NTP
National Toxicology Program

Incorporating Improved Physiological Relevance in Tox21: An Evolution of Resolution

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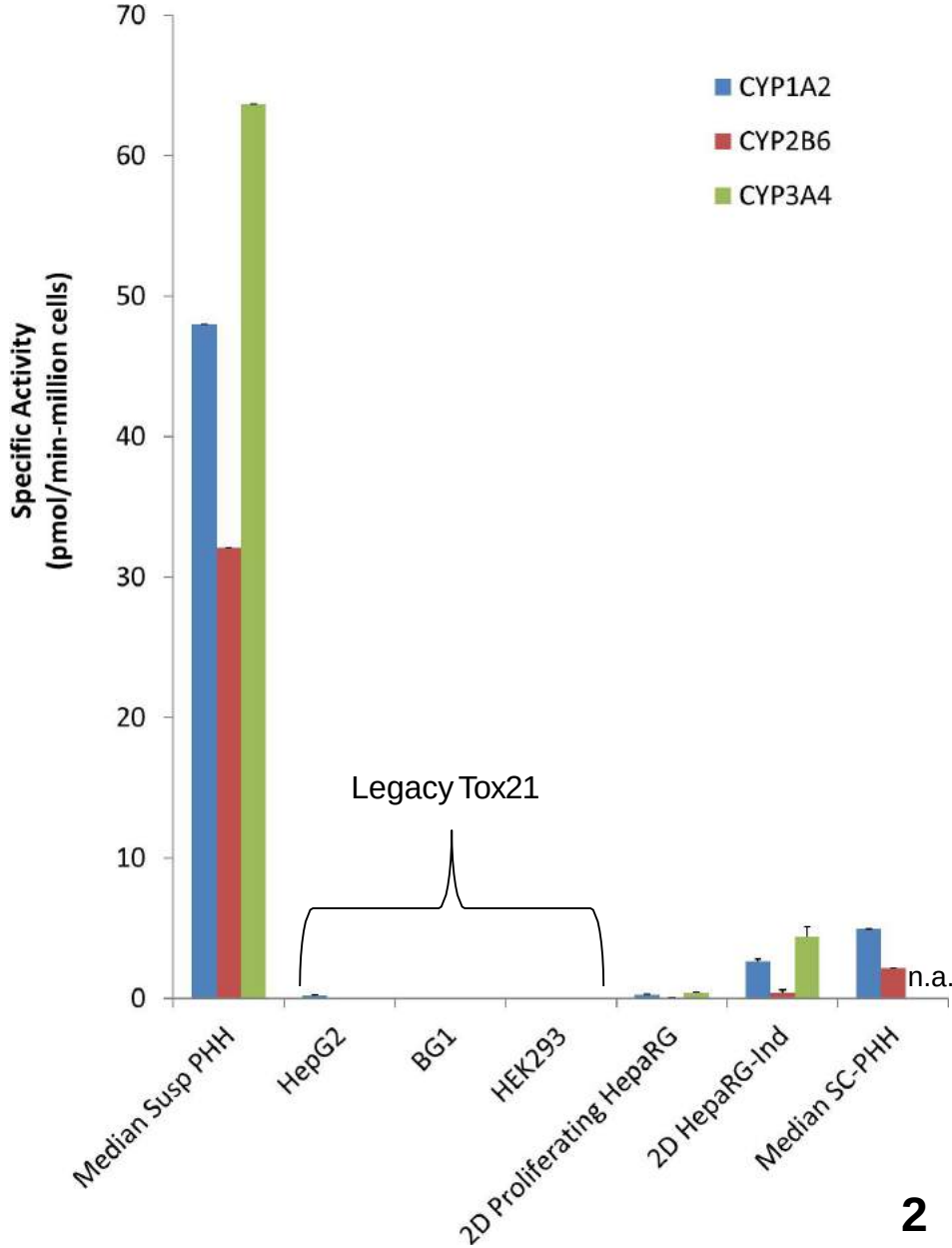
- I have no financial relationships to disclose.
- The statements, opinions or conclusions contained therein do not necessarily represent the statements, opinions or conclusions of NIEHS, NIH or the US government.

