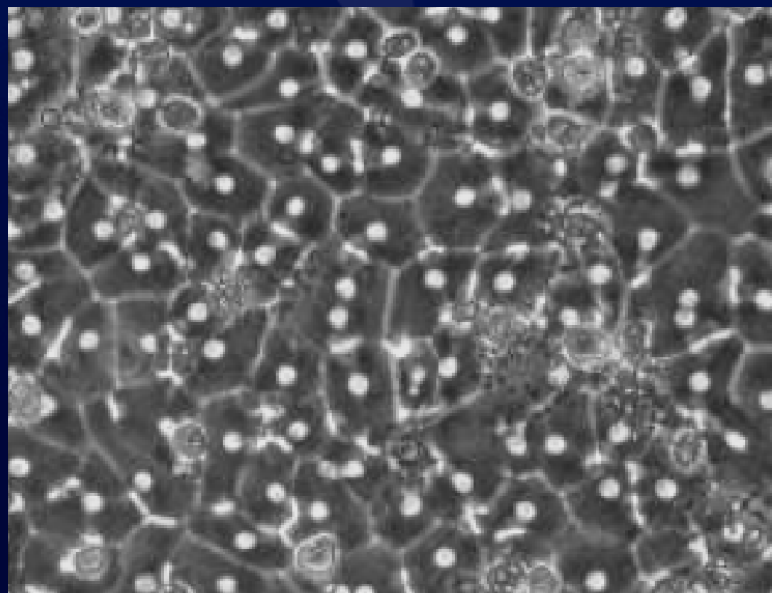
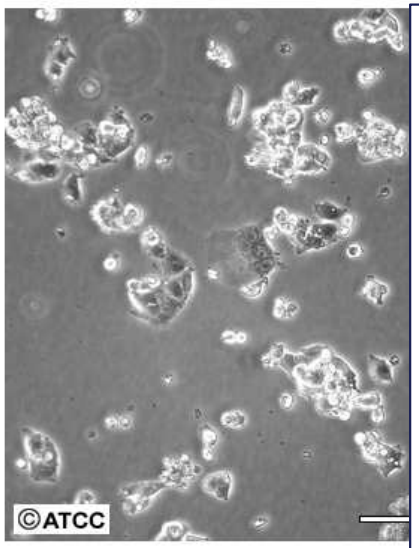
life technologies™

Predictive Hepatic Model Systems from Life Technologies: Strategies in Toxicology & Screening



HepG2 Cells & Other De-Differentiated Liver Cell Lines in Cytotox Screening

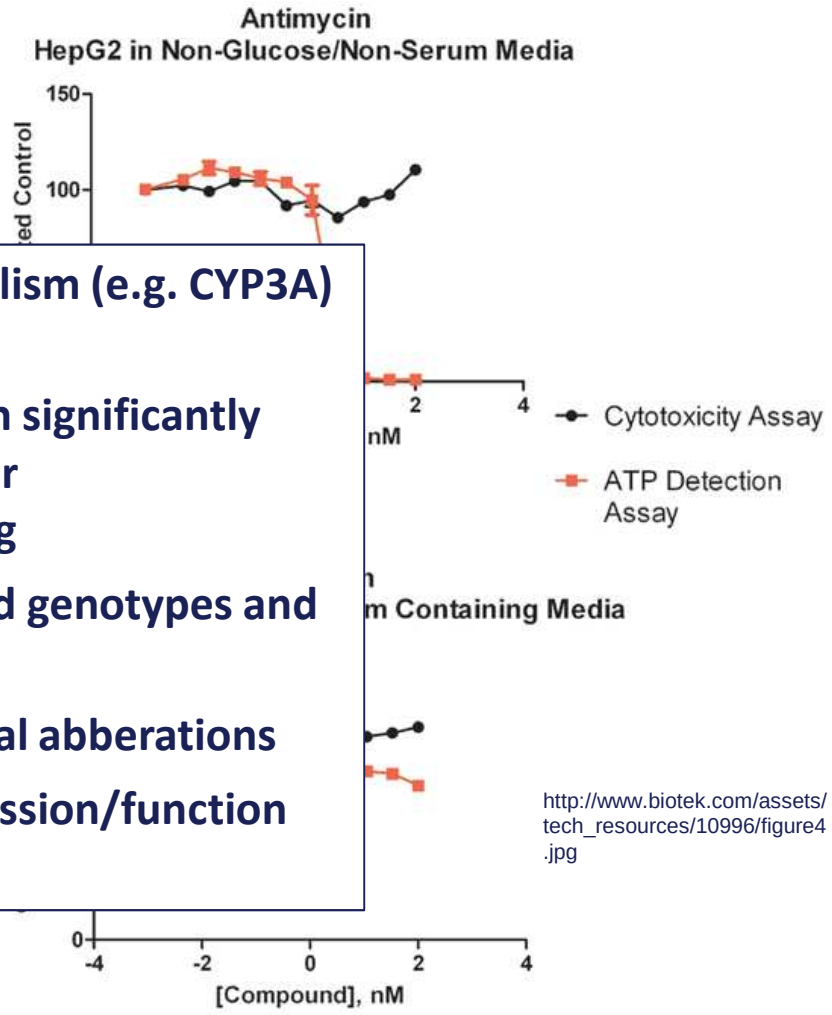
ATCC Number: **HB-8065**
Designation: **Hep G2**



Low Density

Scale Bar = 1

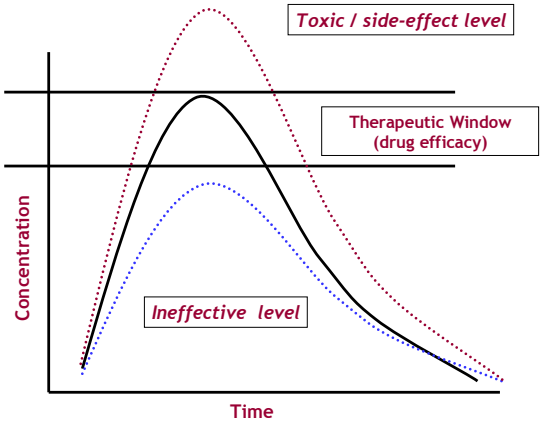
- **Baseline P450 metabolism (e.g. CYP3A) below detection**
- **Transporter expression significantly reduced, lack of proper polarization/trafficking**
- **Fetal, de-differentiated genotypes and phenotypes**
- **Extensive chromosomal abberations**
- **Lack of receptor expression/function (i.e. CAR)**



http://www.biotek.com/assets/tech_resources/10996/figure4.jpg

Predictive In Vitro Biology ‘vs.’ High Throughput Screening

- We need both!
- We want:
  - Identification of predictive pathways and specific molecular assays to assess initial pathway activation potential (e.g. potency, efficacy)
  - Essential in vitro cellular models with dynamic, predictive, in vivo-like responses to chemical exposure for various tissues/systems (e.g. liver)
  - Useful assays as ‘windows’ (e.g. omics) to monitor chemical perturbations (reversible, irreversible) in predictive model systems
  - Acute and ‘longer term’ exposure models
  - Exposure considerations: levels (e.g. intracellular concentration), frequencies, ‘barriers’ (e.g. extracellular matrices), and kinetics (e.g. waves)
  - Metabolism: in vivo-like clearance of parent chemical and generation of in vivo-like metabolites at relevant levels to better assess metabolite effects



Volume Adjusted Concentrations (24 hrs)

[Ritonavir] Added	Quantity in Supernatant (pmol)	Quantity in Monolayer (pmol)	Percent in Monolayer (%)	Supernatant Concentration (µM)	Estimated Monolayer Concentration (µM)‡
0.01 µM	0*	0.0394	100	0*	0.0262
0.1 µM	0.144	22.6	99.4	0.000287	15.1
1 µM	39.5	153	79.5	0.0791	102
10 µM	2670	636	19.2	5.34	424

‡Assumed 4 µL volume per million cells

*below detection limits

PXR binds (K_D) and has EC_{50} to activate CYP3A4 promoter in cell lines @ ~2 µM†

†Dussault, Forman 2001 JBC (276) 33309-33312

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Are We There Yet?

	Arrived?
•Identification of predictive pathways and specific molecular assays to assess initial pathway activation potential (e.g. potency, efficacy)	
•Essential in vitro cellular models with dynamic, predictive, in vivo-like responses to chemical exposure for various tissues/systems (e.g. liver)	
•Useful assays as 'windows' (e.g. omics) to monitor chemical perturbations (reversible, irreversible) in predictive model systems	
•Acute and 'longer term' exposure models	
•Exposure considerations: levels (e.g. intracellular concentration), frequencies, 'barriers' (e.g. extracellular matrices), and kinetics (e.g. waves)	
•Metabolism: in vivo-like clearance of parent chemical and generation of in vivo-like metabolites at relevant levels to better assess metabolite effects	

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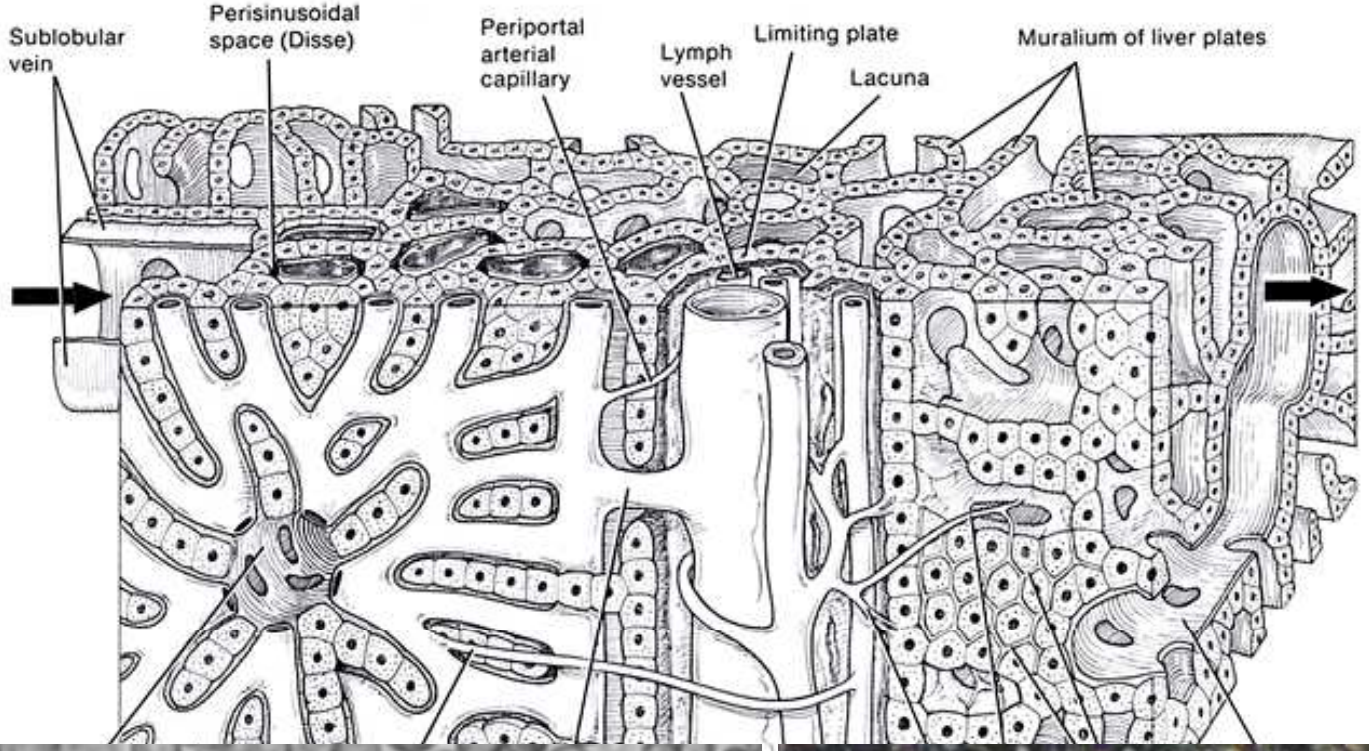
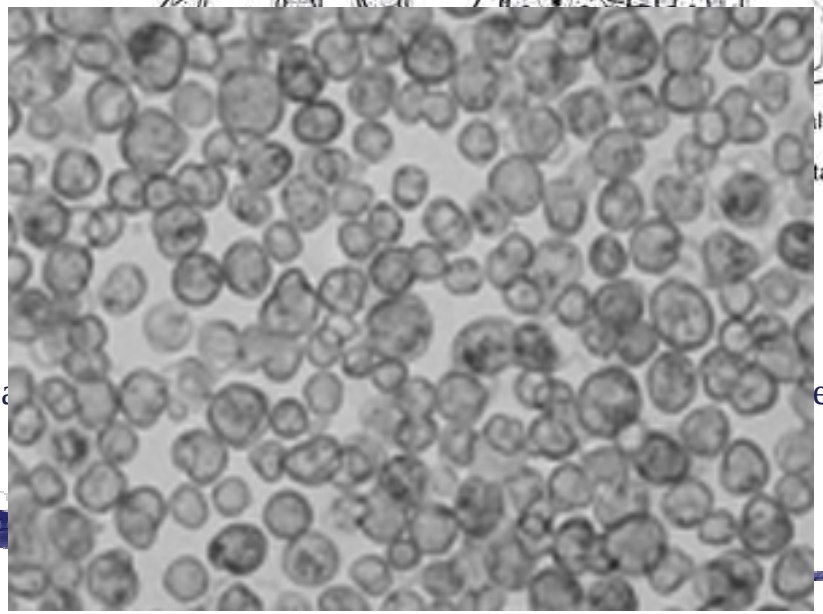
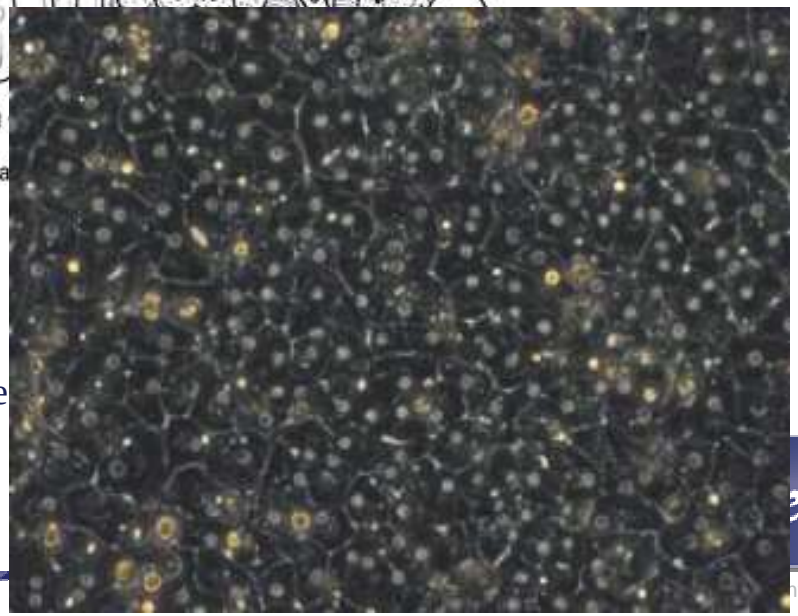
Liver & Important Features to More Predictively Model In Vitro







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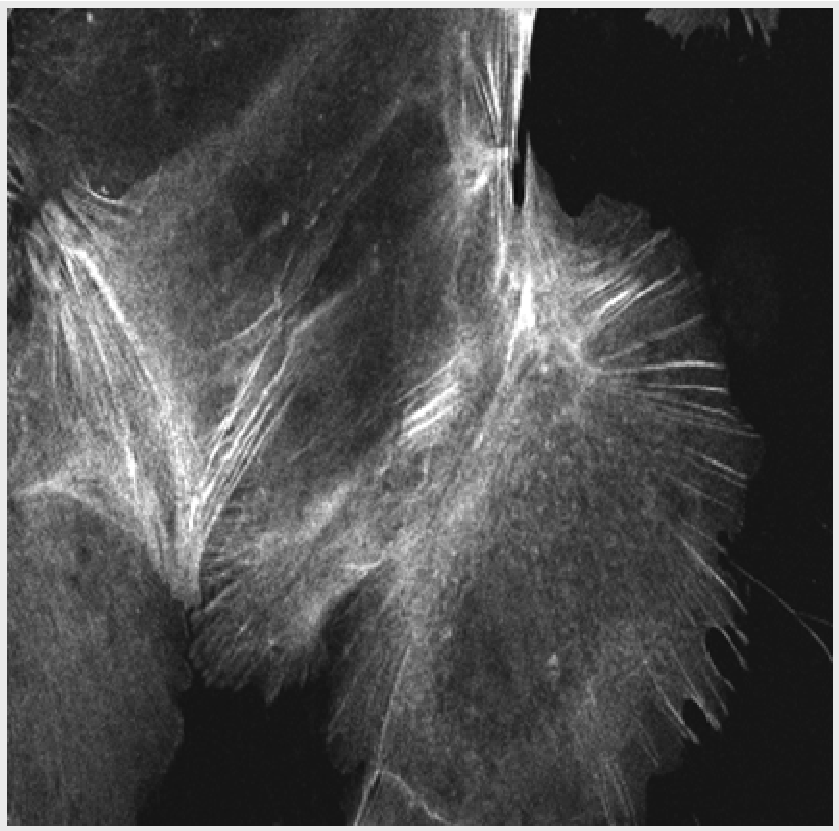
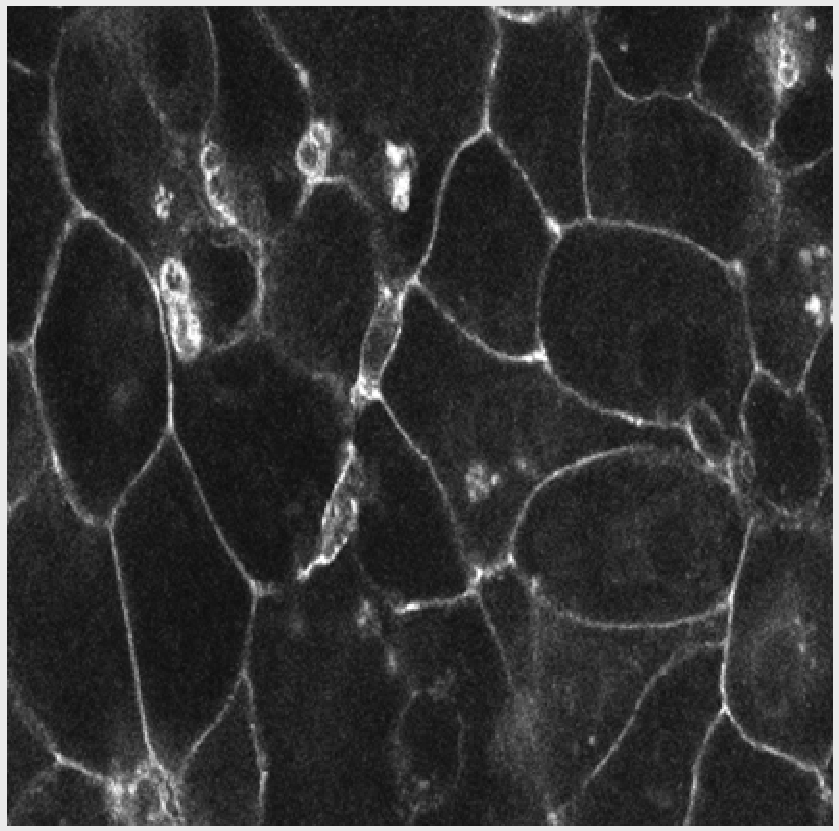
Distribution of Actin Microfilament



Low-density



High-density

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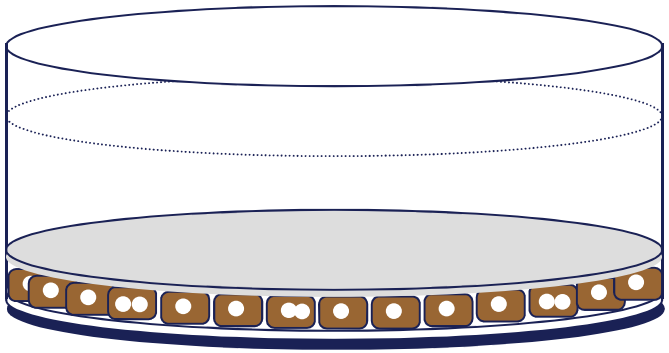
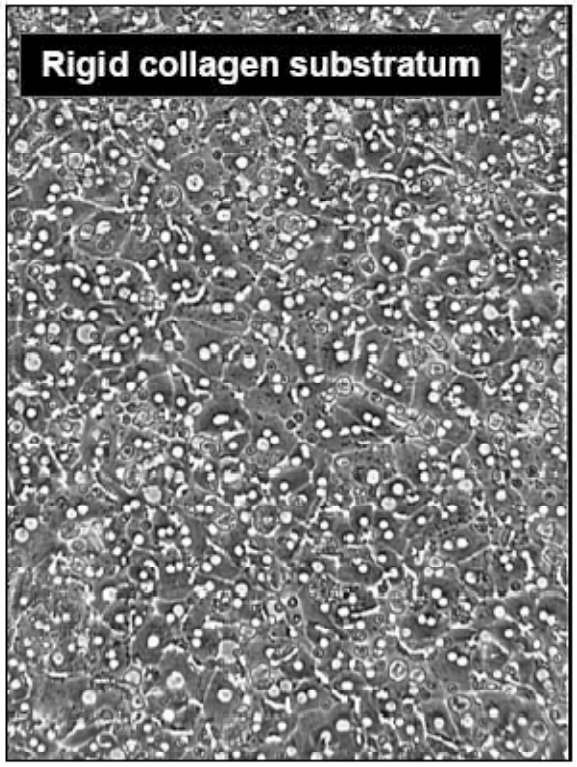
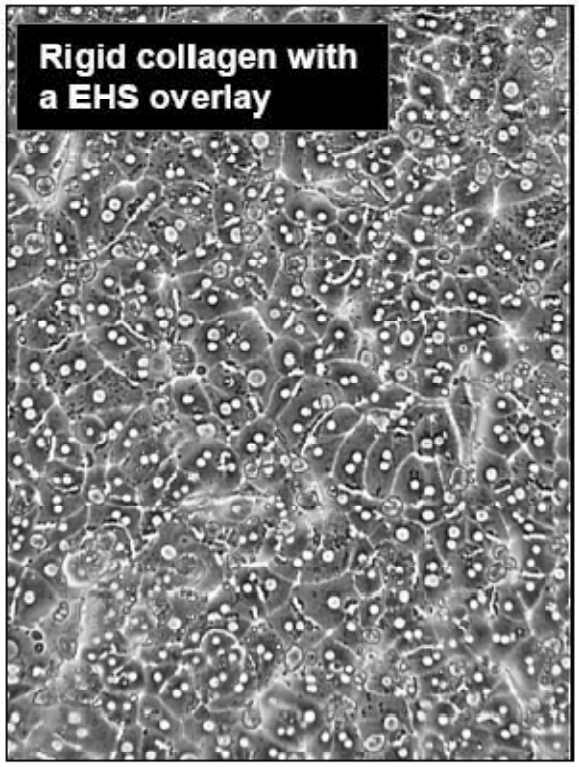
Culture System Configurations



