [3]The Hamner Institutes for Health Sciences, Research Triangle Park, NC 27709

Summary

- Effective in vitro models to predict in vivo metabolism generally employ in vivo-like levels of xenobiotic metabolism competence
- The rapid loss of metabolic competence with suspension primary hepatocytes or subcellular fractions limits our ability to assess lower turnover compounds
- Traditional in vitro assays for metabolic clearance with suspensions of primary hepatocytes do not generally employ physiologically-relevant levels of plasma proteins that may contribute to poorer predictions
- 'Early' PHH cultures and NextGen in vitro liver models have shown promise in improving our ability to predict in vivo metabolism
- Additional research is needed to define metabolic 'competence' grounded in metabolite profiles to reflect stages of cell/tissue differentiation and development (e.g. neonate hepatocytes?)
- In silico tools are emerging, but to date require additional development to cover a broader transformation space

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