Phase I & Phase II of Tox21 (NCATS, EPA, FDA & NTP)

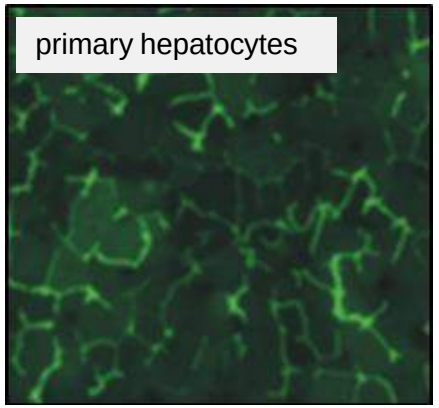
- Phase I (2005-2010)
  - Proof of concept
- Phase II (2011-present)
  - ToxCast Phase II: ~700 compounds, ~700 assays
  - NCGC/NCATS: 10k compound library
    - nuclear receptor
    - cellular stress pathways

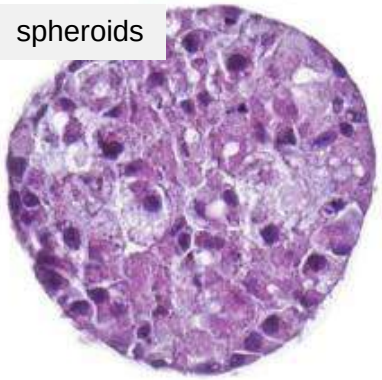
- Limitations with Tox21 Phase II:
  - **Limited capability for xenobiotic metabolism**
  - **Limited pathway coverage**
  - Focus on 'individual' cellular pathways lacking integrated biological/tissue-like functionality
  - Use of immortalized and transformed cell lines
  - Addition-only assays with ≤40h exposure

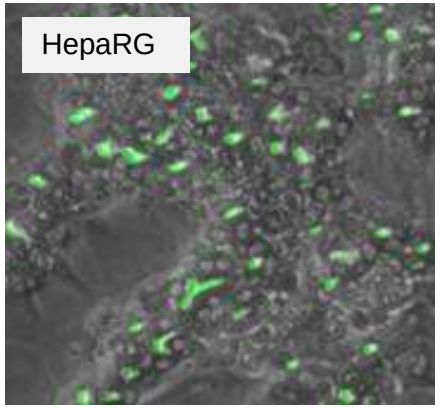


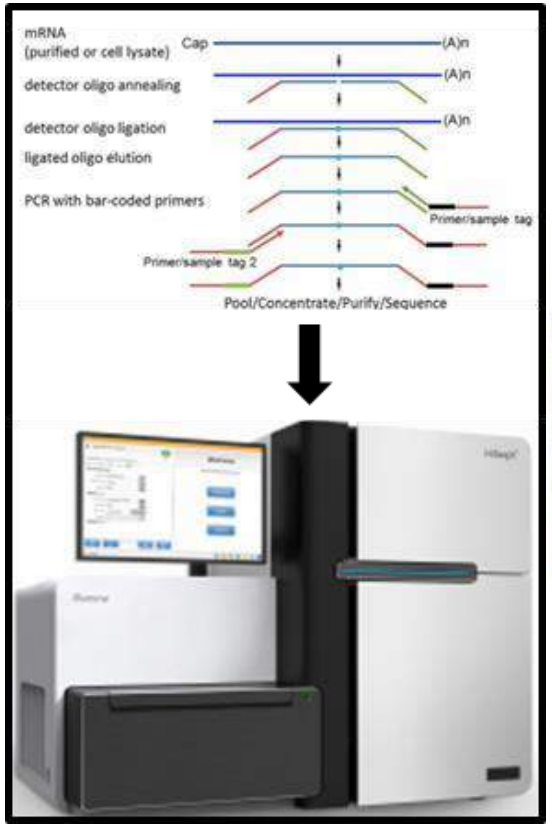
Tox21 Phase III: Predictive Toxicology Screening