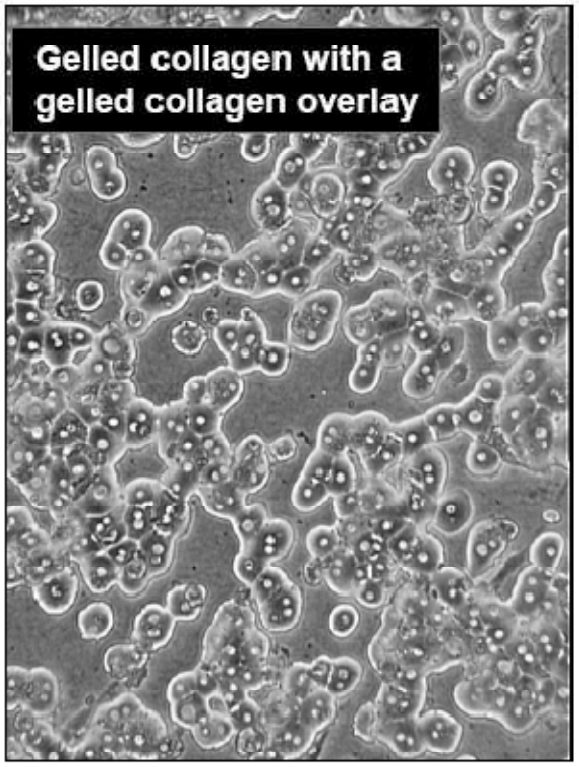
Culture Media
Overlay (e.g. Geltrex®)
Primary Hepatocytes
Basement Matrix (e.g. collagen)



Conventional monolayer

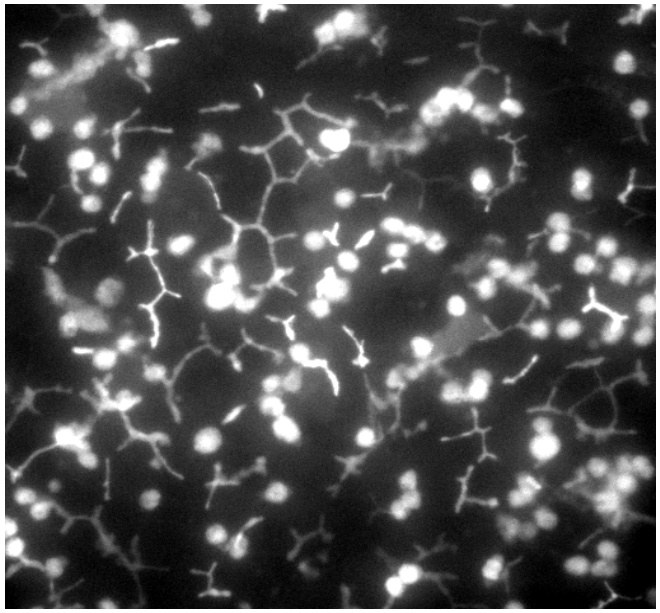


Sandwich culture 1

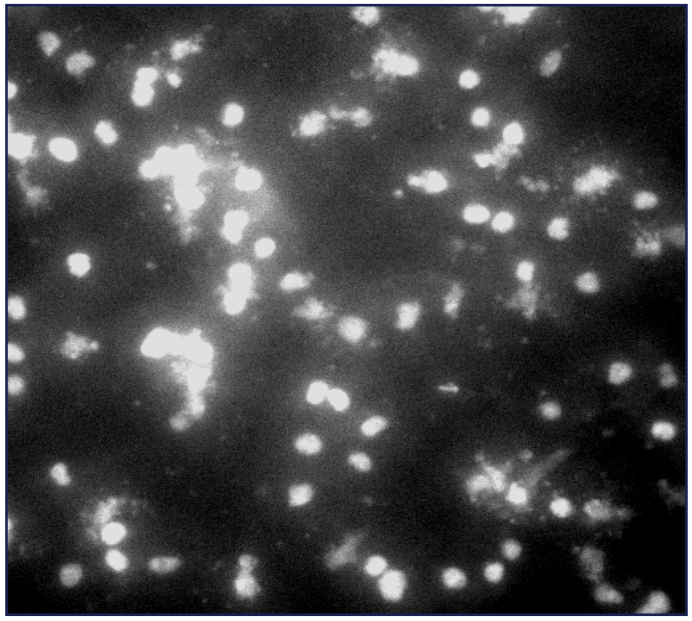


Sandwich culture 2

Primary Cultures of Hepatocytes form Functional Bile Pockets (Canaliculi) Over Time in Culture (days)



CLF



CLF in Ca/Mg-free buffer

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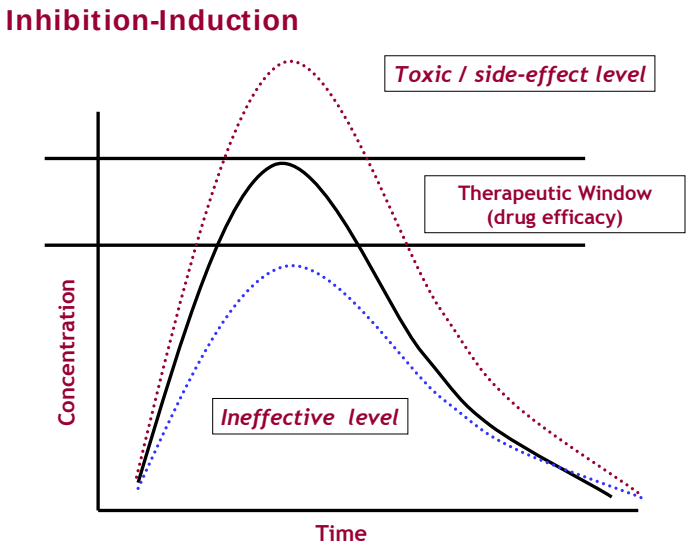
Pharma's Use of Hepatic Models for Prediction of Clearance, DDI, Safety

- Intrinsic Metabolic Clearance (Cl_{INT})
  - Human liver microsomes (HLM) (e.g. 50 donor pools)
  - Human liver S9 fractions
  - Primary human hepatocytes (single donor)
  - Pooled primary human hepatocytes (e.g. 10 donor pools)
- Safety
  - Acute cytotoxicity in cell lines (e.g. HepaG2)
  - P450 transfected cell lines
  - Primary human hepatocytes for cytotoxicity (e.g. HCS, ATP)
  - Cross-species hepatocyte studies
  - HepaRG cells for cytotoxicity
- Metabolism
  - HLM & recombinant P450s in reaction phenotyping
  - Metabolic profiling & major metabolite ID: hepatocytes
- Transport
  - Hepatic uptake clearance (PS_{inf}): hepatocytes (suspension, plated)
  - Biliary excretion clearance (PS_{EFF}): 7-day cultures of hepatocytes
- Drug-drug interactions
  - Induction: primary human hepatocytes & HepaRG
  - Inhibition: HLM, recombinant P450s, hepatocytes
- Pharmacokinetics (brining it all together)
  - Intrinsic clearance, plasma protein binding, absorption, excretion, induction, inhibition



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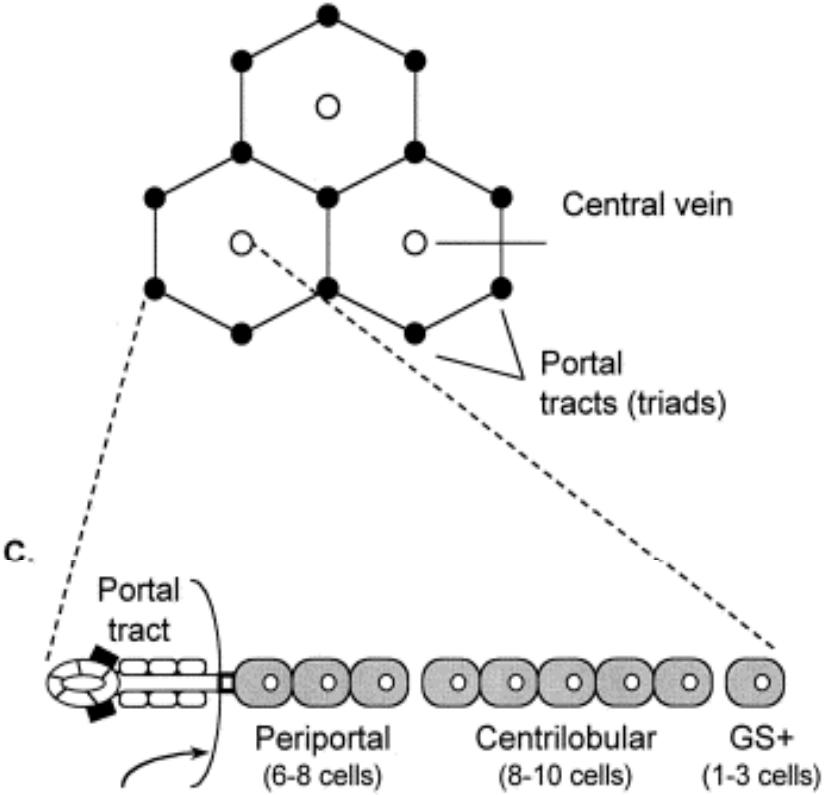
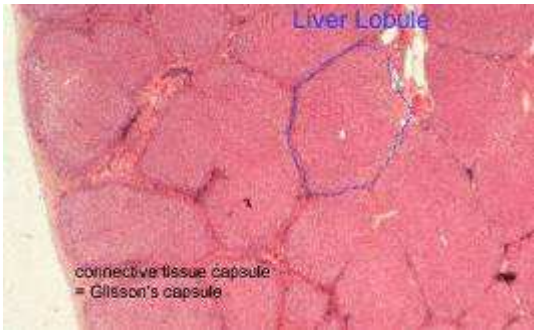
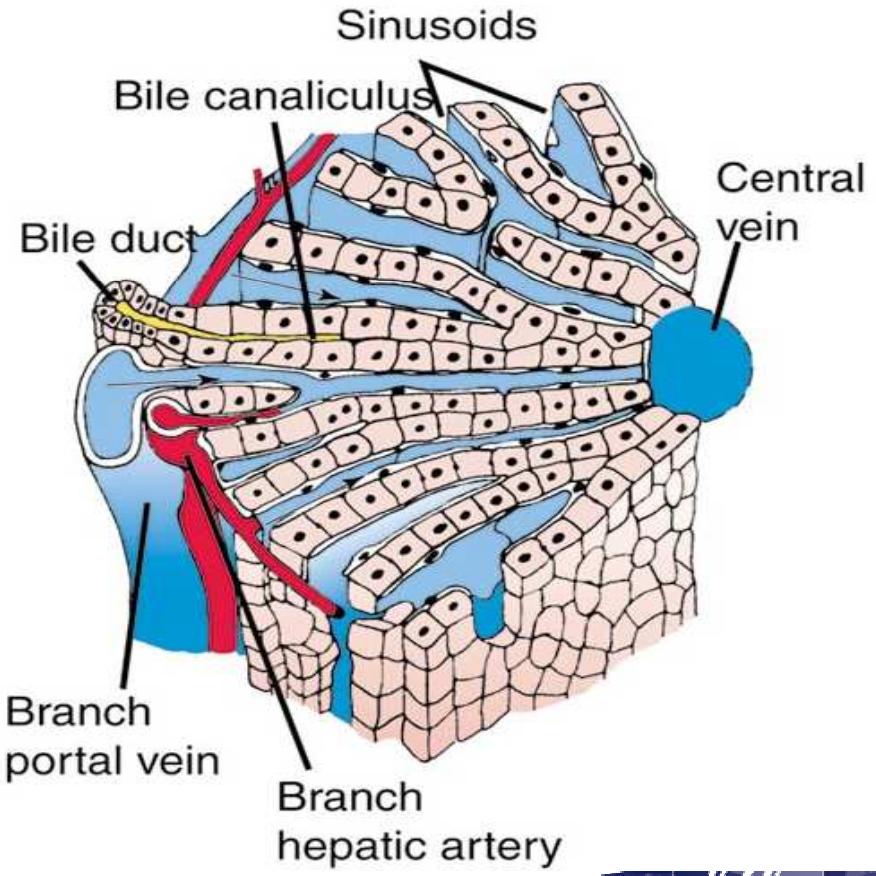
Structure of Liver Lobule







Multiple Cell types: LSECs, Kupffers, Cholangiocytes, Stellate Cells

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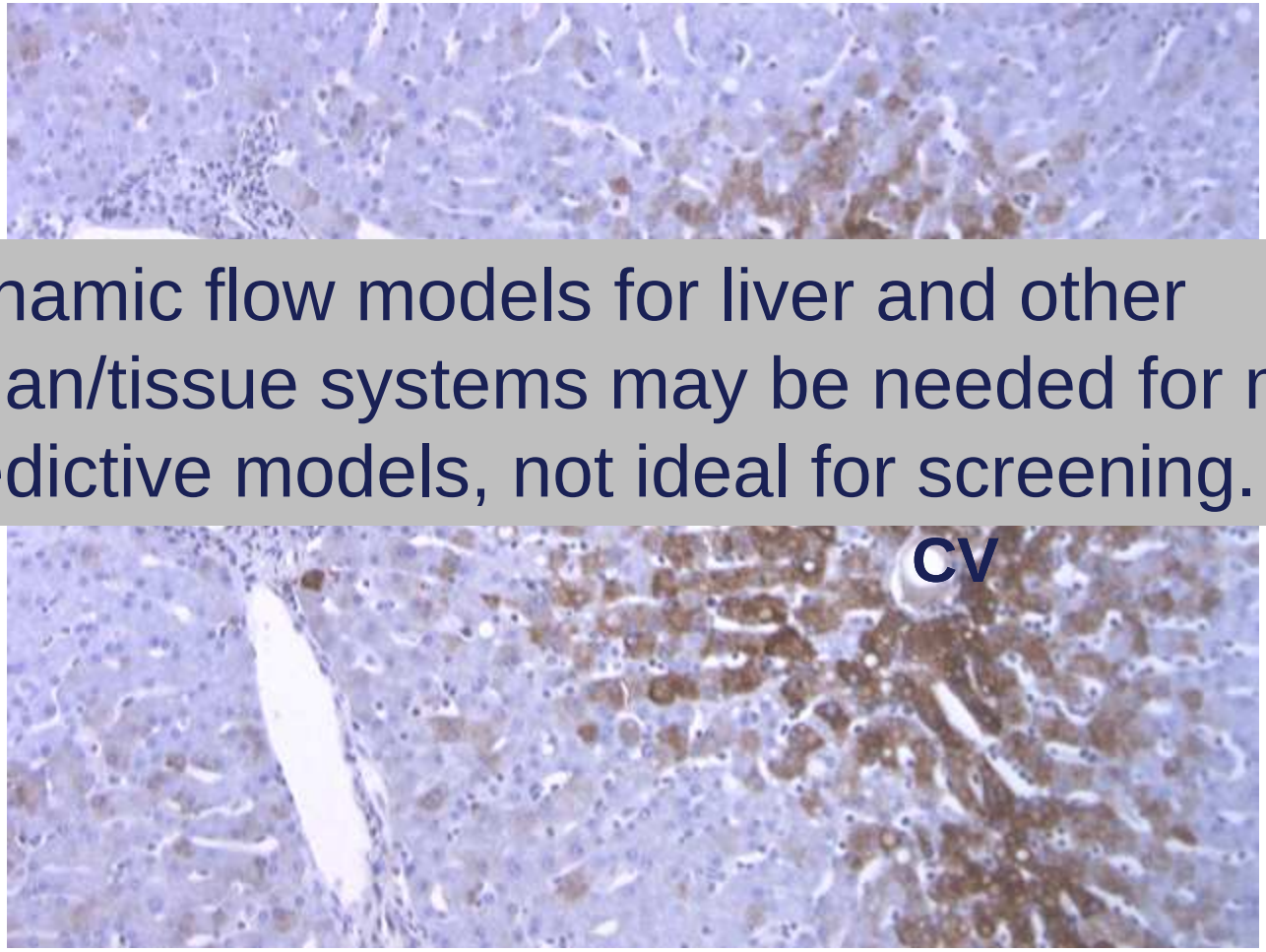
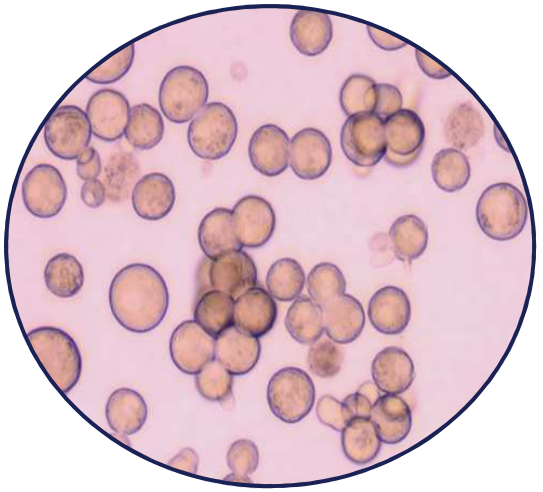
Zonal Distribution of CYP3A4 Expression in Human Liver



Dynamic flow models for liver and other organ/tissue systems may be needed for more predictive models, not ideal for screening.

Clearance Predictions
Intrinsic Clearance (CL_{INT}) from Suspension Hepatocytes



1. Half-life: $t_{1/2}$ (min):

Half-life of disappearance of parent molecule.
Plot ln [S] vs t

$$t_{1/2} = \frac{0.693}{slope\ of\ \ parent\ loss}$$

2. Intrinsic clearance: CL_{int} (mL/min/kg):

Equation holds true only when [S] << Km.

$$CL_{INT} = \frac{g\ liver\ wt}{kg\ body\ wt} \times \frac{ml\ incubate}{cells\ per\ incubate} \times \frac{1.35 \times 10^{6}\ \ cells}{g\ liver\ wt}$$

	average CL_{int} (µL/min/million cells)		
Lot Number	**Midazolam**	**Tolbutamide**	**Dextromethorphan**
Hu 8040	8.0	0.7	0.3
Hu 4049	9.8	0.7	N.D.
Hu 781	10.8	1.3	2.7
Hu 833	10.5	0.8	6.0
Hu 4124	5.8	0.1	0.2
Reference Values	7, 14, 11, 17	1.6, <1	3.1, 16, 15

DMD 26, 216-221 (1998)

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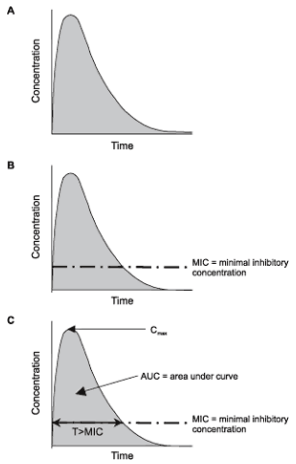
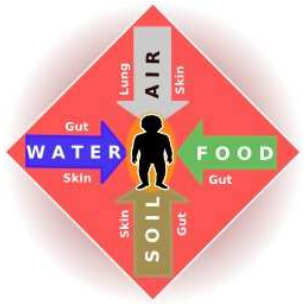
In Vitro Screening of Chemical Metabolism (IV/IV Correlations) (The Hamner, EPA (NCCT), CellzDirect/Life Technologies)





- Thus far, most of the effort has focused on characterizing the biological activity across multiple cellular pathways and processes.
- Little attention paid towards understanding relationship between active concentrations of a chemical *in vitro* and expected concentrations in human populations.
- Pharmacokinetic properties and human exposure characteristics are equally important as the biological activity in determining a chemical's risk to human health.

Slide Courtesy of Rusty Thomas, Barbara Wetmore

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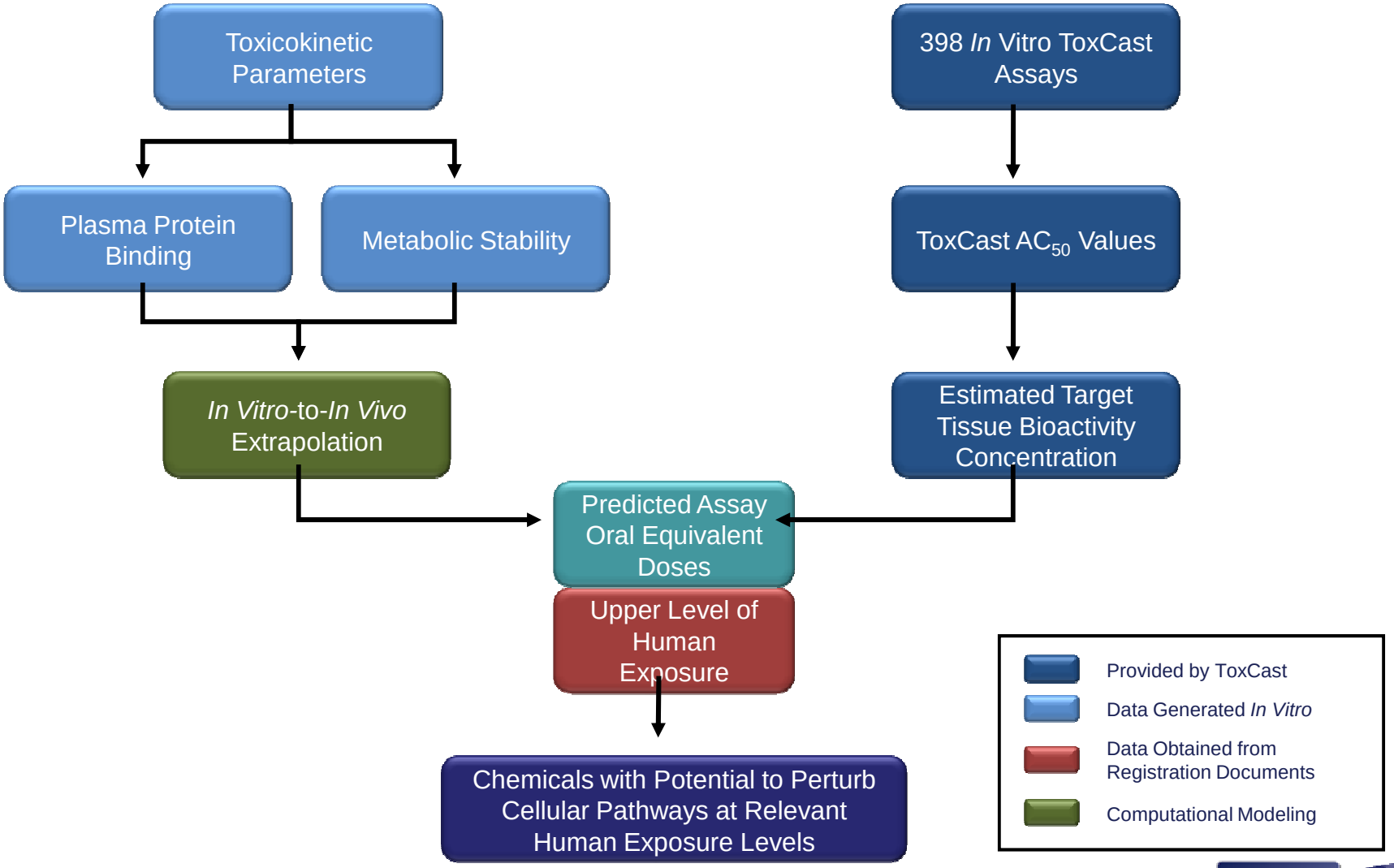
Defining Dosimetry and Exposure in High Throughput Toxicity Screens



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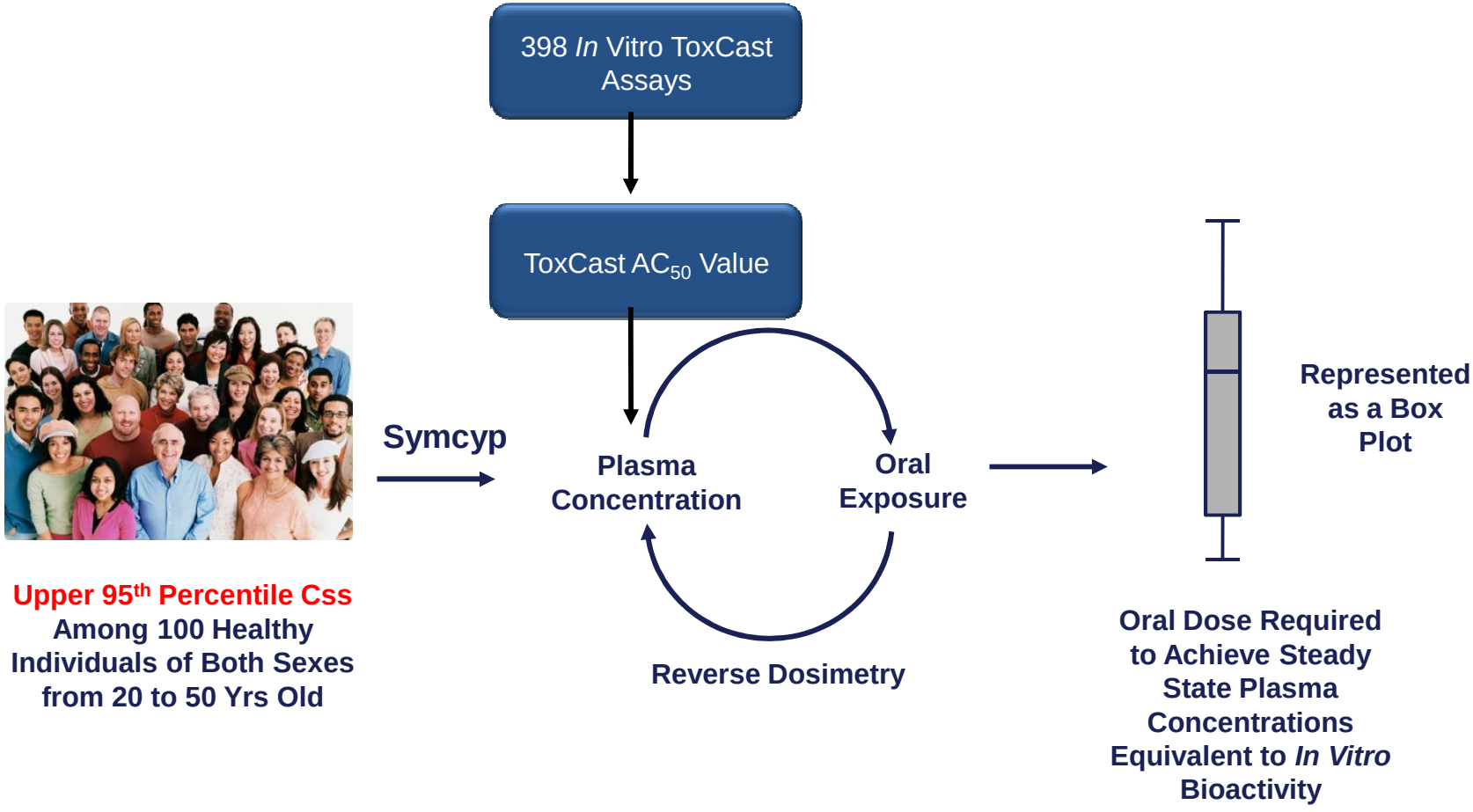
Reverse Dosimetry Modeling for Interpreting *In Vitro* Assay Results



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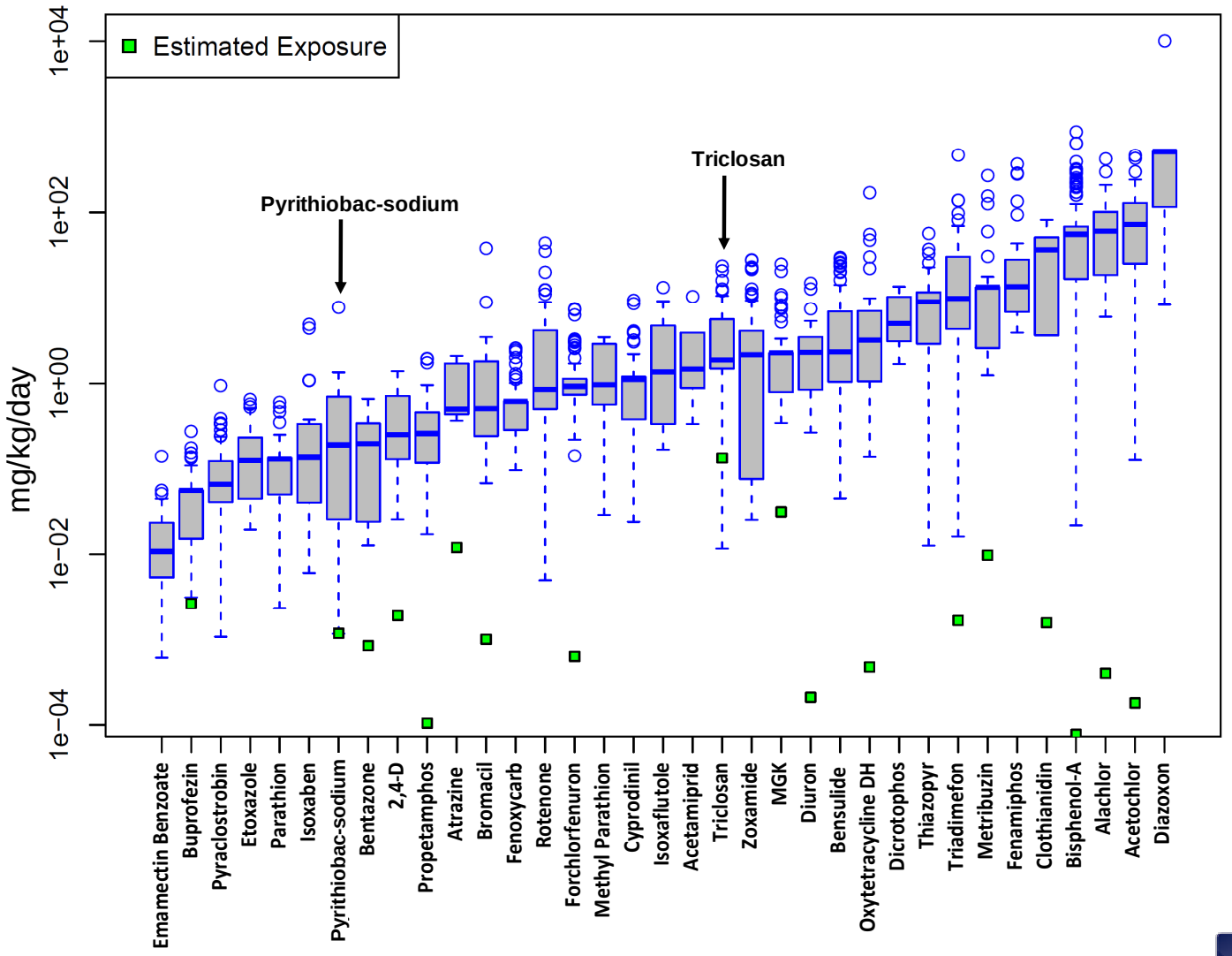
Distribution of Oral Equivalent Values for the ToxCast Assays and Comparison with Exposures: New Consideration for Prioritization?



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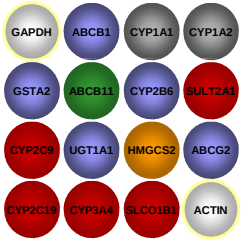
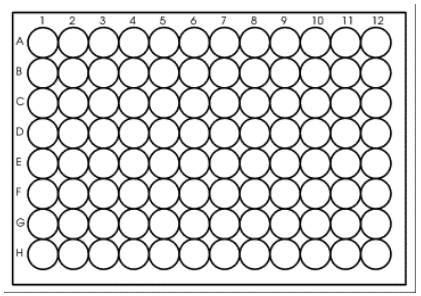
ToxCast™ Collaboration Experimental Design

- Cultures of primary human hepatocytes
- 96-well format
- 6 positive control reference chemicals (known receptor activators)
- ToxCast Phase 1 chemical library
- Endpoints
  - Gene Expression
    - > 16 gene targets relevant for liver biology



  - P450 Enzymatic Activity (CYP1A)
  - Cell morphology (Cell Health)
- Dose-response curves
- Multiple Time Points



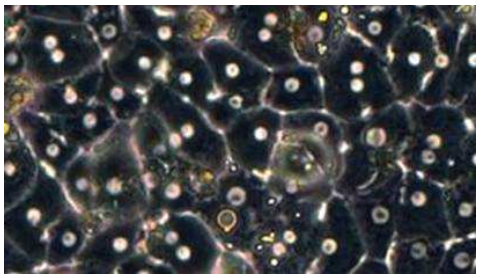


Image cultures

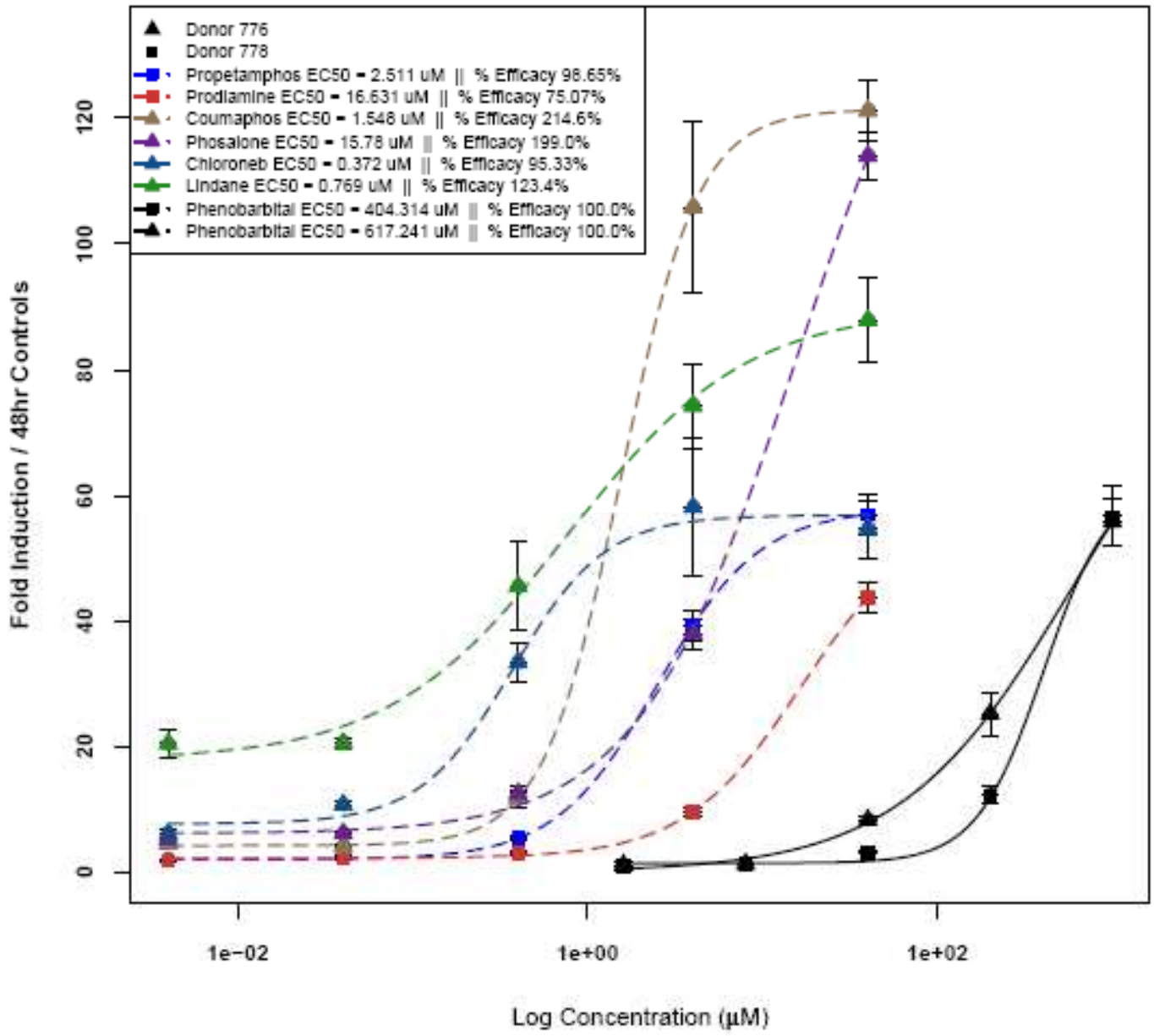
P450 In Situ Assays

Gene Expression Analysis

Data Analysis

Rotroff, et al. J Toxicol Environ Health B Crit Rev. 2010 Feb;13(2-4):329-46.

Representative ToxCast Chemicals for Potency and Efficacy for CYP2B6 at 48hrs



Relative Risk Analysis with Human Hepatocytes/CellzDirect Gene Expression Data & 2-Year NTP In Vivo Animal Studies

Gene	Endpoint	True Positive Count	False Positive Count	True Negative Count	False Negative Count	RR	Sensitivity	Specificity	Permuted p-value
CYP3A4	Rat Thyroid Tumors	22	87	74	7	2.34	0.76	0.46	0.02
CYP2B6	Rat Thyroid Tumors	23	100	61	6	2.09	0.79	0.38	0.04
SULT2A1	Rat Thyroid Hyperplasia	10	42	125	13	2.04	0.43	0.75	0.04
HMGCS2	Rat Proliferative Liver Lesions	7	5	126	52	2.00	0.12	0.96	0.04
SLCO1B1	Rat Liver Tumors	4	4	165	17	5.35	0.19	0.98	0.01
HMGCS2	Rat Liver Tumors	4	8	161	17	3.49	0.19	0.95	0.03
CYP2B6	Rat Liver Hypertrophy	49	74	54	13	2.05	0.79	0.42	0.00
CYP2B6	Rat Liver Apoptosis Necrosis	17	106	63	4	2.32	0.81	0.37	0.03

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Bisphenol A (BPA)

0090-9556/02/3011-1180–1185$7.00
DRUG METABOLISM AND DISPOSITION
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DMD 30:1180–1185, 2002

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METABOLISM OF BISPHENOL A IN PRIMARY CULTURED HEPATOCYTES FROM MICE, RATS, AND HUMANS

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ABSTRACT:

Studies have shown that in the rat, bisphenol A (BPA) is metabolized and eliminated primarily as a monoglucuronide, a metabolite without estrogenic activity. The purpose of this study was to determine the extent of monoglucuronide formation in monolayers of hepatocytes from rats, mice, and humans. Noncytotoxic concentrations of BPA (10, 20, and 35 μM; 1.0 μCi), as assessed by lactate dehydrogenase leakage, were incubated with isolated hepatocytes for 0–6 h. Media were collected and analyzed for metabolites by radiochemical high performance liquid chromatography and liquid chromatography-tandem mass spectrometry. The metabolites identified include a monoglucuronide (major metabolite), a sulfate conjugate, and a glucuronide/sulfate diconjugate (minor metabolites). In hepatocytes of male Fischer-344 rats, the predominate metabolite was the diconjugate (glucuronide/sulfate). Under these conditions, the extent of metabolism by 3 h was similar in all species tested because all BPA was converted to conjugates by 3 h. Initial rates of metabolism in hepatocytes followed the order of mice > rats > humans. However, when extrapolated to the whole liver (i.e., cells per liver), the hepatic capacity for BPA glucuronidation is predicted to be humans > rats > mice. This research was supported in part by The Society of Plastics Industry Inc., and Southwest Environmental Health Science Center (ES 06694).

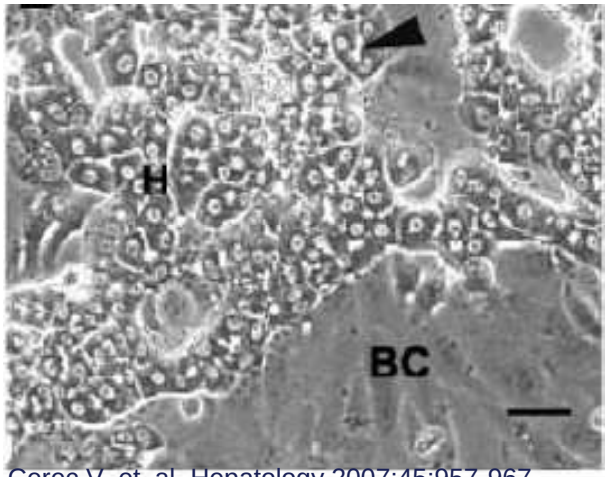
Primary Human Hepatocytes: Strengths & Limitations

Model System	Biological Relevance	Advantages	Limitations
Primary Hepatocytes (Sandwich Culture) "Gold Standard"	• **High** > Maintain Liver microstructure - polarized epithelium > Functional Phenotypes - Metabolism - Transport - Nuclear Receptor Pathways (e.g. CAR) > Proper culture methodologies critical for mature phenotypes	**Effective Models for In Vitro/In Vivo Correlation**	•**Technically Demanding** • **Limited Availability** > Tissue sourcing > Tissue quality > Ethical/legal issues •**High Cost** •**High Inter-Individual Variation** > Large donor-donor variability • **Phenotype Instability** > Short culture life-span ≤ 10 days

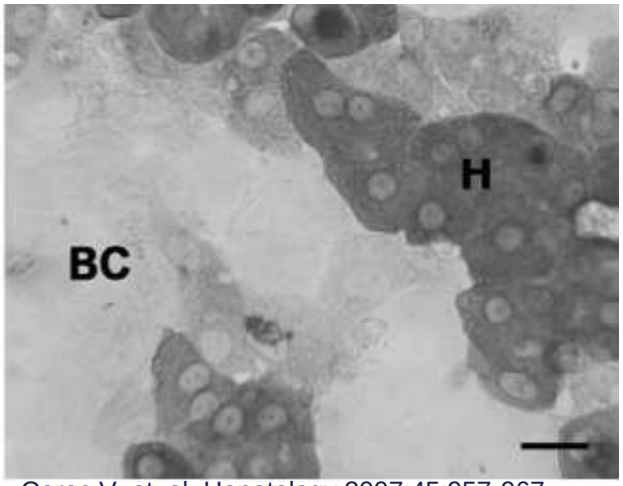
1105 2. **Design of In Vitro Drug Induction Studies**
1106
1107 At this time, the most reliable method to study a drug's induction potential is to quantify the
1108 enzyme activity of primary hepatocyte cultures following treatments including the potential
1109 inducer drug, a positive control inducer drug (see Table 5), and vehicle-treated hepatocytes
1110 (negative control), respectively. Freshly isolated human hepatocytes or cryopreserved
1111 hepatocytes that can be thawed and cultured are the preferred liver tissue for these studies;
1112 immortalized liver cells are acceptable if it can be demonstrated with positive controls that
1113 CYP3A4 and CYP1A2 are inducible in these cell lines.
1114

Introducing Cryopreserved HepaRG™ Cells

- Liver Progenitor Cell Line
  - > Differentiates into two distinct cell populations
- Hepatocyte-like Cell Population
  - > Adult phenotype similar to PHH
    - ~50% of cell population
    - P450 expression (i.e. CYP3A4)
- Biliary-like Cell Population
  - > CYP3A4 expression undetected
  - > Biliary markers
    - CK19
    - α6 Intergrin