- Physiologically-relevant in vitro screening models
  - **xenobiotic metabolism** 'competence'
  - longevity to address longer-term exposures (weeks/months)
- Data-rich, high dimensional assay platforms as windows into chemical-induced perturbation cascades
  - **high throughput transcriptomics** (time & concentration)
  - high content imaging (i.e., stress pathways)
  - metabolomics (xenobiotic & endogenous)
- Quantitatively link chemicals & metabolites to biological response pathways
  - C_{max}/EC_{50} ratios
  - AUC correlations
  - IVIVE
- Extend 'normal' models to susceptibility models & extrahepatic
  - inflamed liver, fatty liver, cholestatic liver
  - extrahepatic tissues (i.e., kidney, intestine)
  - developmental tissue models (i.e., neonatal, stem cells)









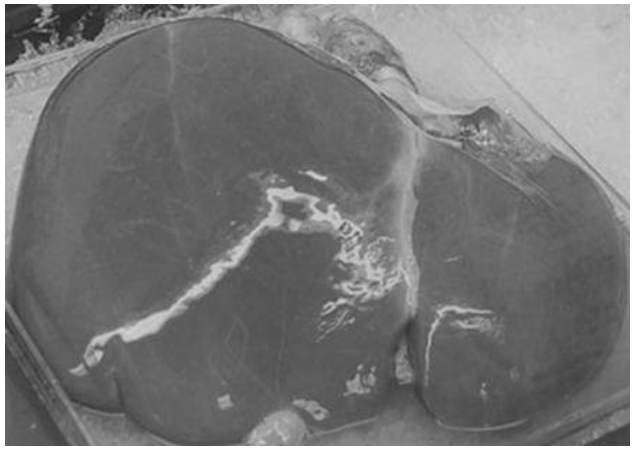
Outline

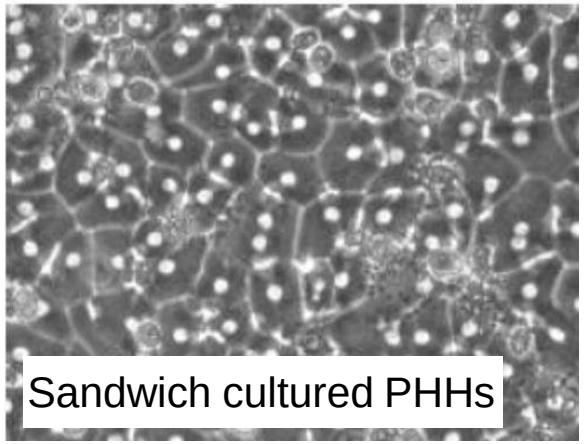
- **Sandwich cultures of primary human hepatocytes to explore:**
  - Ginkgo biloba extract (GBE) biological response equivalence across 29 lots
  - Predict their potential for liver enzyme induction in humans
  - Link GBE constituents with response phenotypes: liver enzyme induction/receptor pathway activation, cytotoxicity & oxidative stress

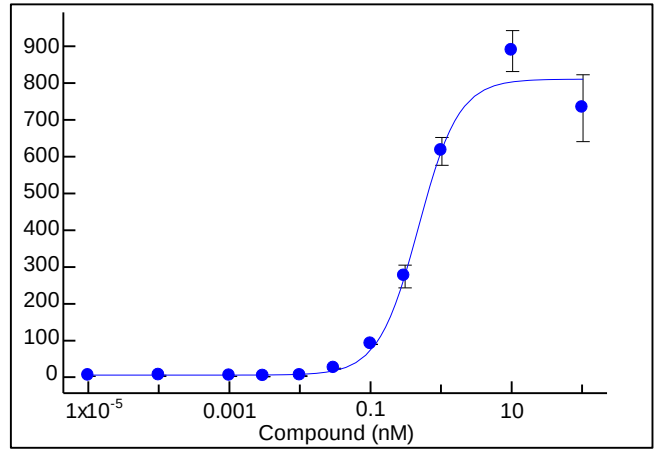
- **3D organotypic spheroid cultures of HepaRG cells to:**
  - Screen chemicals with physiologically-relevant xenobiotic metabolism
  - Support functional liver enzyme induction pathways
  - Identify liver injury potential with chemicals