Cerec V. et. al. Hepatology 2007;45:957-967

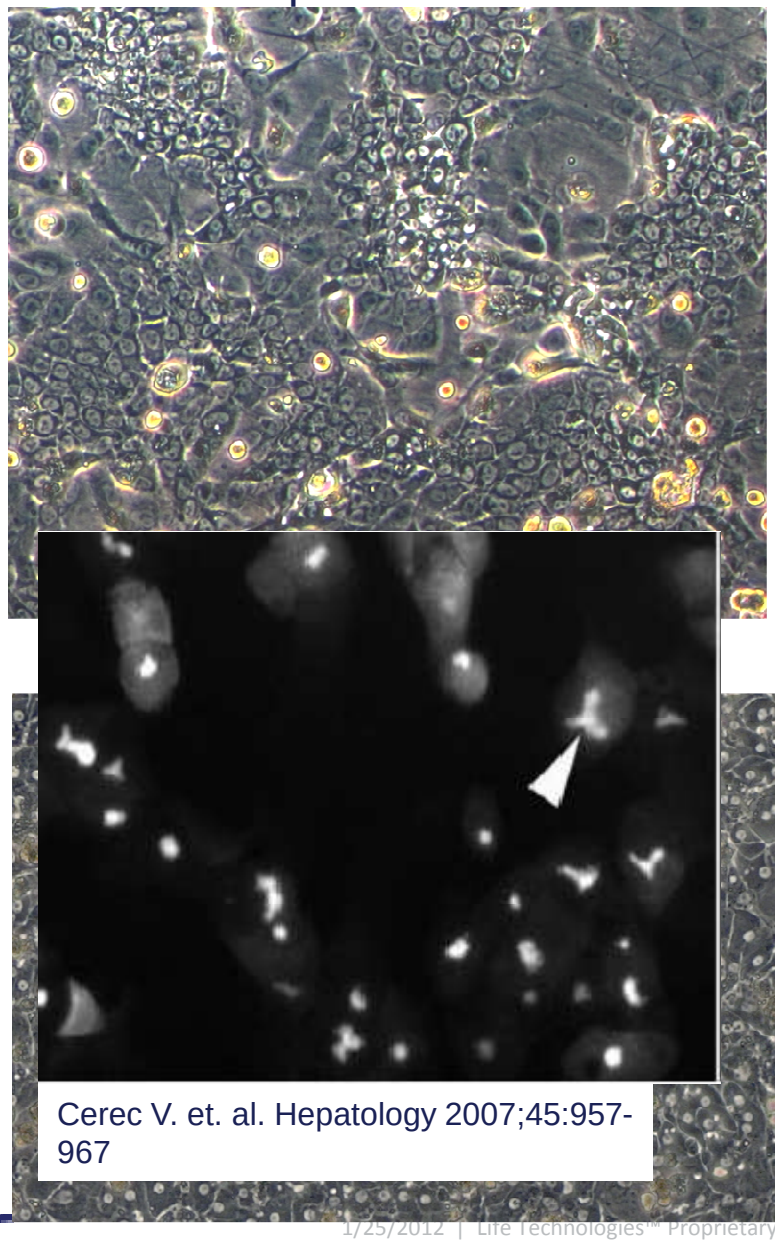


Cerec V. et. al. Hepatology 2007;45:957-967

HepaRG: A Primary Hepatocyte-Like Alternative

- HepaRG™ Cells
  - > Organize in an physiologically relevant manner
    - – Polarized epithelium
  - > Integrated DME & Drug transport System
    - Transporters
      - Uptake (e.g. OATP, NTCP)
      - Efflux (e.g. MRPs, MDR)
    - DME
      - Phase I (e.g. P450, FMO)
      - Phase II (e.g. UGTs, SULT)
    - Receptor Pathways
      - Functional CAR, PXR, AhR
      - Induction of DMEs and Transporters

HepaRG™ Cells



Cerec V. et. al. Hepatology 2007;45:957-967

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HepaRG™ Cells Timeline: A Short History

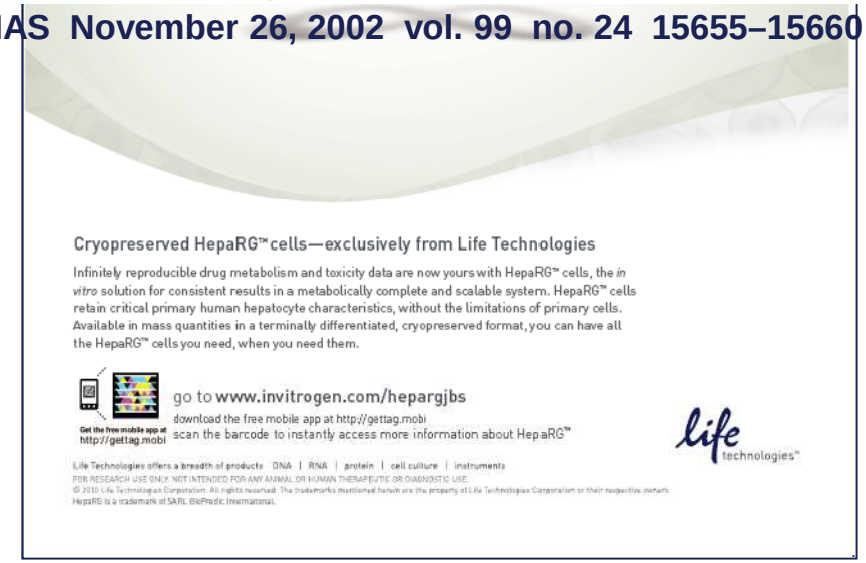
- 1999
  - > Isolation
  - > Development
  - > Institut National de la Sante' Recherche Medicale (INSERM), France
- 2002
  - > 1st publication
- 2004
  - > Commercially available in Europe
  - > Fresh Product
- 2011
  - > Life Technologies
  - > Cryo-preserved HepaRG™ Cells

Infection of a human hepatoma cell line by hepatitis B virus

Philippe Gripon*†, Sylvie Rumin*†, Stephan Urban‡¶, Jacques Le Seyec*, Denise Glaise*, Isabelle Cannie*, Claire Guyomard||, Josette Lucas**, Christian Trepo*, and Christiane Guguen-Guillouzo*

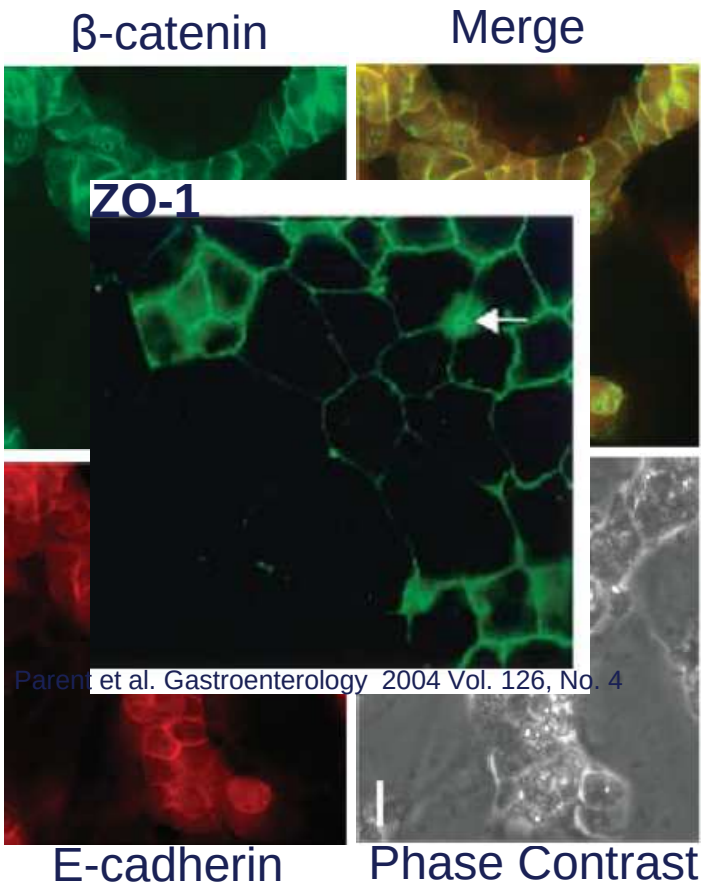
*Institut National de la Santé et de la Recherche Médicale (INSERM) U522, Hôpital de Pontchaillou, 35033 Rennes, France; ‡Zentrum für Molekulare Biologie, Universität Heidelberg, 69120 Heidelberg, Germany; ||BIOPREDIC, 14 Rue Jean Pecker, 35000 Rennes, France; **Laboratoire de Génétique et Biologie Cellulaire, Hôpital de Pontchaillou, 35033 Rennes, France; and †Institut National de la Santé et de la Recherche Médicale (INSERM) U271, 151 Cours Albert Thomas, 69424 Lyon Cedex 03, France

PNAS November 26, 2002 vol. 99 no. 24 15655–15660



HepaRG™ Cells Form Cell-Cell Interactions Analogous to Primary Human Hepatocytes

- Cell-Cell Interactions
  - > Co-localization of adhension molecules
    - β-catenin
    - E-cadherin
  - > Formation of tight junctions
    - ZO-1
- Establishing a polarized epithelium
  - > As observed in PHH culture

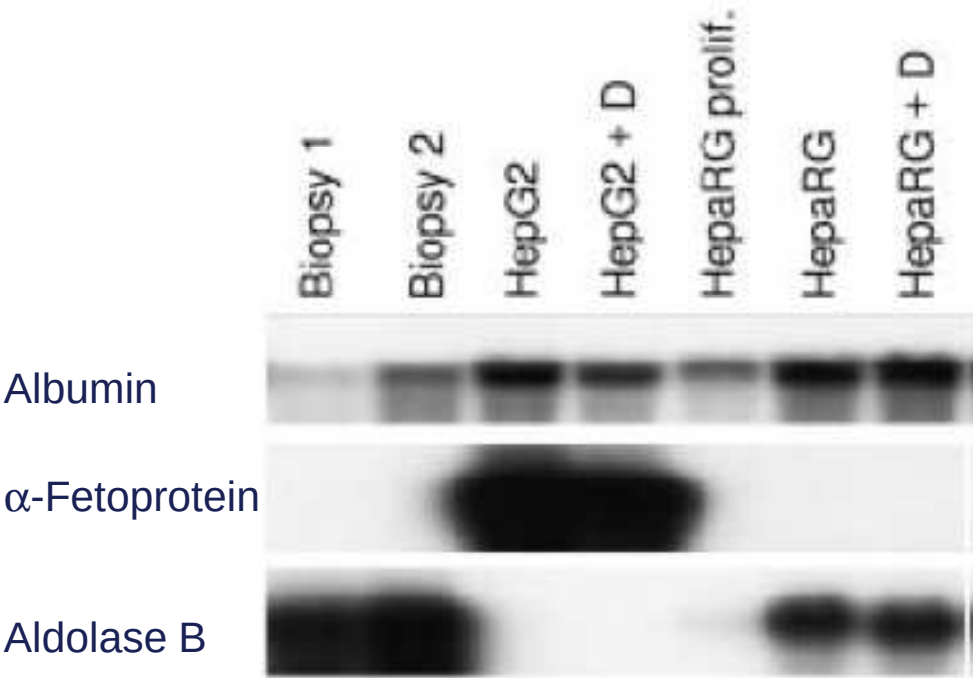


Parent et al. Gastroenterology 2004 Vol. 126, No. 4

Cerec V. et. al. Hepatology 2007;45:957-967

HepaRG™ Cells: Exhibits Adult Hepatic Phenotype

- Adult Liver Tissue
  - > α-fetoprotein undetectable
    - **Fetal** hepatocyte marker
  - > Aldolase B highly expressed
    - **Adult** hepatocyte marker
- HepG2
  - > Aldolase B undetectable
  - > α-fetoprotein highly expressed
- HepaRG™ Cells
  - > Express Aldolase B
  - > α-fetoprotein is undetectable



Gripon, P. et. al. PNAS 2002; 99:15655-15660

HepaRG™ Cell Production

HepaRG™
Growth & Differentation

28 days

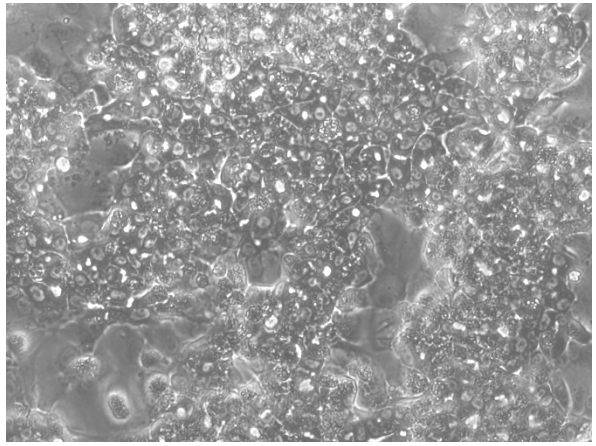
14 days each phase

HepaRG™
Cryopreservation

Terminally
Differentiated

Culture
(Post-Thaw)

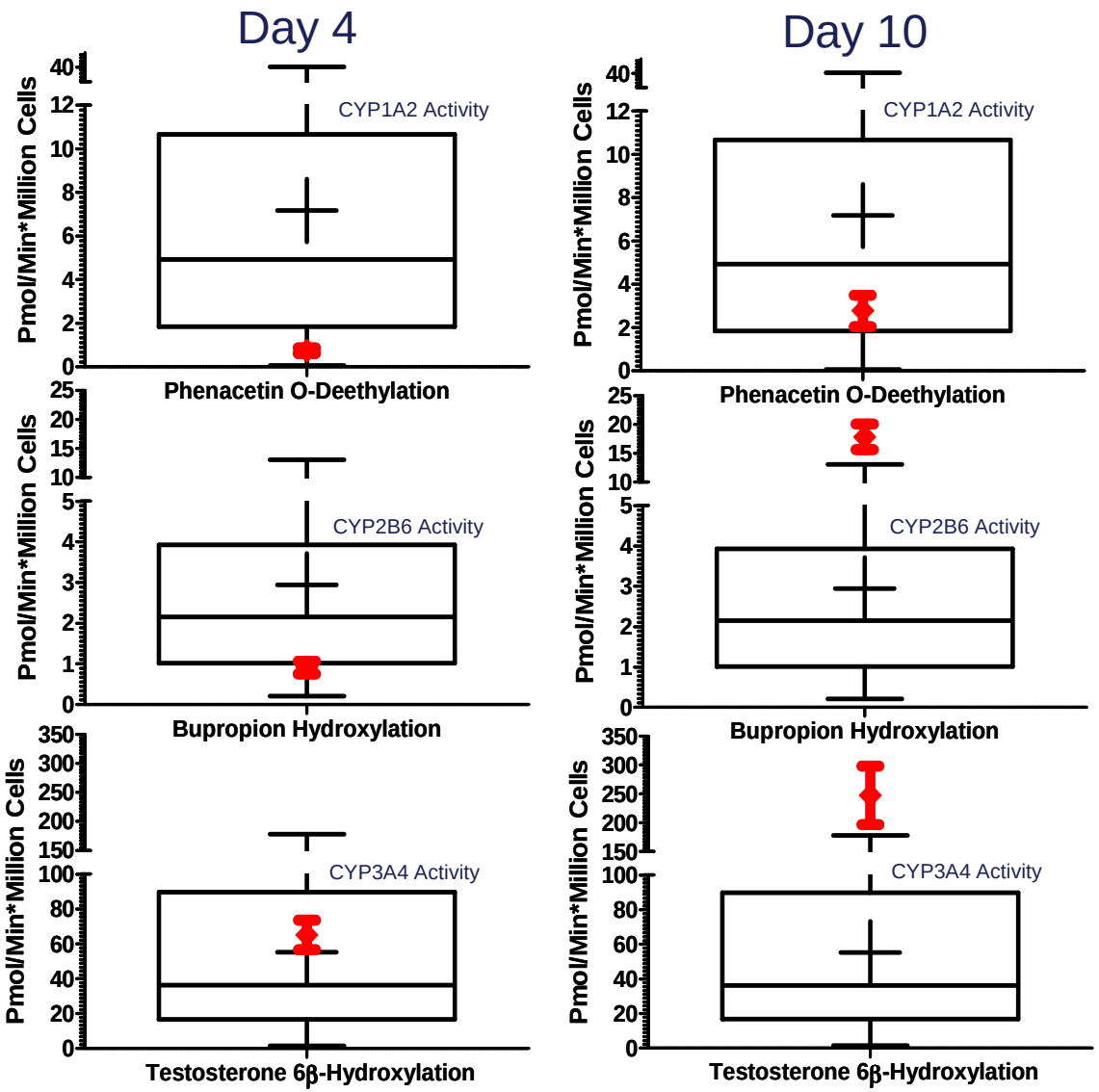
Sub-culturing of Differentiated HepaRG™ Cells is not supported post-thaw.



HepaRG™ Cells
(Post-thaw)

P450 Metabolism in HepaRG™ Cells

- PHH Activity (Box & Whisker Plots)
  - > N = 52
  - > Large donor to donor variability
- HepaRG™ Cells Activity in 720 Metabolism Media) (Red Points)
  - > Across 3 different 96-well plates
  - > Cultured in metabolism media
- Comparable P450 Activity
  - > HepaRG™ Cells vs. PHH



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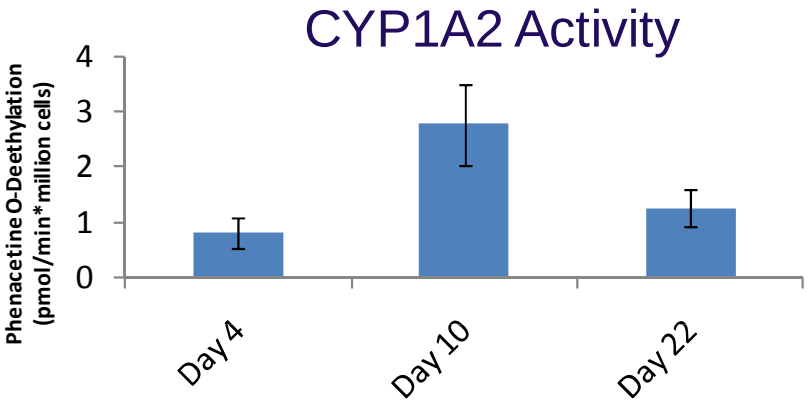
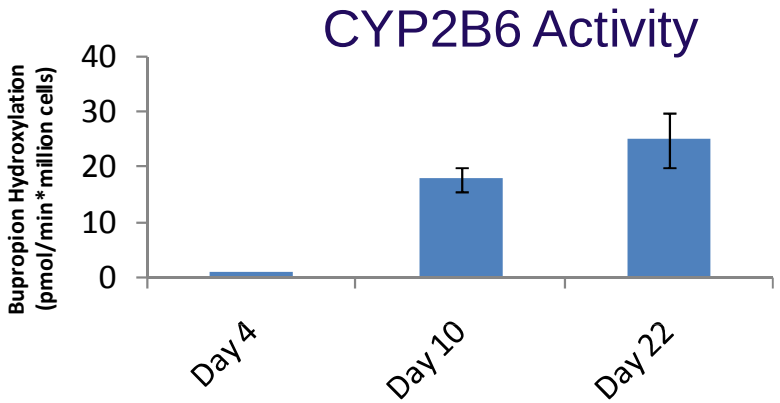
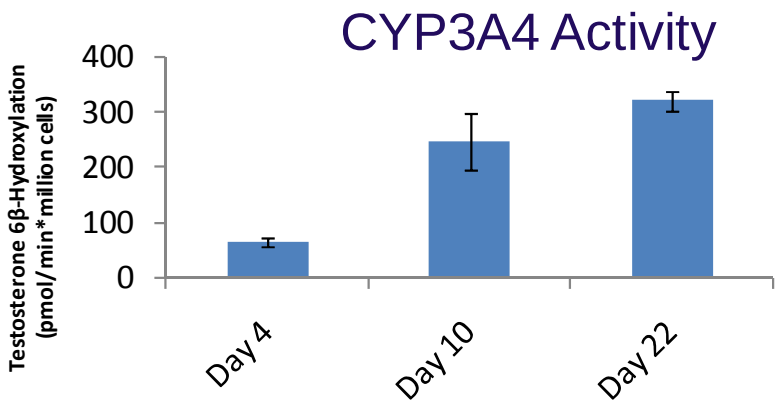
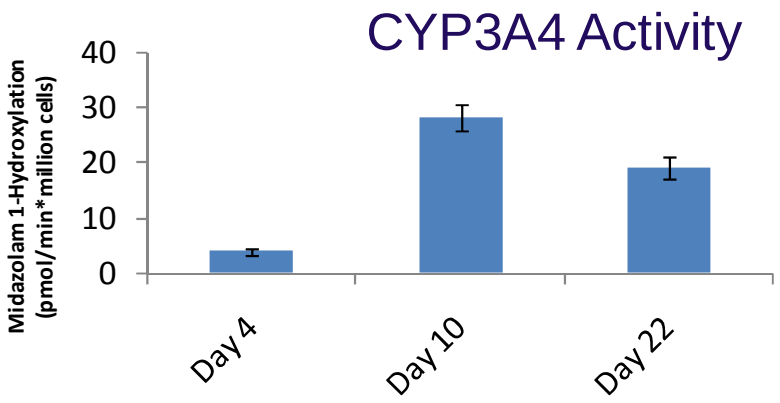
HepaRG™ Cells: Long-Term Metabolic Compentence

- **'Long-term' culture** ≥ 22 Days
- Metabolically active
- Attractive for chronic dosing strategies









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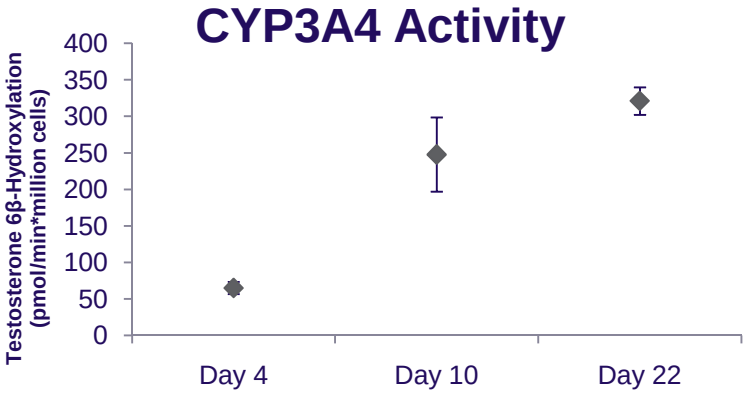
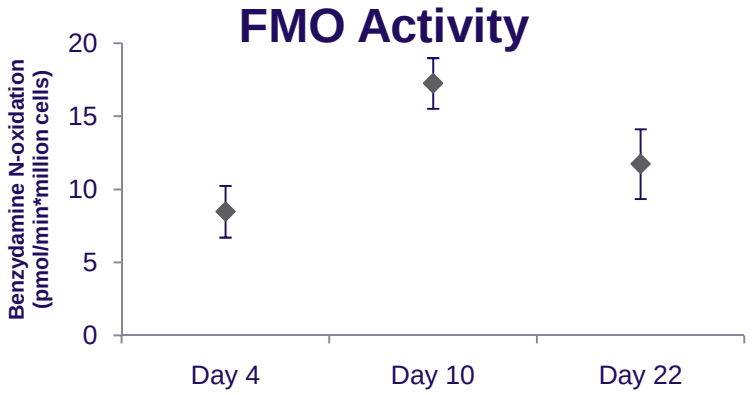
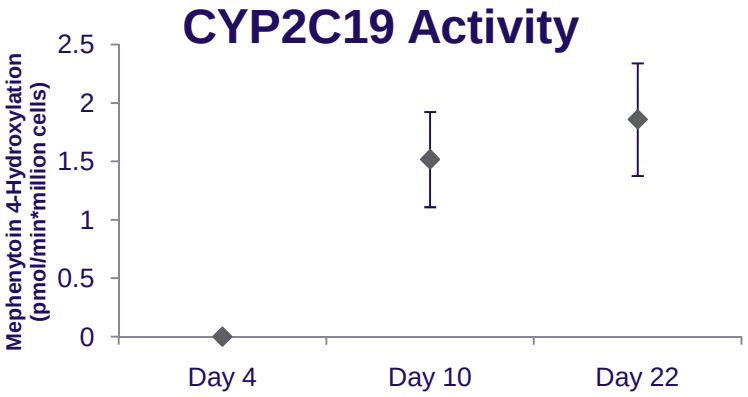
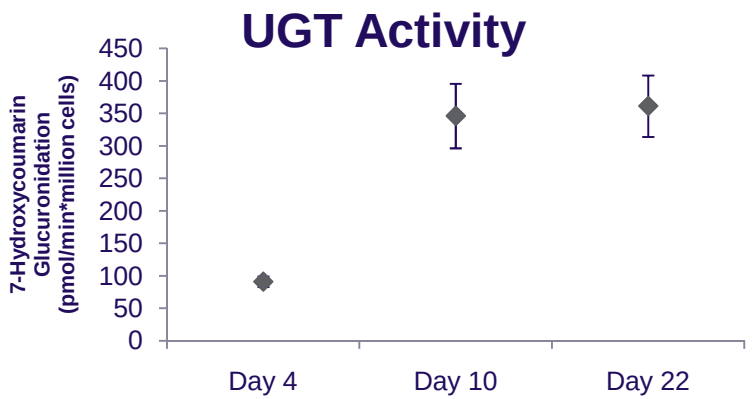
Metabolism in HepaRG™ Cells









Metabolism in HepaRG™ Cells

	Enzyme	Substrate	Marker Metabolite	Donor	Specific Activity (pmol/min*million cells)	Std Dev
Phase I DME	CYP1A2	Phenacetin	Acetaminophen	HepaRG PHH (N=52)	2.78 0.072-40.3	0.73
	CYP2A6	Coumarin	7-Hydroxycoumarin	HepaRG PHH (N=9)	0.85 0.12-9.87	0.16
	CYP2B6	Bupropion	Hydroxybupropion	HepaRG PHH (N=52)	17.9 0.21-13.1	2.21
	CYP2C8	Paclitaxel	6α-Hydroxypaclitaxel	HepaRG PHH (N=7)	0.22 0.064-0.24	0.04
	CYP2C9	Diclofenac	4′-Hydroxydiclofenac	HepaRG* PHH (N=3)	3.94 8.44-31.2	0.38
	CYP2C19	Mephenytoin	4′-Hydroxymephenytoin	HepaRG PHH (N=13)	1.52 0.10-23.5	0.41
	CYP2D6	Dextromethorphan	Dextrorphan	HepaRG** PHH (N=2)	0.40 3.93-14.0	0.06
	CYP3A4	Testosterone	6β-Hydroxytestosterone	HepaRG PHH (N=52)	248 1.47-178	50.9
	CYP3A4	Midazolam	1-Hydroxymidazolam	HepaRG PHH (N=0)	28.4 NA	2.39
	FMO	Benzydamine	Benzydamine N-oxide	HepaRG PHH (N=0)	17.3 NA	1.74
Phase II DME	UGT	7-Hydroxycoumarin	7-Hydroxycoumarin Glucuronide	HepaRG PHH (N=0)	346 NA	49.7
	SULT	7-Hydroxycoumarin	7-Hydroxycoumarin Sulfate	HepaRG PHH (N=0)	9.23 NA	2.92

* CYP2C9 Genotype in HepaRG = *2/*2
** CYP2D6 Genotype in HepaRG = *2/WT and *9/WT

HepaRG™ Cells were cultured for 10 days in HepaRG™ Metabolism Media. Drug metabolism enzymes were evaluated using in situ incubations with prototypical substrates.

PHH: Primary Human Hepatocytes; NA: Not Available

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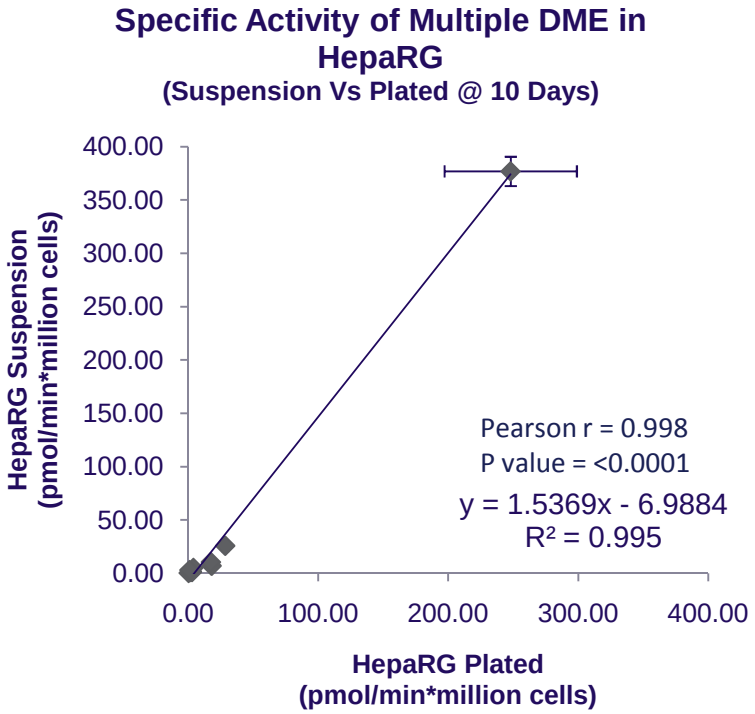
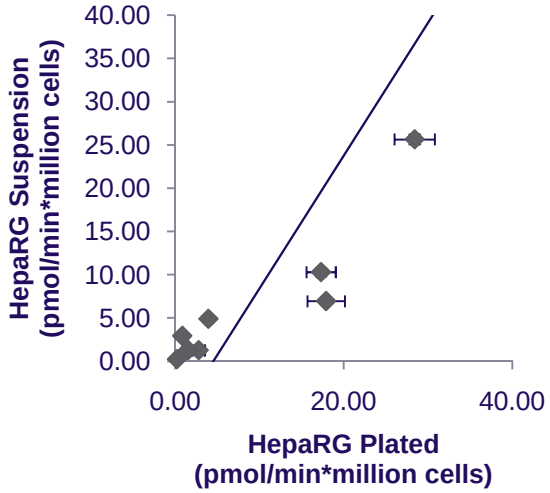
Metabolism in HepaRG™ Cells Suspension vs Plated

Marker Meta bolites	Plated	Suspension	Std Dev Plated	Std Dev Suspension
APAP	2.78	1.26	0.73	0.09
OHBP	17.90	6.95	2.21	0.30
MDZ	28.40	25.65	2.39	0.58
6BT	248.00	376.85	50.90	13.72
6OHTAX	0.22	0.23	0.04	0.02
4OHDC	3.94	4.89	0.38	0.38
4HMPN	1.52	1.27	0.41	0.04
FMO	17.30	10.30	1.74	0.50
7OHCMN	0.85	2.92	0.16	0.13





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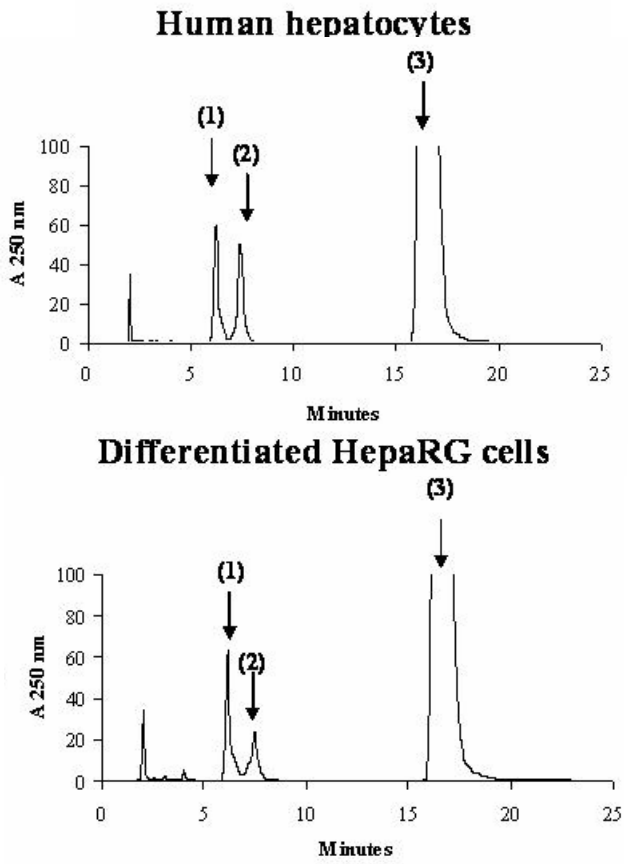
Metabolism in HepaRG™ Cells

- Metabolic Profile of APAP
  - > Similar in PHH & HepaRG
  - > Exhibits Phase II Enzyme Activity



(1) glucuronide acetaminophen, (2) sulfate acetaminophen and (3) acetaminophen.

Aninat C *et al*, DMD 2006 p.75-83

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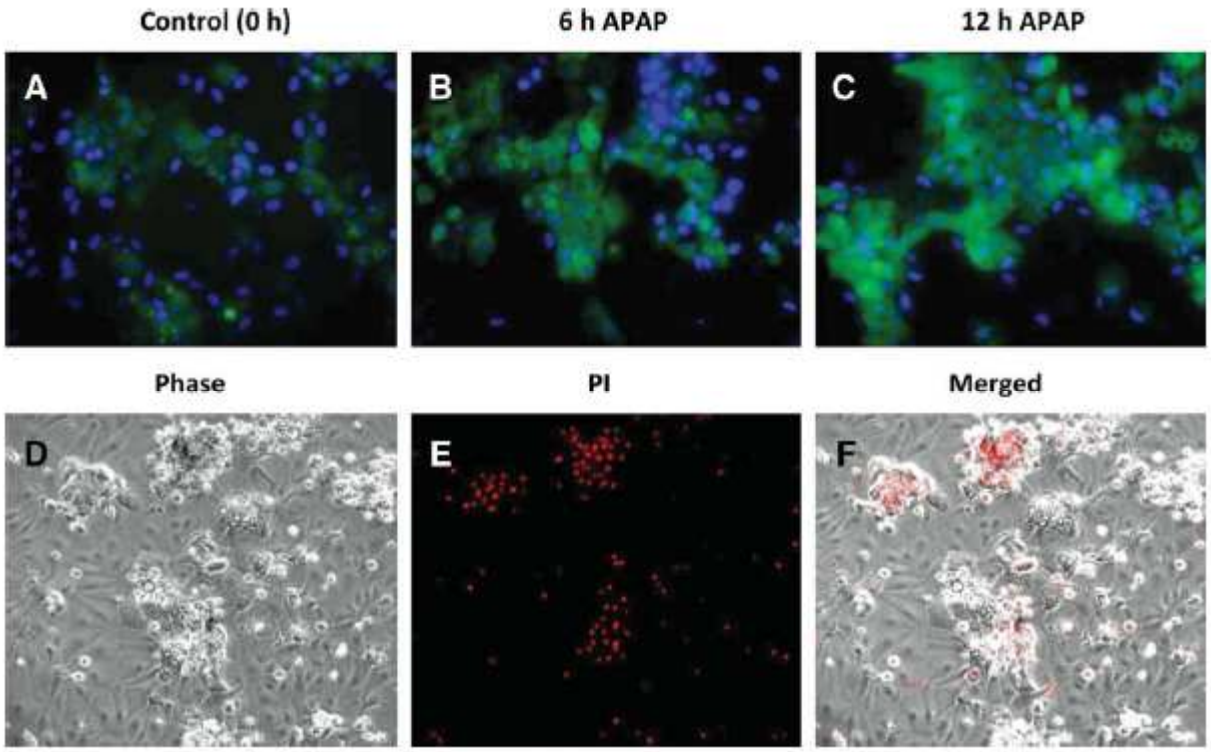
HepaRG™ Cells: Hepatotoxicity Model

HepaRG Oxidative Stress

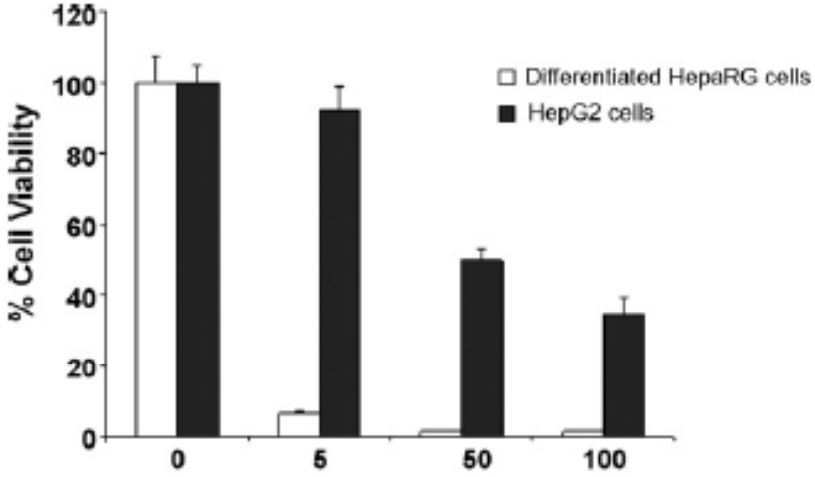
HepaRG Necrosis



M. McGill et al. Hepatology 53(3):974-982, 2011

HepaRG™ Cells: Aflatoxin B1 Hepatotoxicity

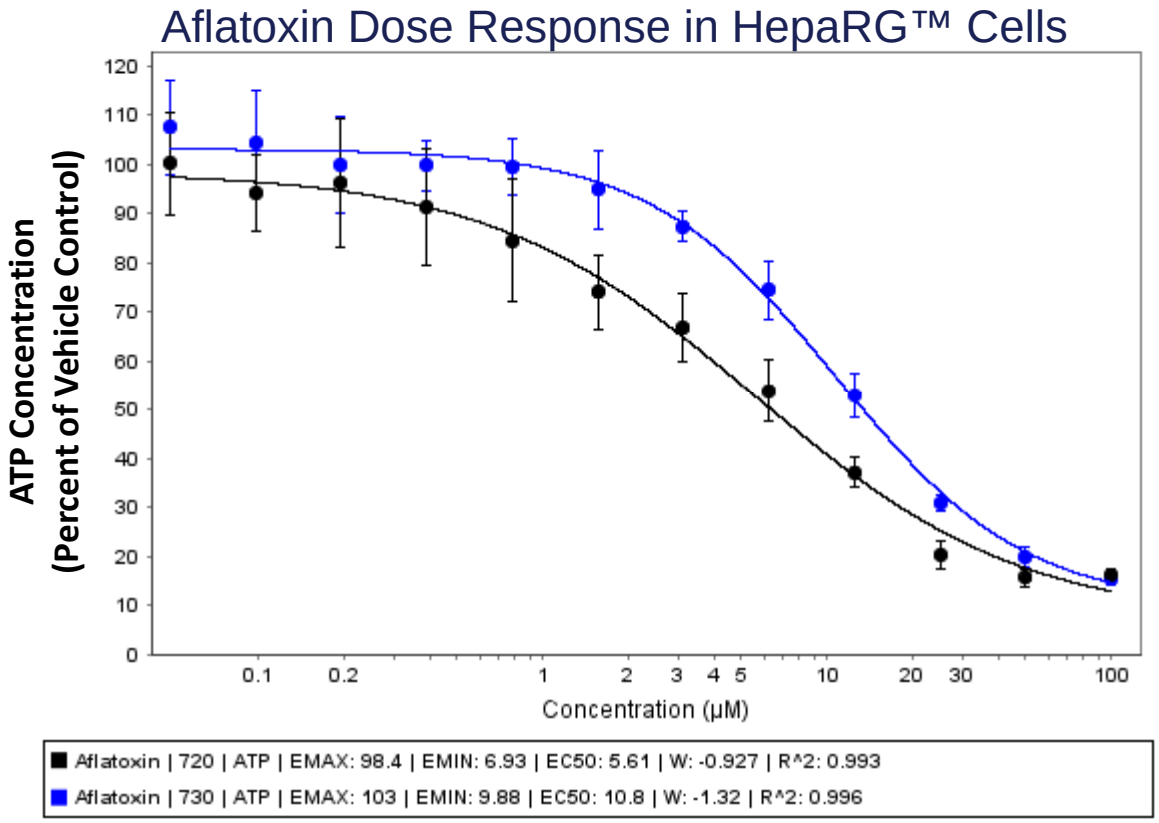
- HepG2
  - > Minimal DME Activity
- HepaRG™ Cells
  - > Metabolically competent
  - > Bioactivation of aflatoxin
- Metabolism Affects
  - > Efficacy & potency
  - > Greater in a metabolically competent system



A. Guillouzo et al. / Chemico-Biological Interactions 168 (2007) 66–73

HepaRG™ Cells: Hepatotoxicity Model

- HepaRG™ Cells
  - > Modulate P450 Activity
    - Media Supplements
  - > HPRG720
    - Supports High P450 Activity (Black Line)
  - > HPRG730
    - Supports Lower P450 Activity (Blue Line)
  - > Metabolism Affects
    - Efficacy & potency



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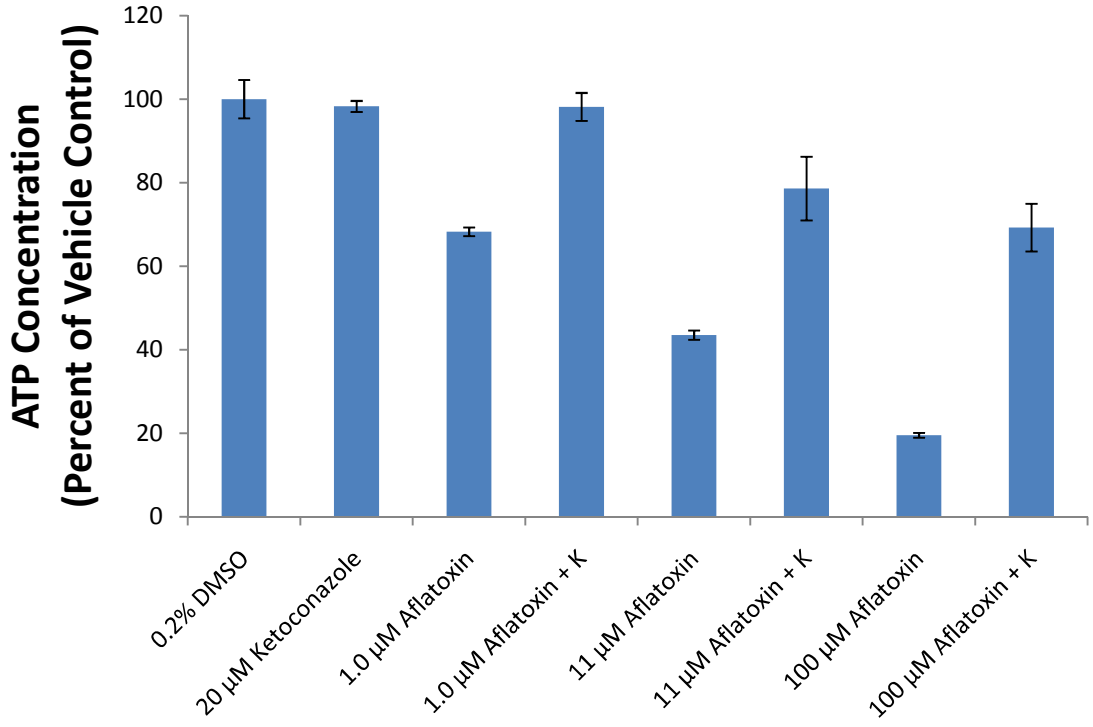
HepaRG™ Cells: Hepatotoxicity Model

- HepaRG™ Cells
  - > HPRG720
    - Supports High P450 Activity (Black Line)
  - > Inhibition of CYP3A4
    - Ketoconazole
    - Abolishes aflatoxin metabolism-dependent toxicity



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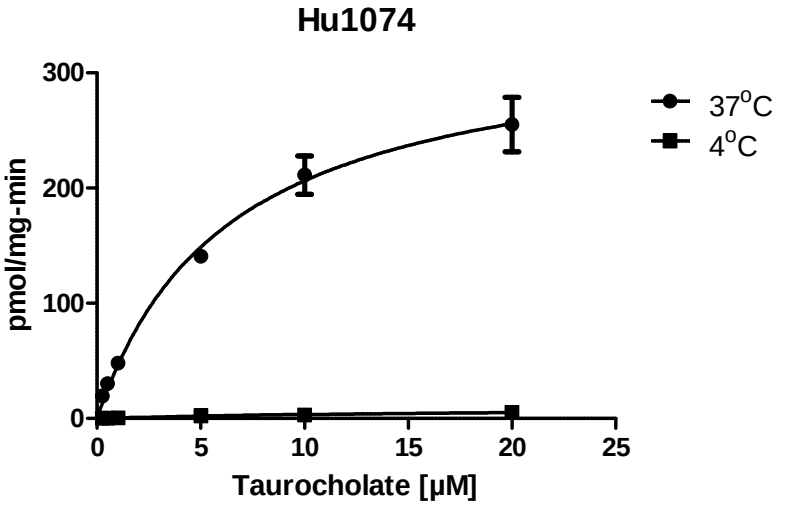
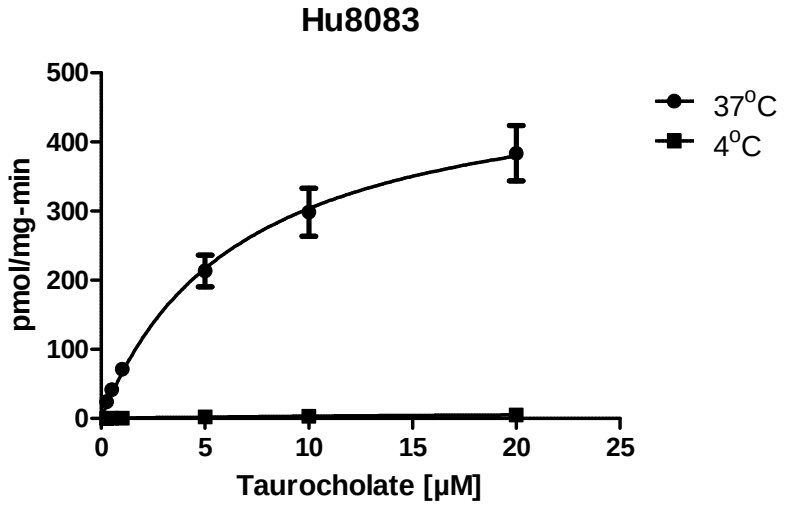
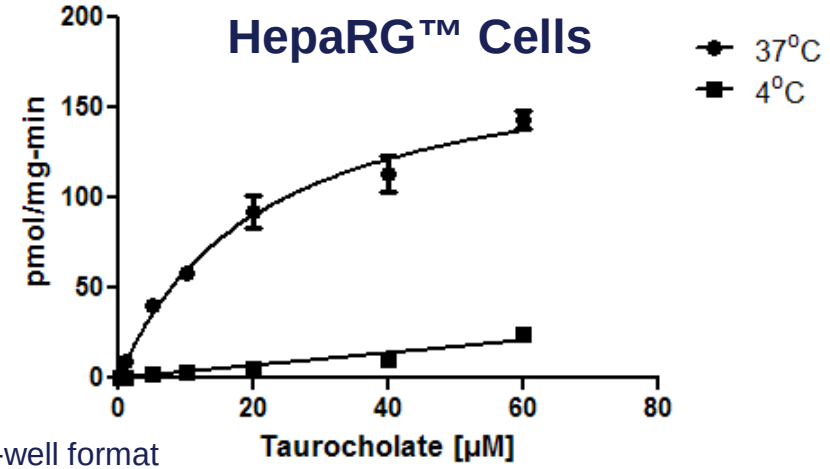
Plated Uptake in Cultures of Primary Hepatocytes & HepaRG™ Cells