- **High throughput transcriptomics paired with in vitro liver models to:**
  - Explore tissue model functionality, chemical-biological interaction gene & pathway dynamics
  - Characterize the impact of cell culture configuration
  - Dose-response modeling of benchmark dose & biological pathway perturbations

Prediction of Drug Metabolism & Drug-Drug Interactions with In Vitro Models



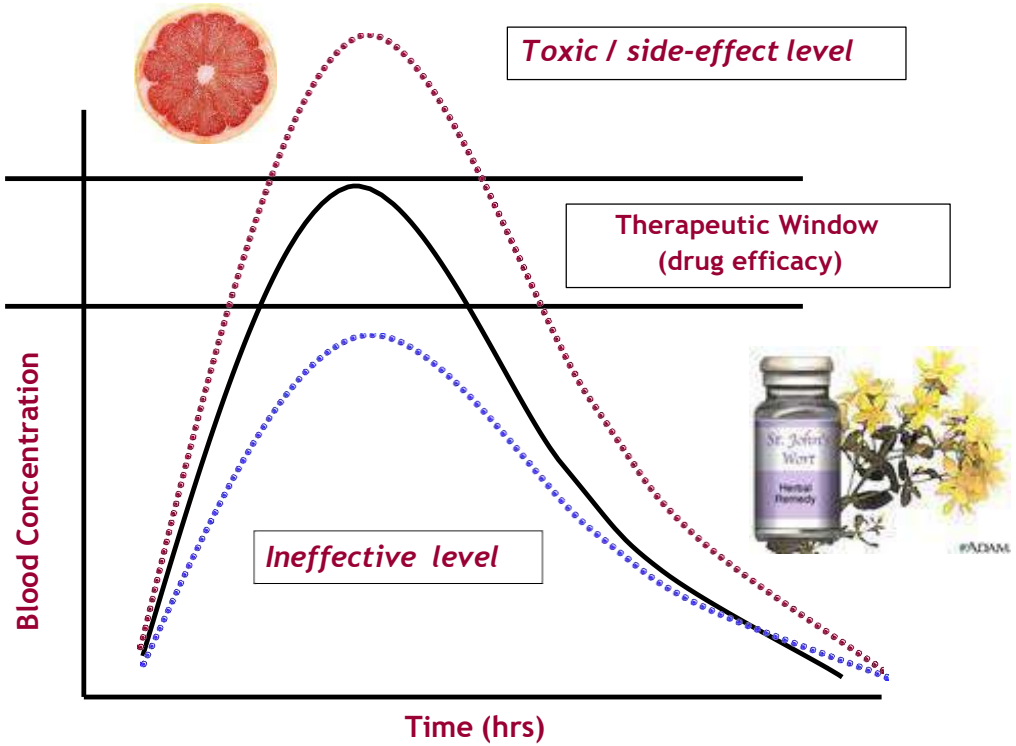




Drug Labelling

negative

positive

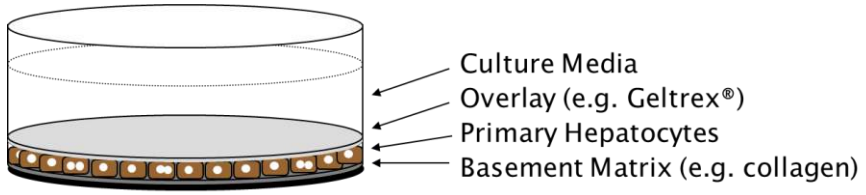


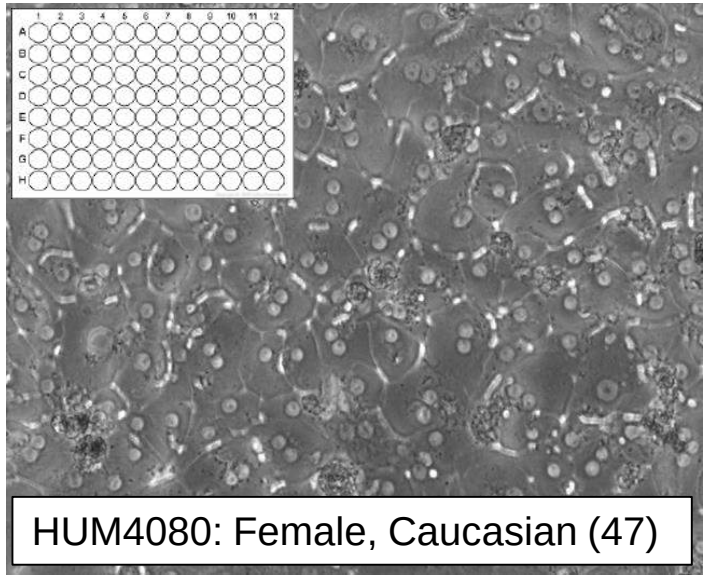
IVIVE

Translation of in vitro responses to clinical effects

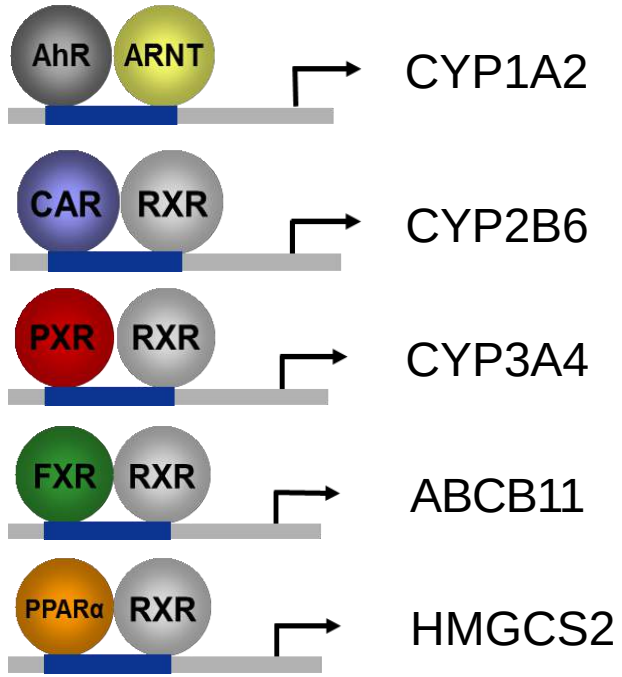
Ginkgo Biloba Extract Study Design Summary

- Sandwich cultures of PHHs (SC-PHHs)