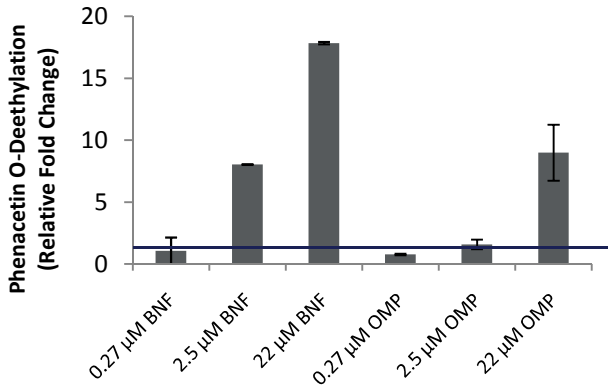


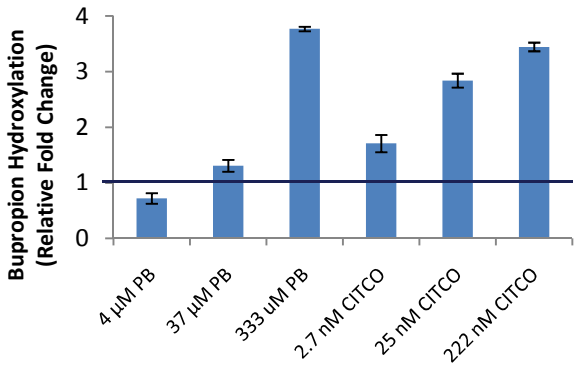
Model System	Km	SE	Vmax	SE
Hu8083-37°C	6.68	0.564	506.2	16.65
Hu1074-37°C	6.32	0.799	336.6	16.20
HepaRG-37°C	**21.09**	**3.084**	**185.6**	**11.05**
Hu8083-4°C	16.45	5.039	9.279	1.566
Hu1074-4°C	17.49	5.347	9.627	1.656
HepaRG-4°C	**NA**	**NA**	**NA**	**NA**

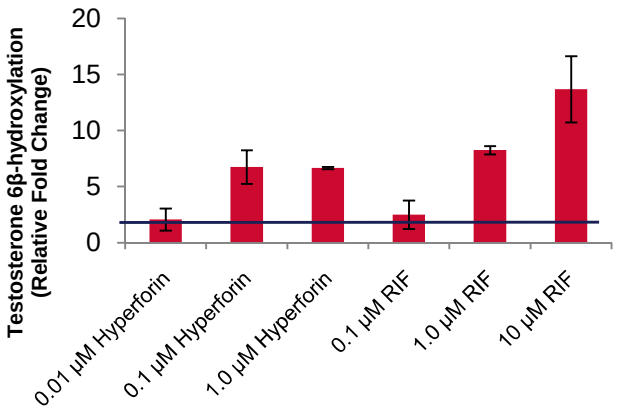


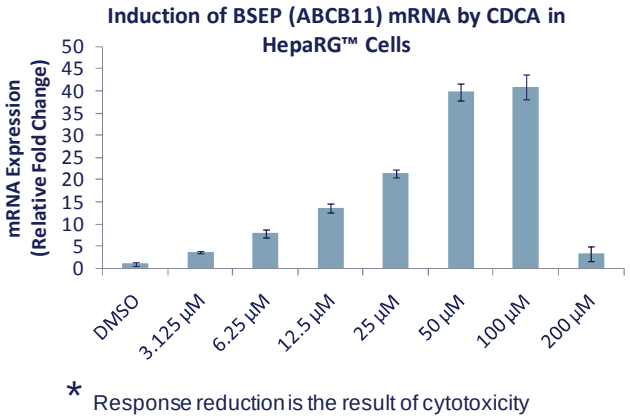
- Uptake in PHH determined 24 hrs after plating in 24 well format
- Uptake in HepaRG™ Cells determined 10 days after plating in 96-well format

HepaRG™ Cells: Functional Xenobiotic Activated Signaling Pathways









* Response reduction is the result of cytotoxicity

CYP1A
Xenobiotic metabolism

PAHs, Xenobiotics, Steroids
(Prototypical Inducer: OMP)

CYP2B
Xenobiotic, Steroid metabolism

PB, Steroids, Xenobiotics
(Prototypical Inducer: PB)

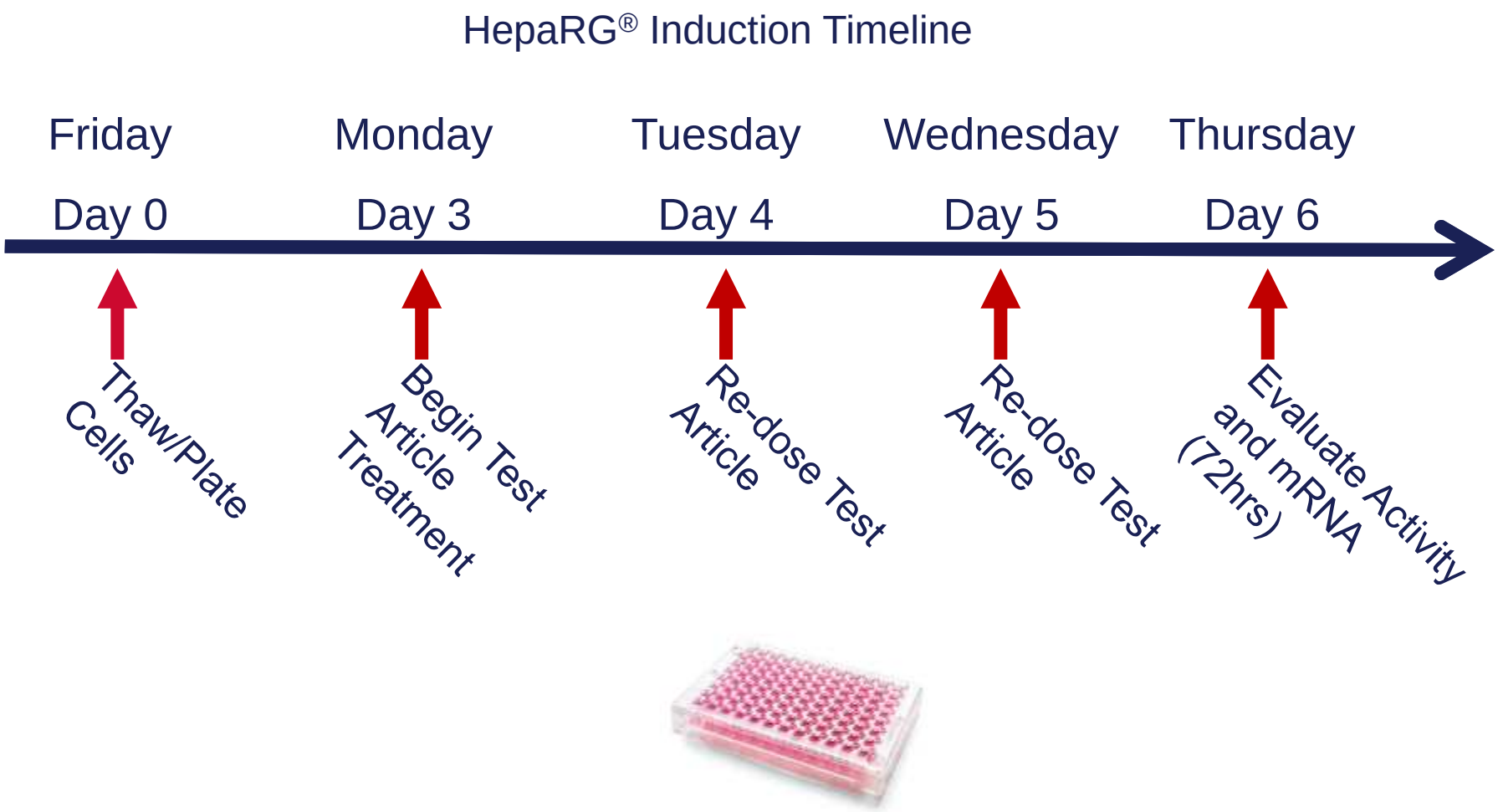
CYP3A
Xenobiotic, Steroid metabolism

Bile Acids, Steroids, Xenobiotics
(Prototypical Inducer: RIF)

BSEP/ABCB11
Efflux Transporter Bile Salts

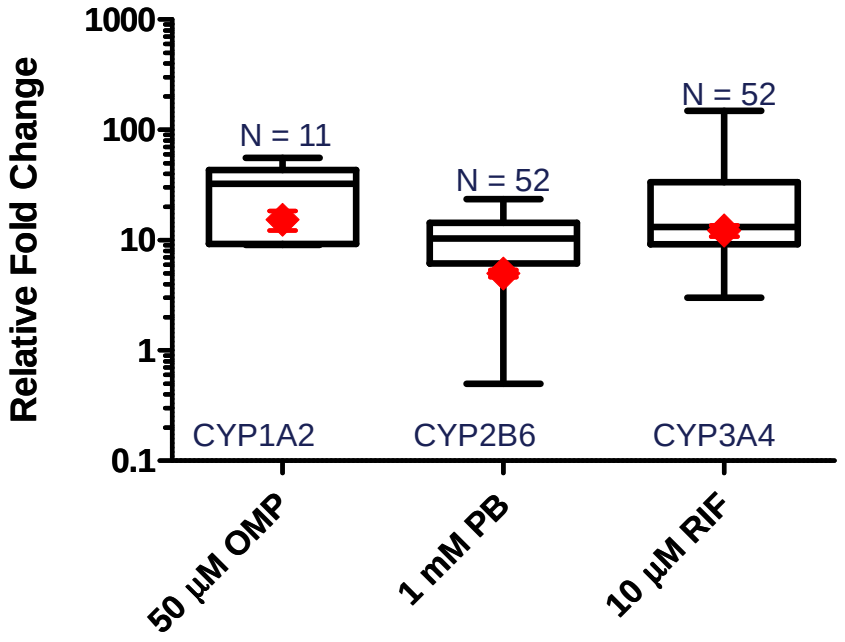
Bile Acids, Steroids, Xenobiotics
(Prototypical Inducer: RIF)

HepaRG®: Easy to Use



HepaRG™ Cells: P450 Activity Induction Responses

- Large Variation in PHH Responses
  - > Box and whisker plots = PHH
    - OMP: N of 11
    - PB & RIF: N of 52
- HepaRG™ Cells
  - > Single donor source
    - Low variation
- All Cells Treated 72 hrs
  - > In situ incubations
  - > Activities compared to Vehicle
- **Comparable Induction Responses**
  - > HepaRG™ Cells vs. PHH
  - > Qualifies as an acceptable donor
    - FDA DDI In vitro Drug Induction Study per 2007 FDA Guidance



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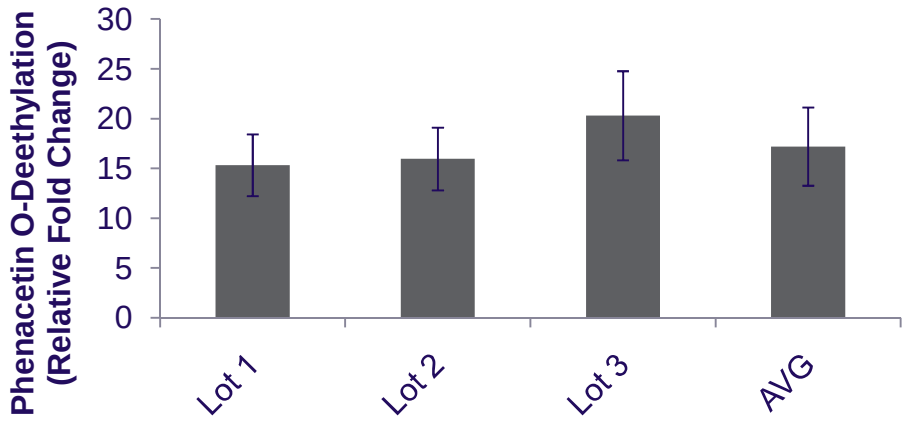
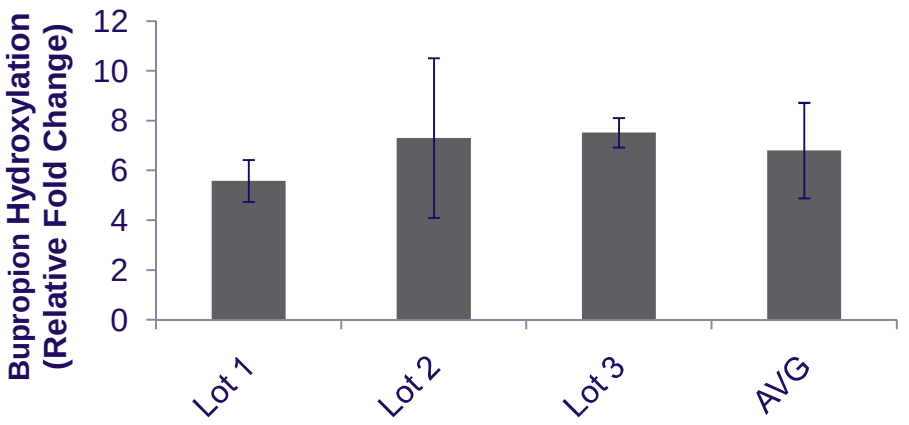
HepaRG™ Cells: Induction Reproducibility

Inter-Lot Variation

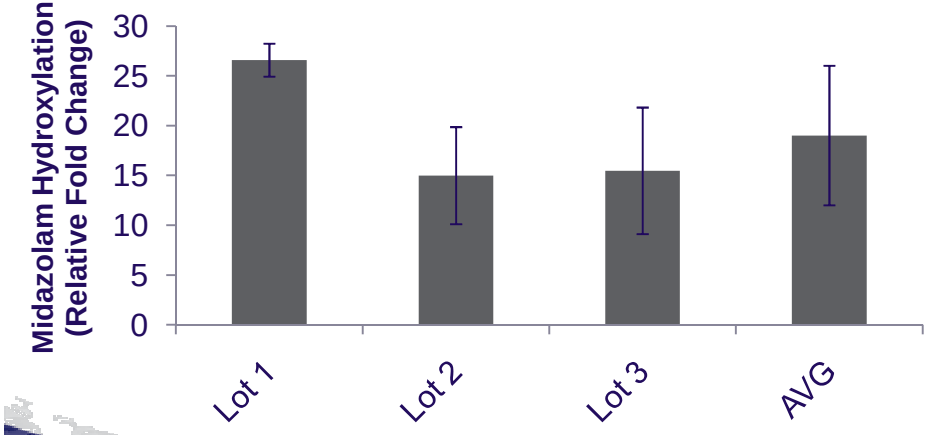
CYP1A2 Activity (3 Independent Plates Per Lot)



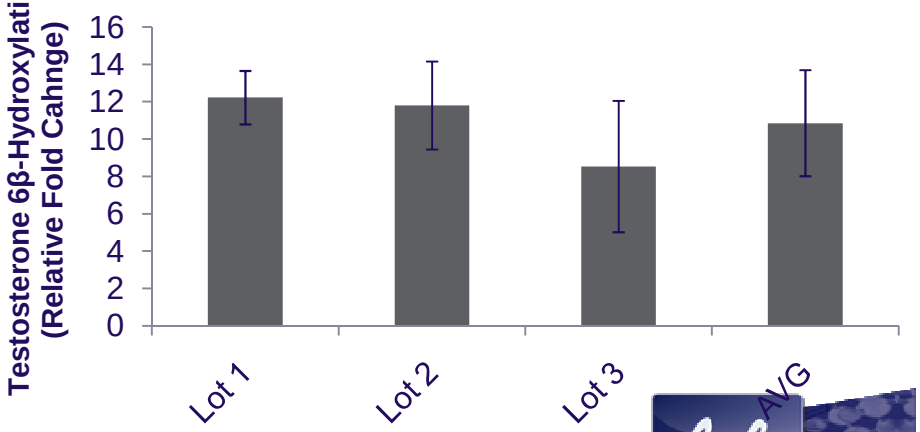
CYP2B6 Activity (3 Independent Plates Per Lot)



CYP3A4 Activity (3 Independent Plates Per Lot)

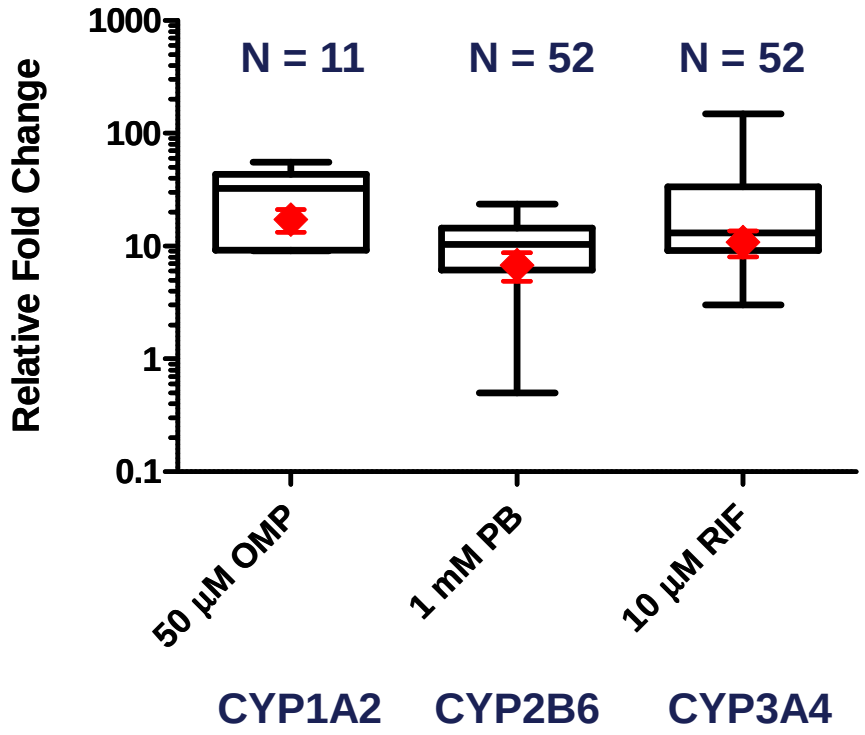


CYP3A4 Activity (3 Independent Plates Per Lot)



HepaRG™ Cells: Induction Reproducibility

Inter-Lot Variation



Three Independent Lots of HepaRG™
3 Independent Plates Per Lot

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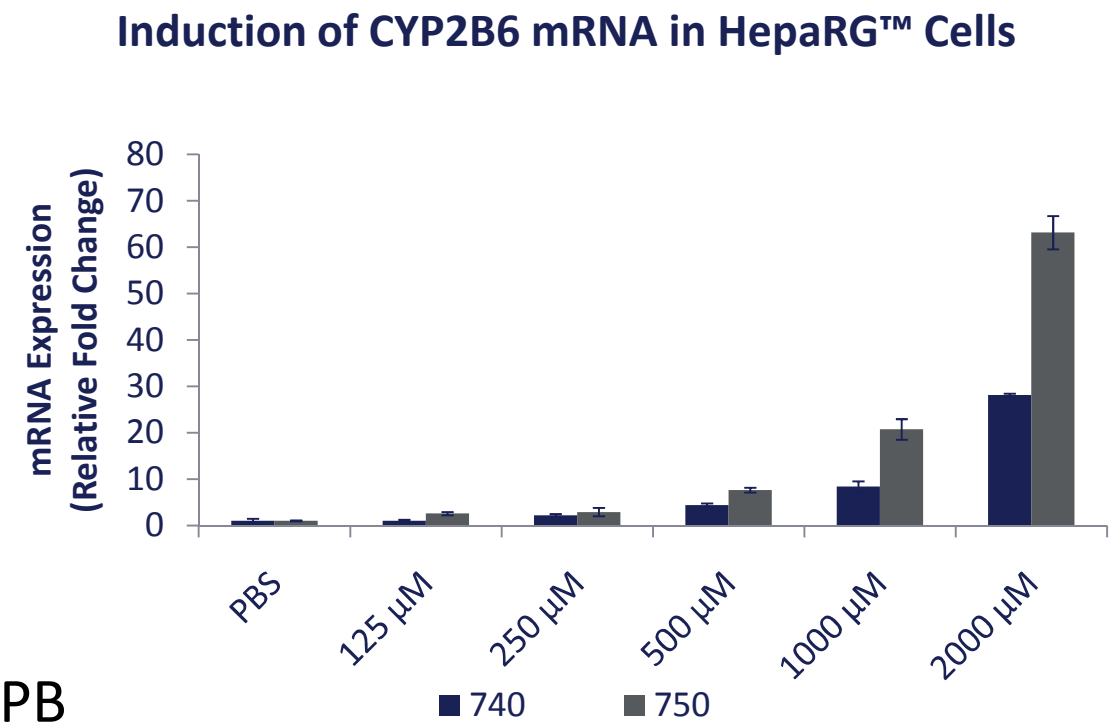
Induction Media: Serum or Serum-Free

- Media Supplements
  - Base Media
    - > WEM
    - > GlutaMAX™
  - HPRG740
    - > Serum
  - HPRG750
    - > Serum-free
- 72 hrs treatment with PB



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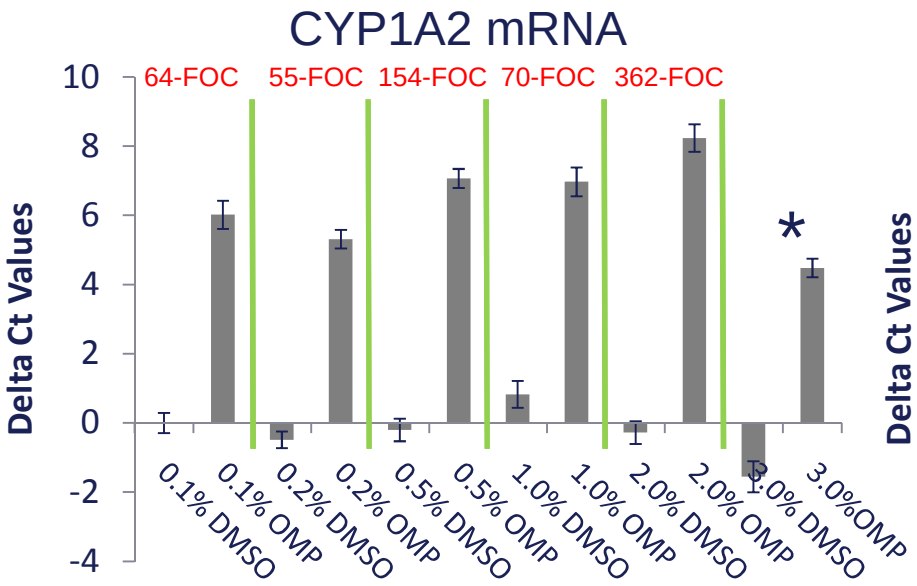
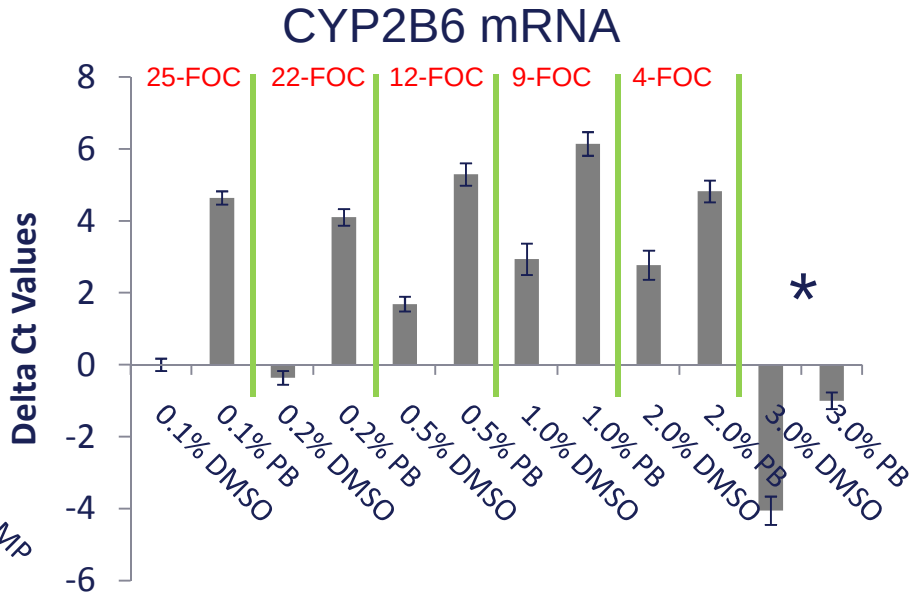
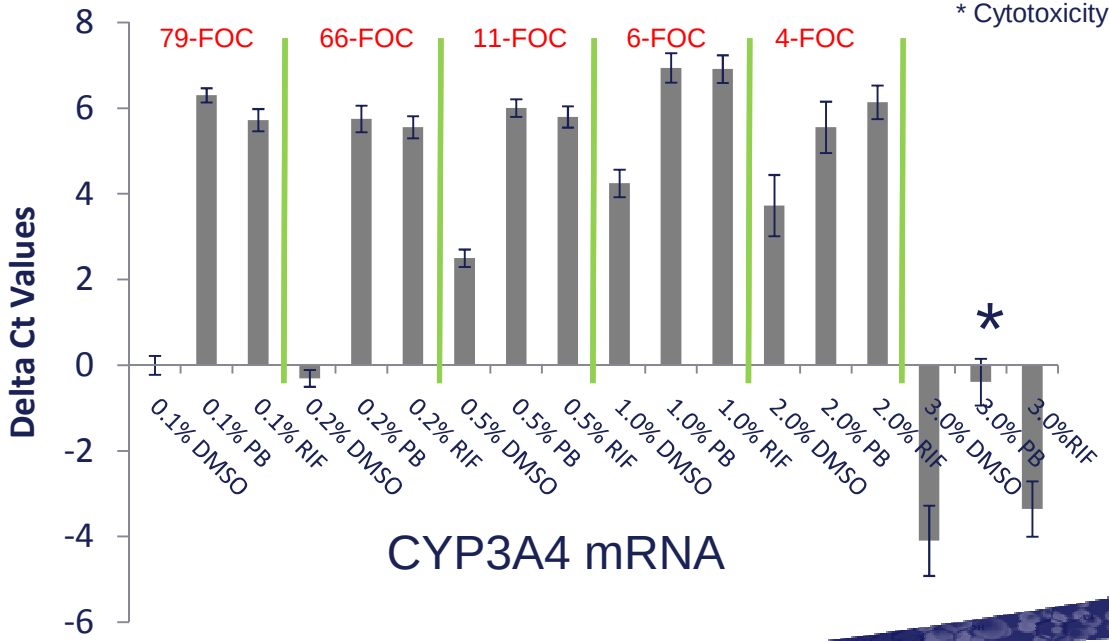
DMSO Tolerance: What Amount is Too Much

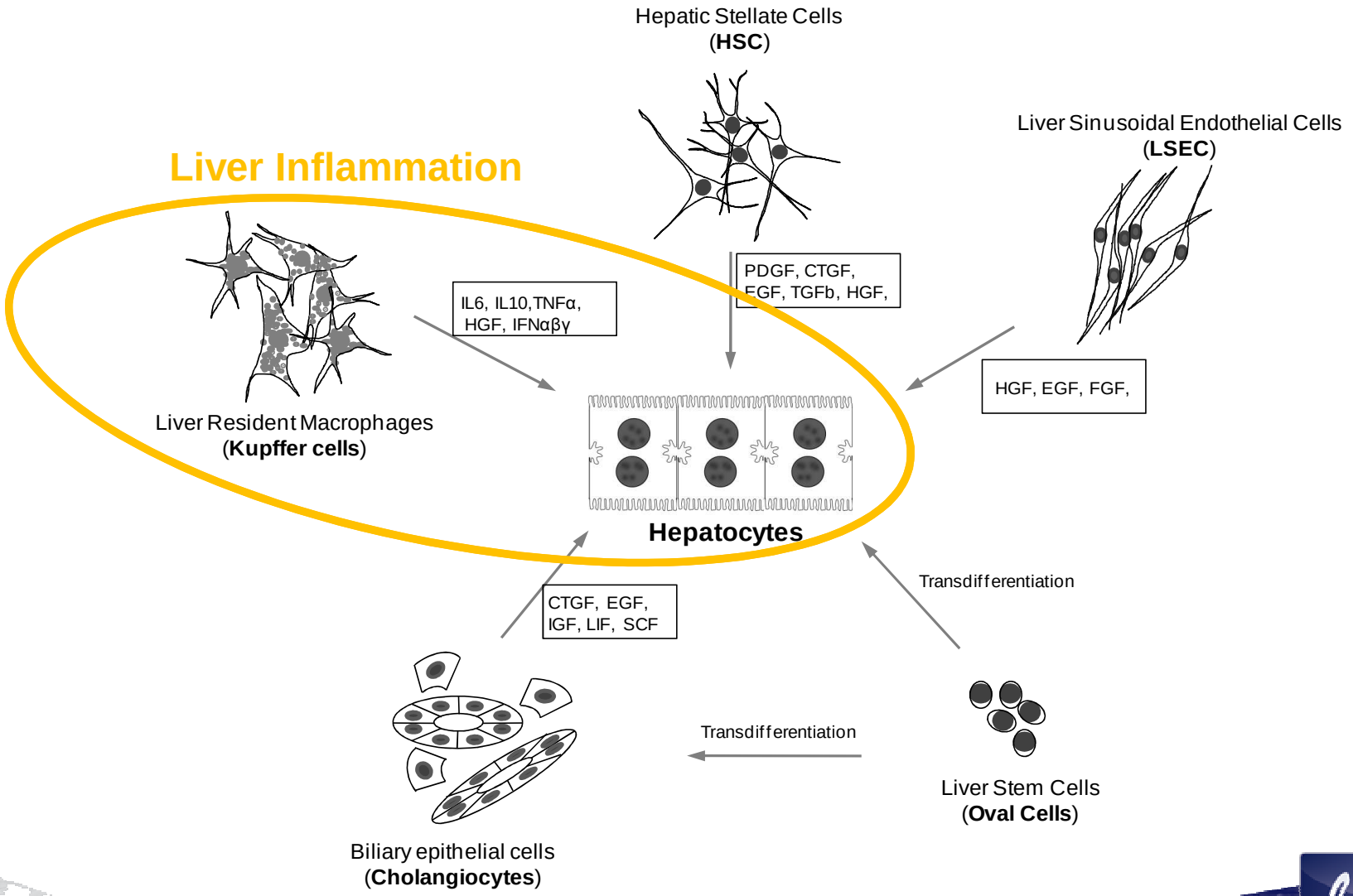




* Cytotoxicity observed in cultures

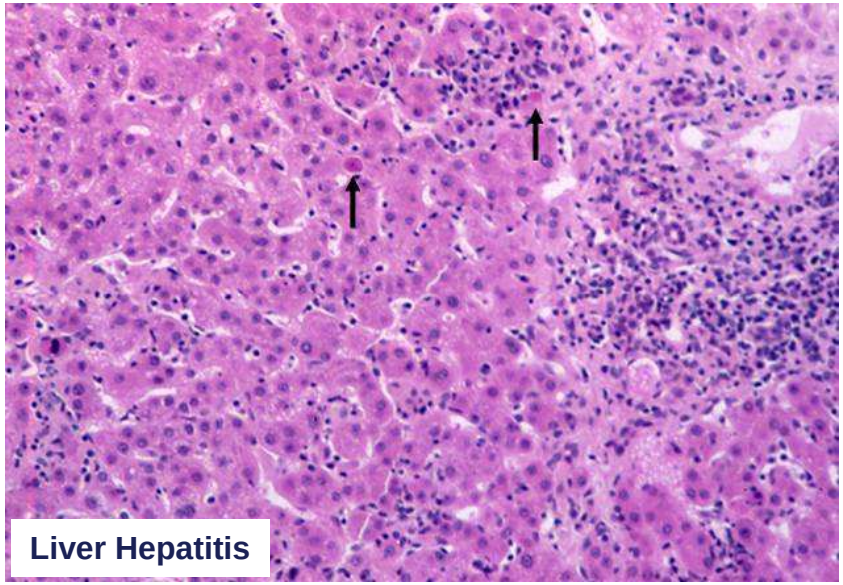


Non-parenchymal Cells (NPC)

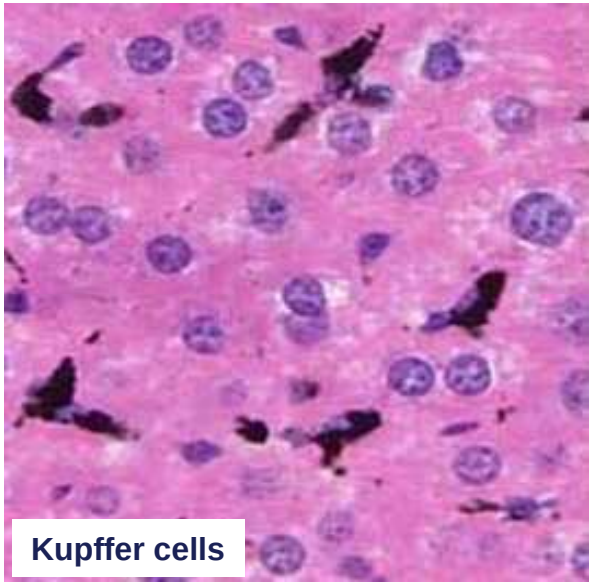


Liver Diseases with progressive Inflammation

- **Hepatitis** – viral HBV and HCV, autoimmune,
- **Alcoholic and Non-alcoholic fatty liver disease (NAFLD)** – may lead to steatohepatitis and cirrhosis
- **Primary biliary cirrhosis (PBC)** or **Primary sclerosing cholangitis (PSC)**



Liver Hepatitis



Kupffer cells

Obtained form Gastrointestinal Pathology for Medical II Students, Online Article

Most hepatic modeling is with 'healthy' cells, and clinical trials done in 'healthy' patients treated with a single drug. Diseased patients in the general population may be at risk to idiosyncratic toxicity due to lack of disease model focus (e.g. diminished clearance capacity, potential 'overdose')

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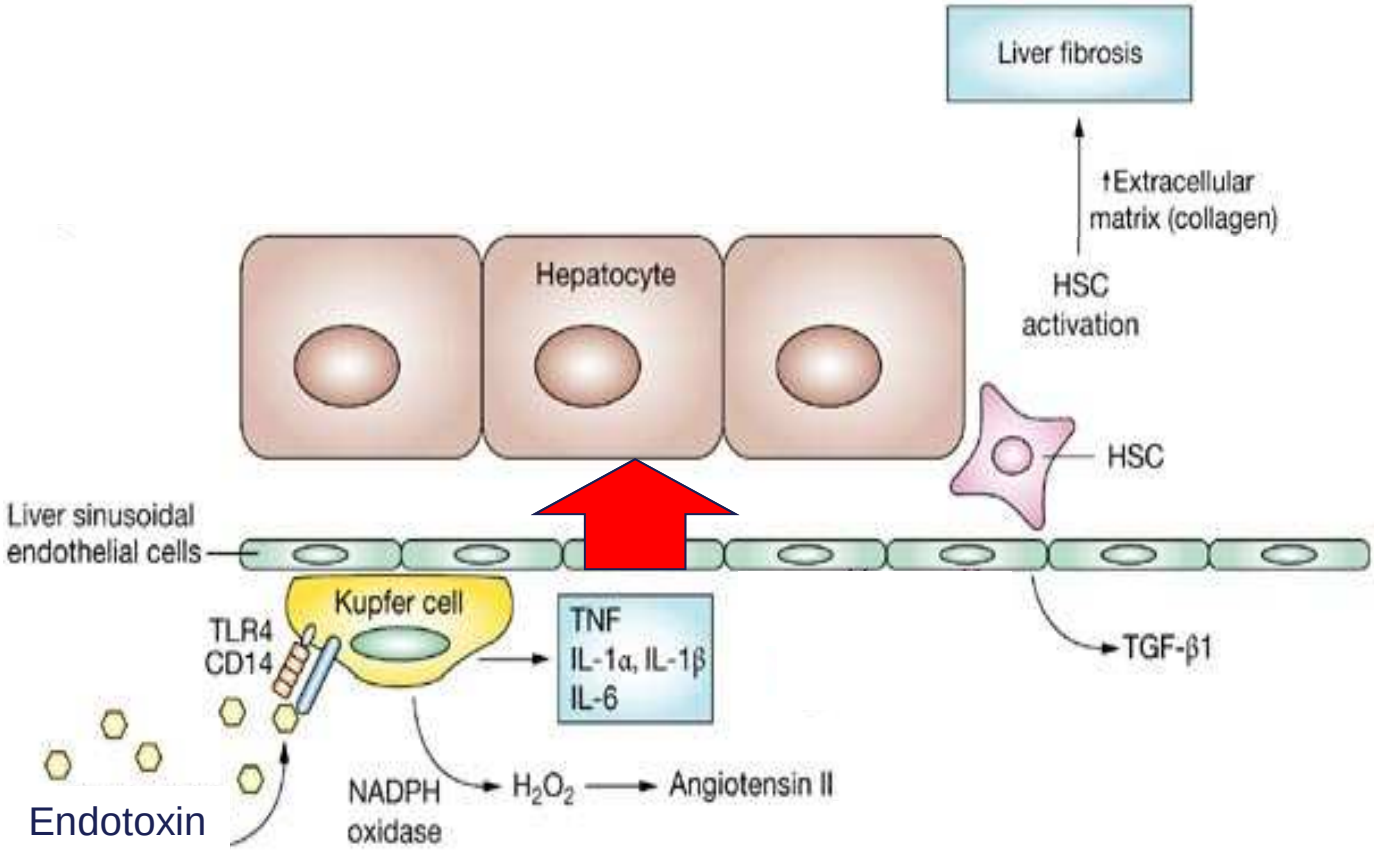
Liver Disease & Inflammation



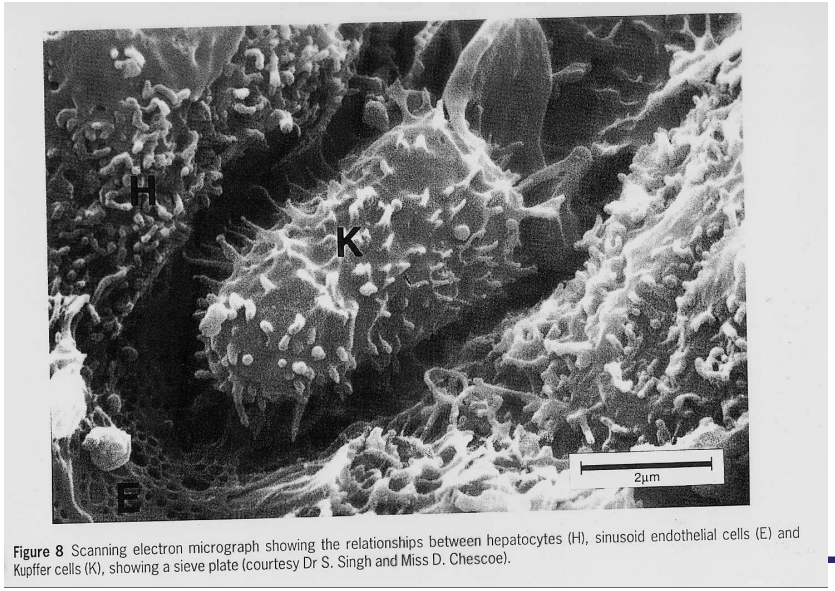


Figure 8 Scanning electron micrograph showing the relationships between hepatocytes (H), sinusoid endothelial cells (E) and Kupffer cells (K), showing a sieve plate (courtesy Dr S. Singh and Miss D. Chescoe).

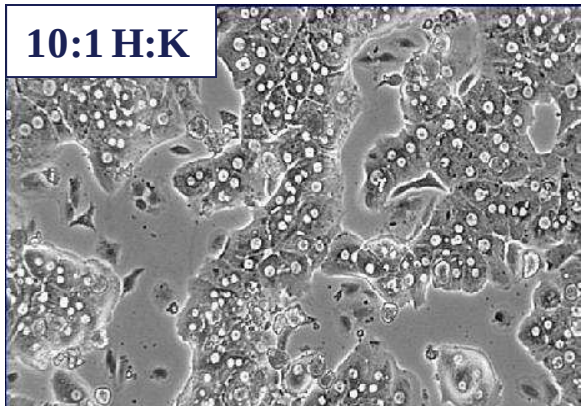
Effect of Inflammatory cytokines on P450 activities

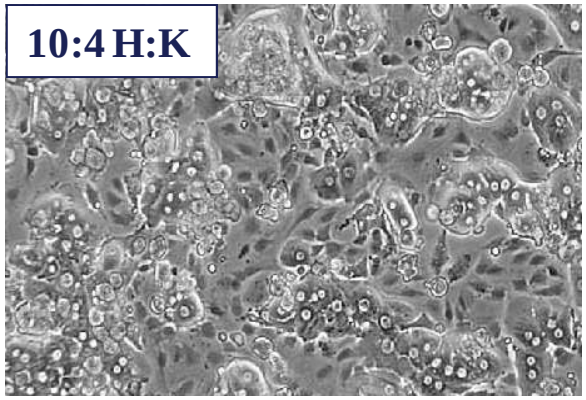
Table 1 List of CYP enzymes with altered activities (decreased, unless noted[a,b]) in the presence of specific cytokines, cytokine modulators, and human growth hormone, based on *in vitro* and/or *in vivo* studies in humans

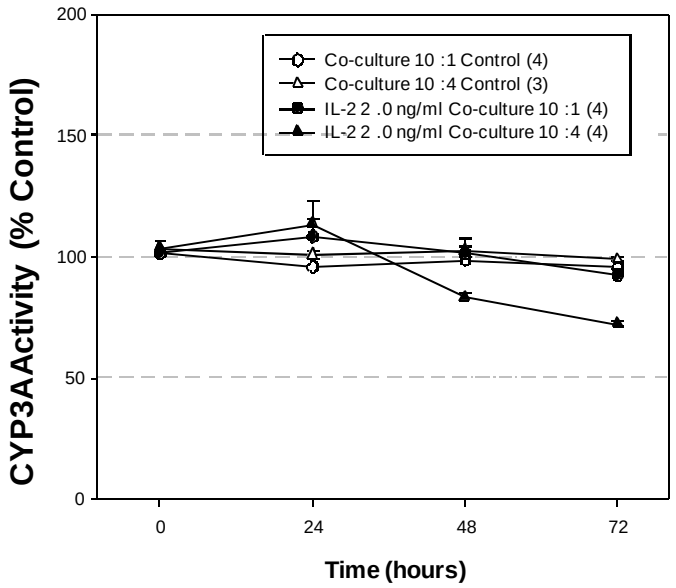
CYP enzyme	Cytokines/cytokine modulators
CYP1A2	IFN-α, IFNα-2b, IFN-β, IL-2, IL-6, hGH[a]
CYP2C8	IL-1
CYP2C9	IL-2, IL-10
CYP2C19	Tocilizumab[b], IFNα-2b, FN-β, IL-2, TNF-α, IL-6, hGH
CYP2D6	IFNα-2b
CYP2E1	IL-2, IFNα-2b
CYP3A	Basiliximab, muromonab-CD3, tocilizumab[b], IL-1, IL-2, IL-6, IL-10

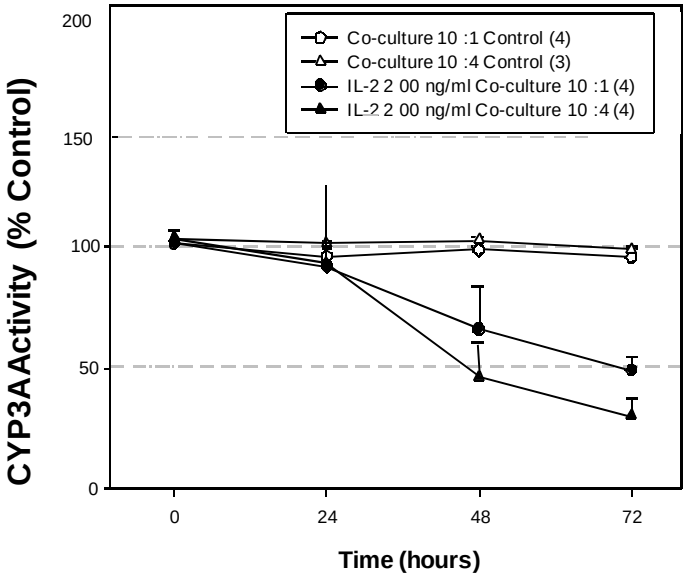
Huang SM, Zhao H, Lee JI, Reynolds K, Zhang L, Temple R, Lesko LJ. **Therapeutic protein-drug interactions and implications for drug development**. Clin Pharmacol Ther. 2010 Apr;87(4):497-503. Epub 2010 Mar 3.