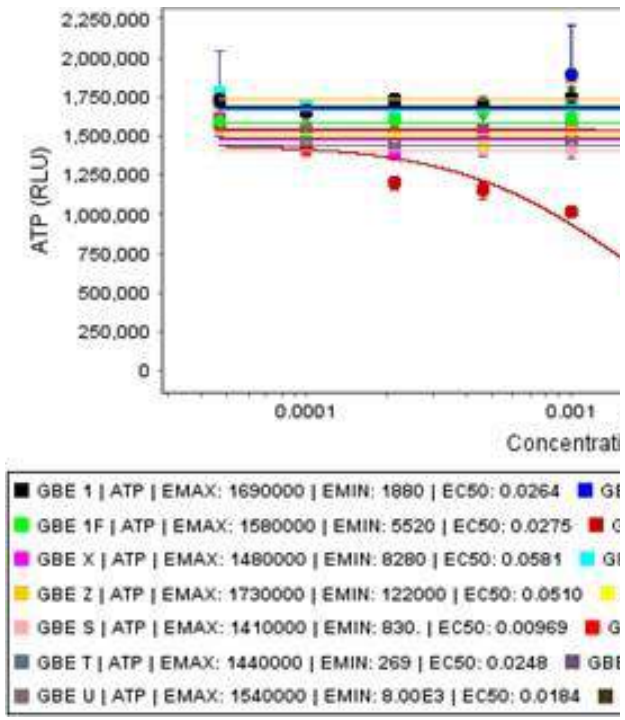




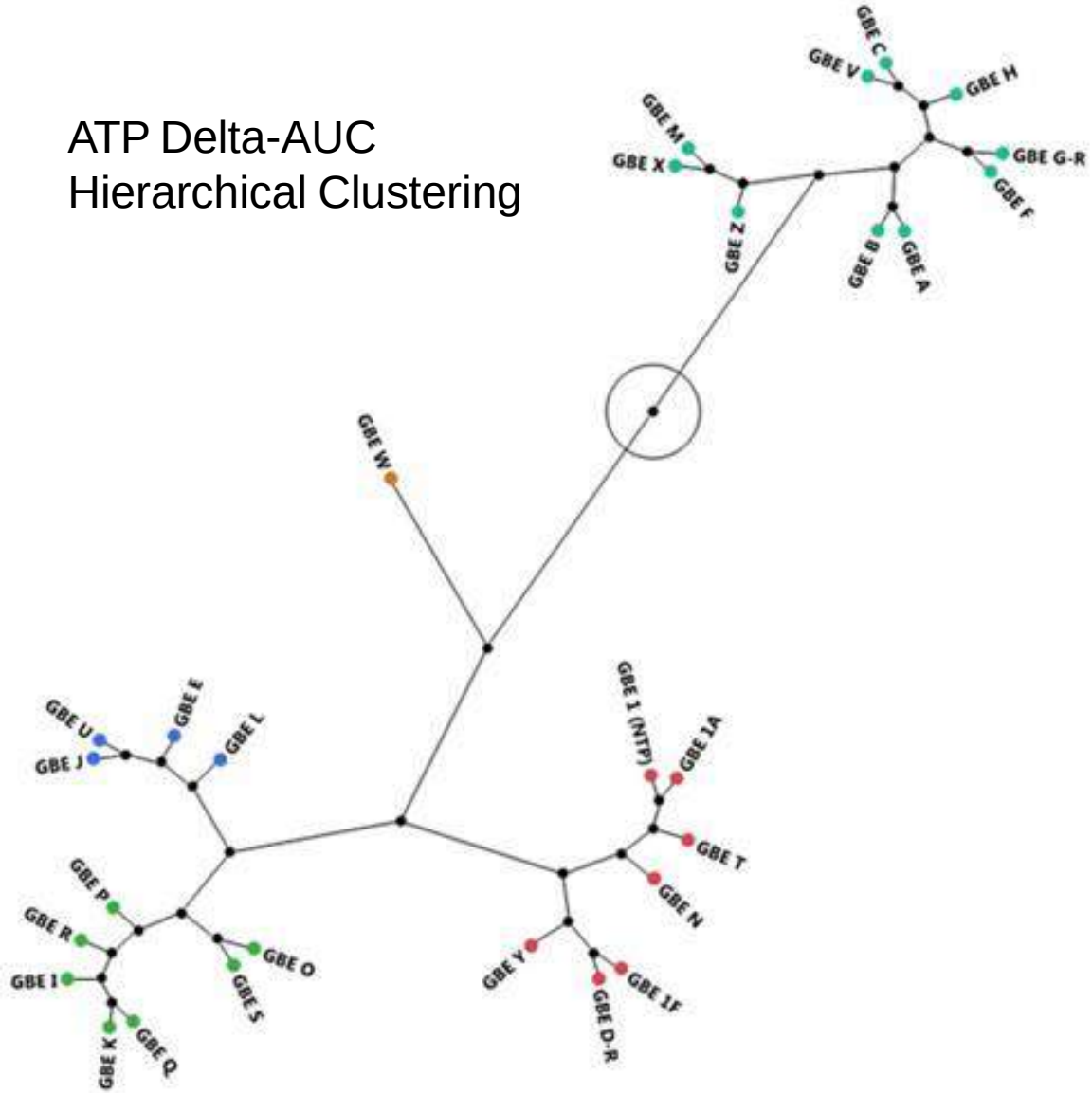
- Endpoint Assays:
  - Cell Health:
    - Photomicrographs of each culture (Incucyte)
    - ATP Depletion (CellTiter-Glo)
    - Reactive Oxygen Species (H_2O_2, ROS-Glo)
  - Liver enzyme induction/receptor activation
    - mRNA (TaqMan)
      - 5 target genes, sentinels to hepatic receptor pathways
      - 3 Endogenous Ctrls (PSMB6, GAPDH, ß-actin)
    - Liver enzymatic activity
      - CYP1A2 (phenacetin to acetaminophen)
      - CYP2B6 (bupropion to hydroxybupropion)
      - CYP3A4/5 (midazolam to 1-hydroxymidazolam)
  - Samples frozen for future transcriptomics/metabolomics