Human Hepatocytes Co-cultured with Kupffer Cells: Effect on Liver Enzyme Expression









Sunman et al., DMD 32(3):359-63, 2004

Co-cultures: Direct contact vs. Transwell approach

Hoebe KH, Witkamp RF, Fink-Gremmels J, Van Miert AS, Monshouwer M.
Direct cell-to-cell contact between Kupffer cells and hepatocytes augments endotoxin-induced hepatic injury.
Am J Physiol Gastrointest Liver Physiol. 2001 Apr;280(4):G720-8.

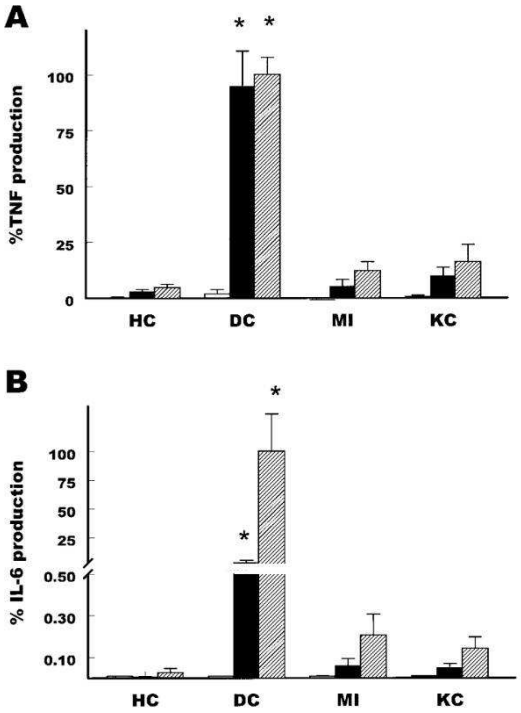


Table 1. *Effect of LPS on biotransformation capacity in HC cultures, DC cocultures, and MI cocultures*

	LPS, μg/ml	Testosterone 6β-OH	Testosterone 2β-OH	1-NG
HC culture	control	100 ± 10	100 ± 6	100 ± 10
	1	86 ± 8*	87 ± 8*	37 ± 19*
	10	73 ± 7*	73 ± 12*	34 ± 20*
DC coculture	control	59 ± 6†	62 ± 5†	45 ± 10†
	1	38 ± 8*	41 ± 15*	8 ± 2*
	10	22 ± 5*	27 ± 9*	2 ± 0.7*
MI coculture	control	80 ± 12†	85 ± 12†	65 ± 13†
	1	65 ± 15	71 ± 17	36 ± 8*
	10	57 ± 11*	65 ± 13*	27 ± 3*

HC – Hepatocyte cultures **DC** – Direct contact co-cultures **MI** – Transwell co-cultures

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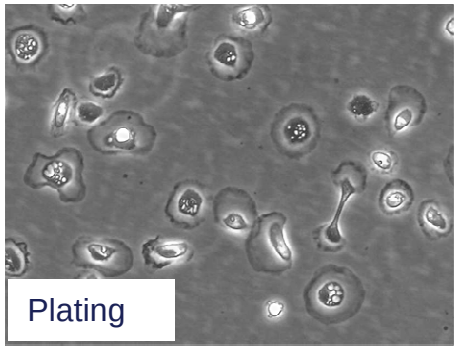
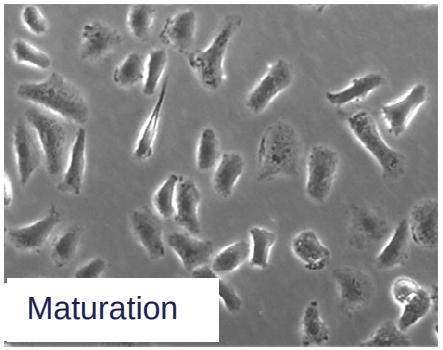
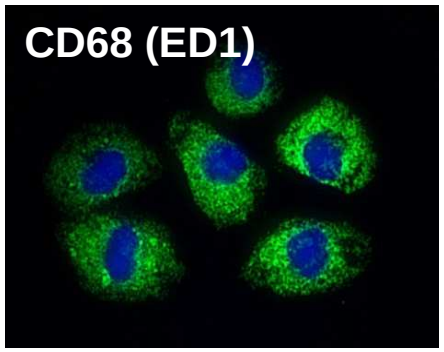
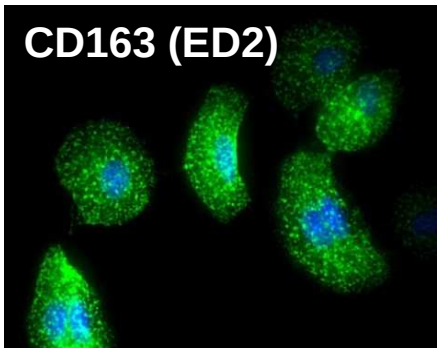
Cryopreserved Kupffer Cells from Life Technologies









New Product Availability: Rat Cryopreserved Kupffer cells (**now**), Human Cryopreserved Kupffer cells (**custom product currently, launch expected mid-2012**)

Potential Applications: Inflammatory Co-cultures for toxicology screening, and cells for basic and clinical research

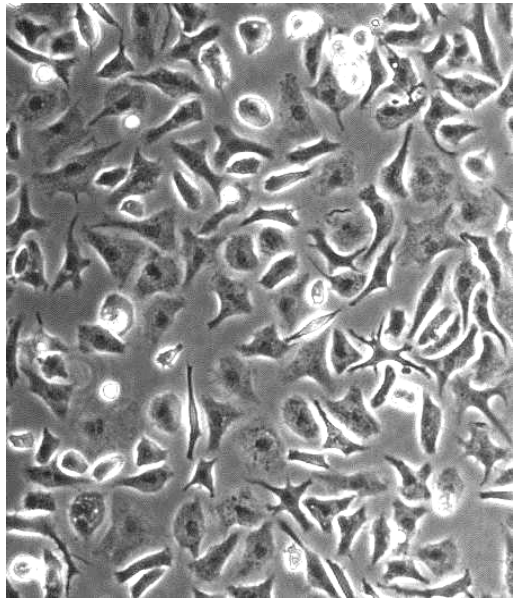
Monocultures

- One cell type
- No need for self assembly
- Does not mimic *in vivo* interactions
- Can produce false results in toxicology screening since in the liver, multiple cell types are involved

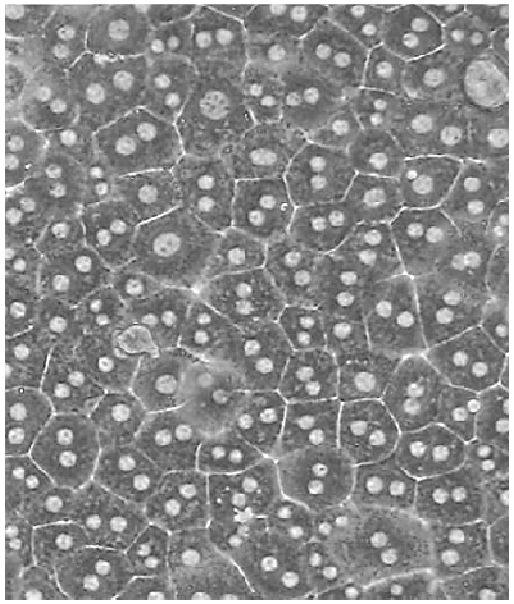
Vs.

Co-cultures

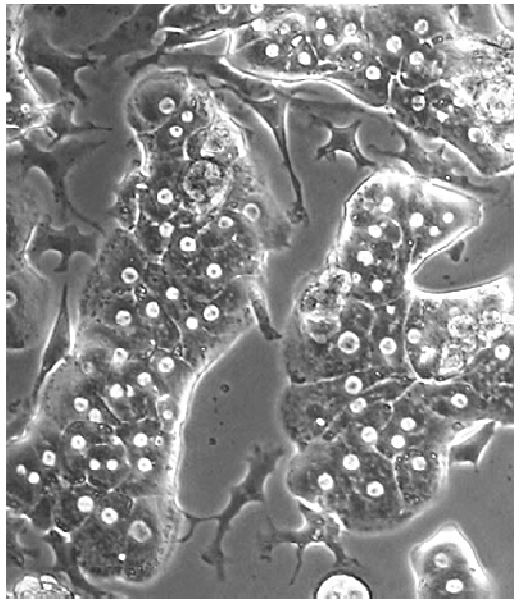
- Multiple cell types
- Require self assembly to function
- Mimic *in vivo* interactions
- More appropriate for toxicology and drug screening since they can be prepared to mimic liver disease state



Monoculture:
Kupffer cells



Monoculture:
Hepatocytes



Co-Culture:
Kupffer cells and Hepatocytes

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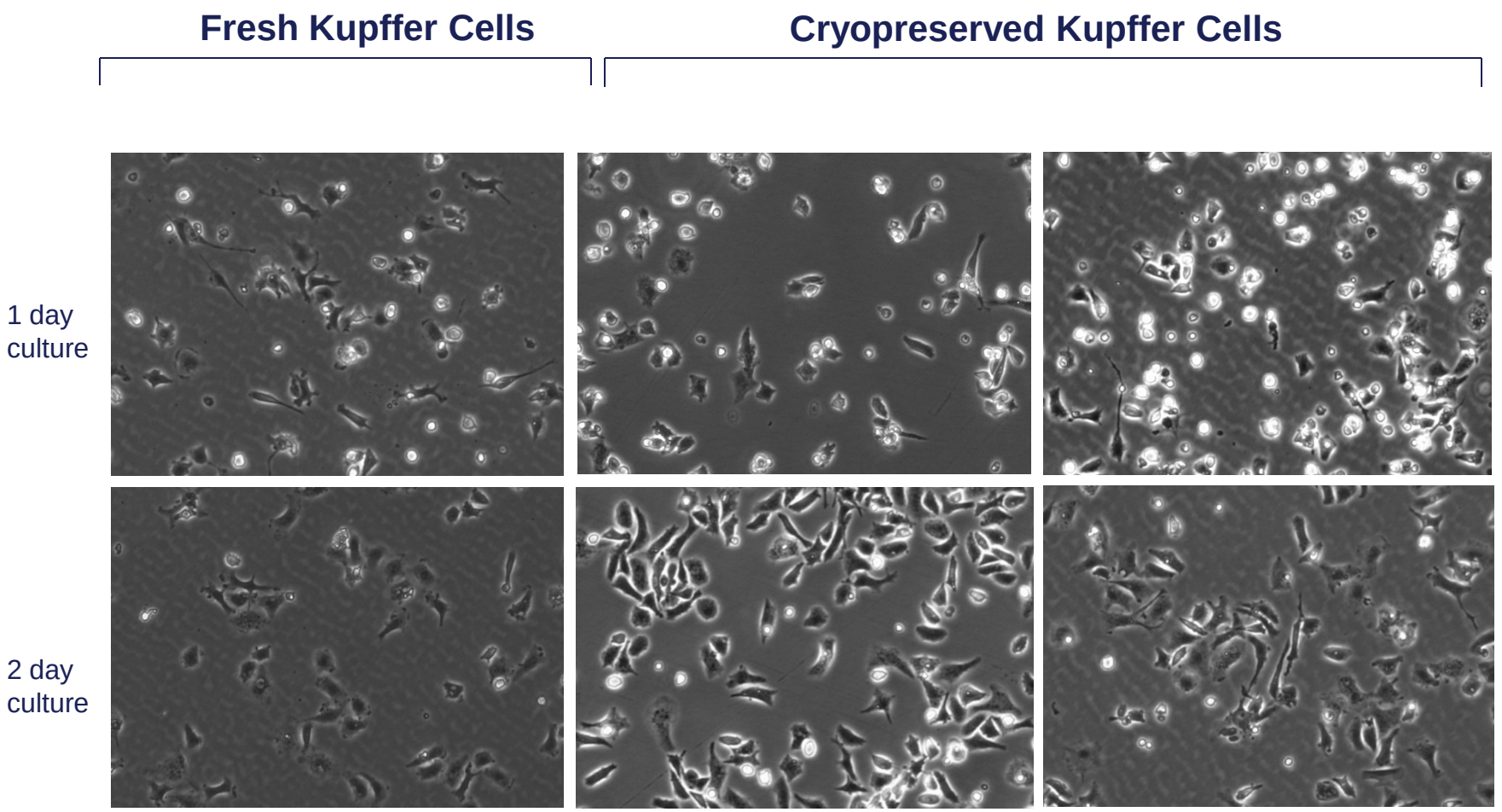
Kupffer cells: Fresh vs. Cryo-preserved – Morphology



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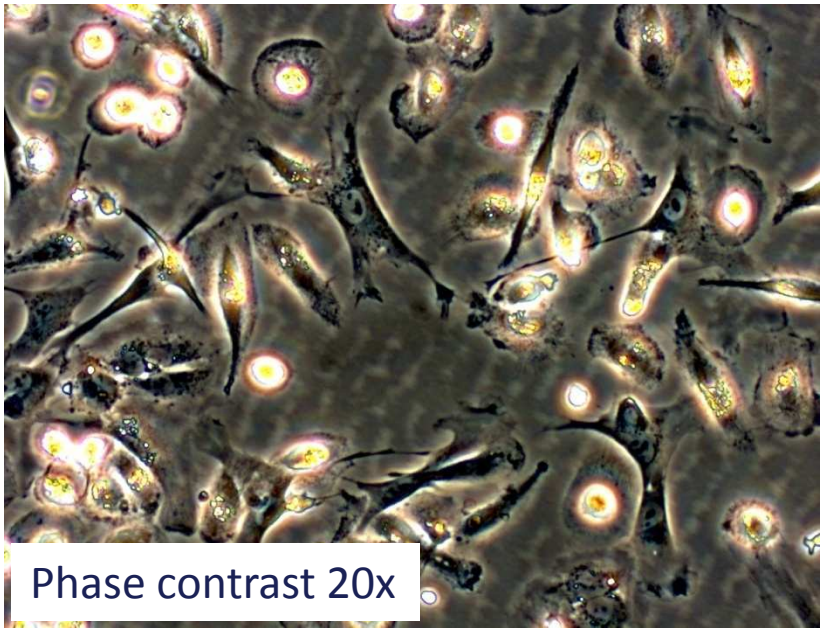
Kupffer cells: Fresh vs. Cryo-preserved – Cytokine Expression

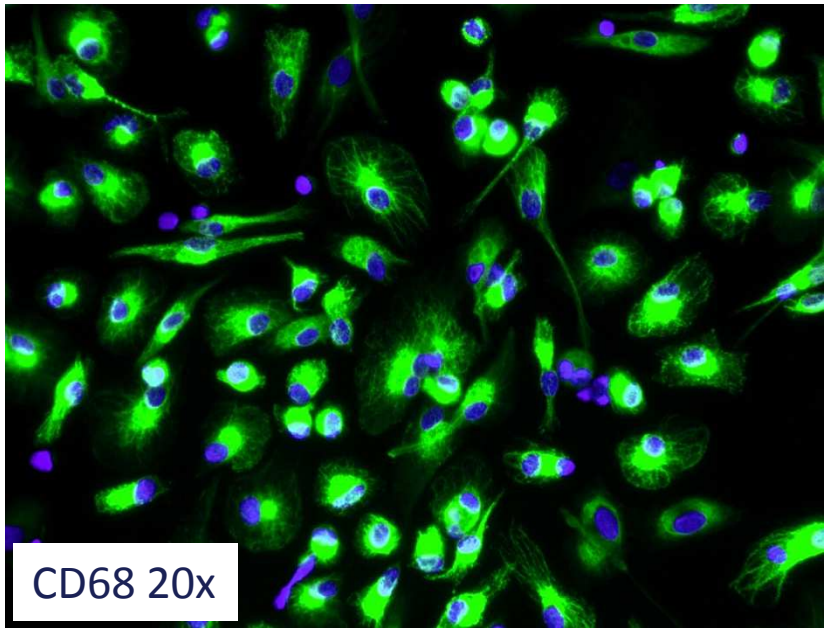
	IL1a	IL1b	IL2	IL4	IL6	IL10	IL12	IL13	IFNy	TNFa	GM-CSF	RANTES (CCL5)
Fresh Kupffer cells	+	+++	-	-	+++	+	-	-	-	+++	-	++
Cryo Kupffer cells	+	+++	-	-	+++	+	-	-	-	+++	-	++

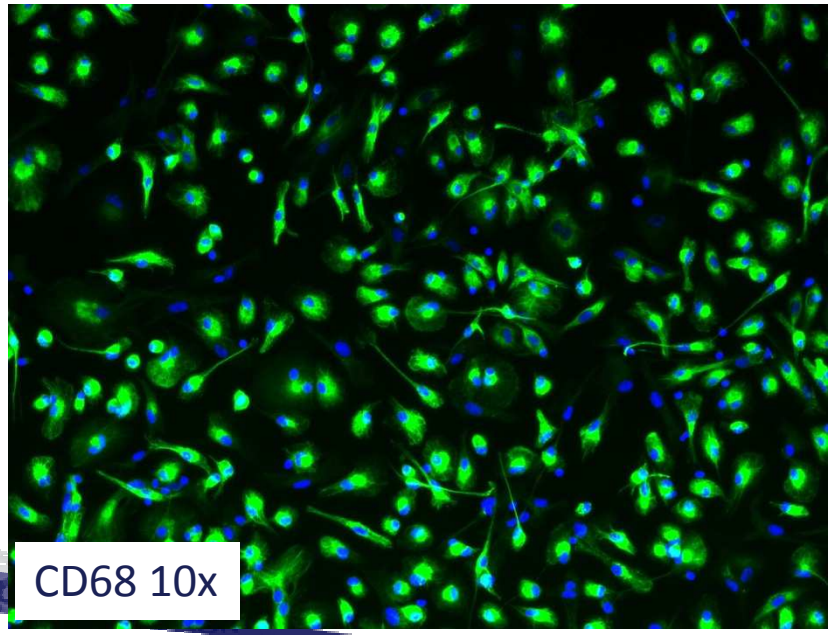
Legend

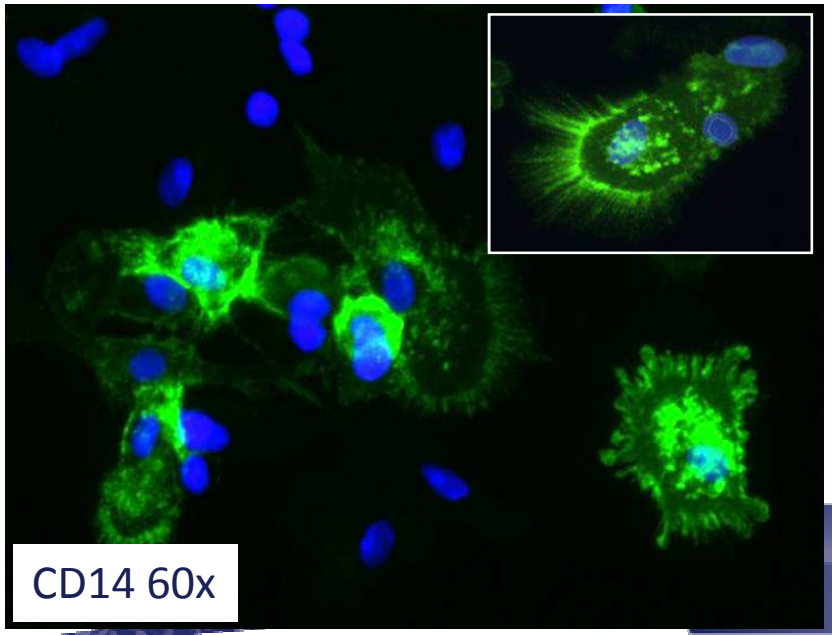
-	<5% of control	++	25-75% of control
+	5-25% of control	+++	>75% of control

Human Kupffer cells Hu8137 (~92% Purity, 25 vials out of 1/5 of liver material)

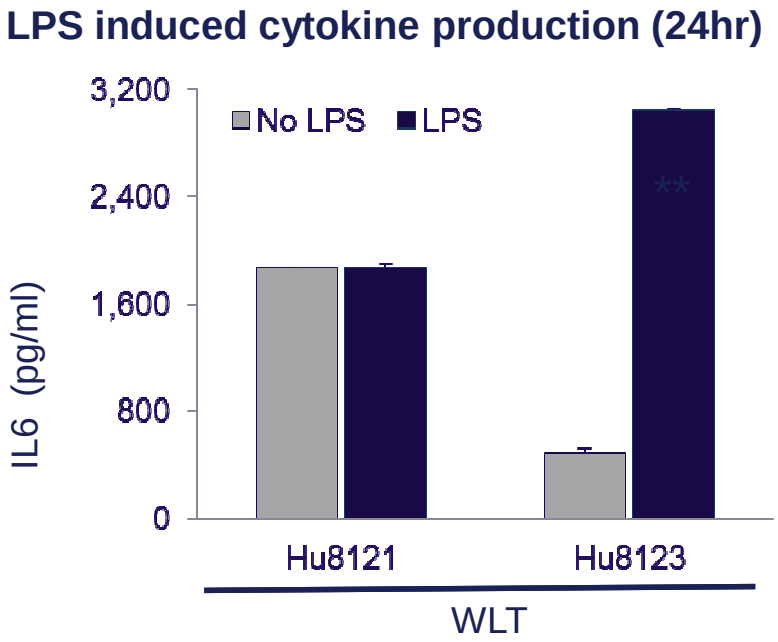








Human Kupffer cells – donor variations



	IL6 Basal Level	Increase in IL6 levels due to LPS response
WLT: Hu8121	++++	-
WLT: Hu8123	+	++++
Resections: Hu1362	+++	+
Resections: Hu1387	+++	N/A

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ADME-Tox R&D Team at Life Tech (formerly CellzDirect) 2011