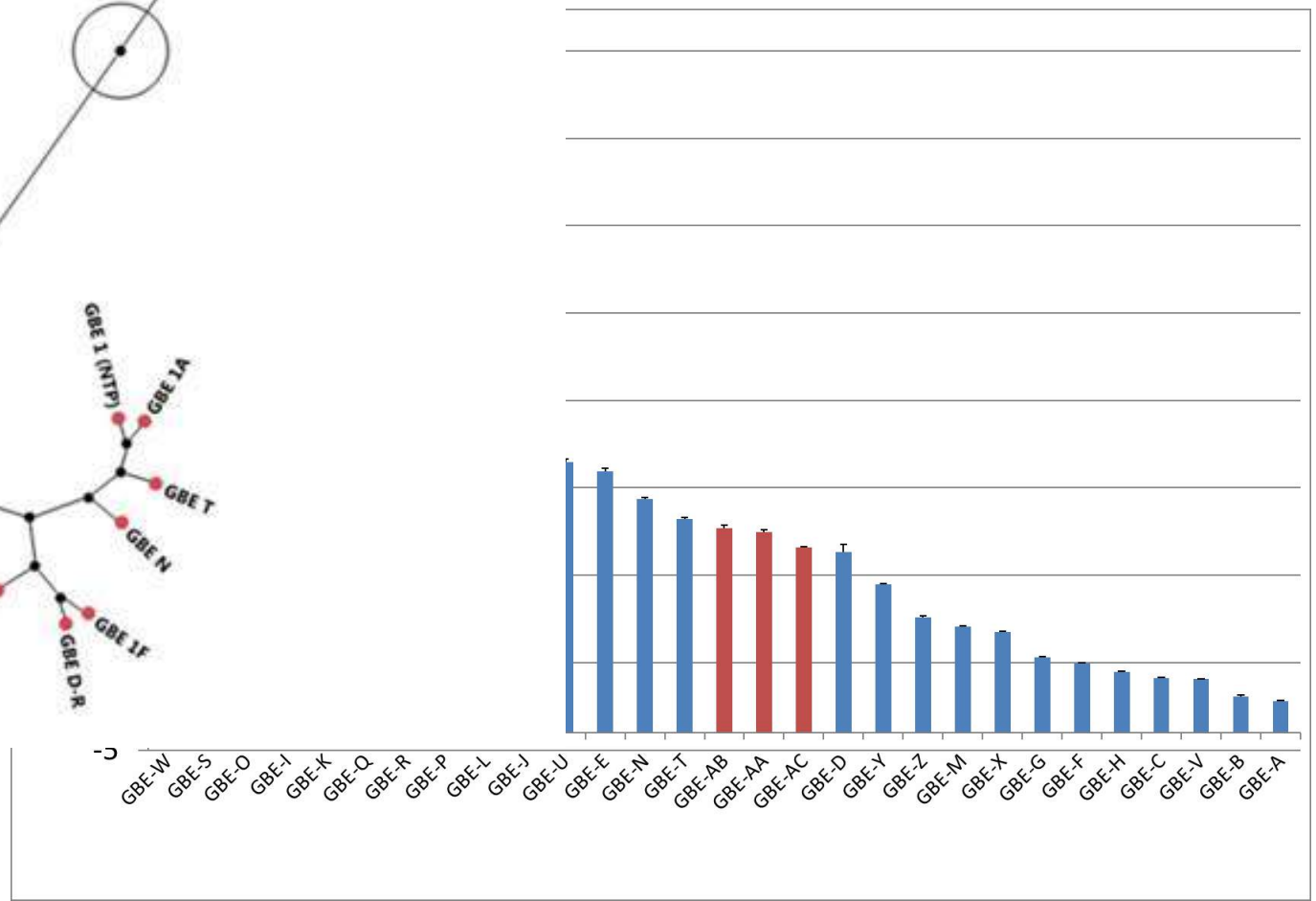


ATP Depletion Assays with SC-PHHs & GBEs

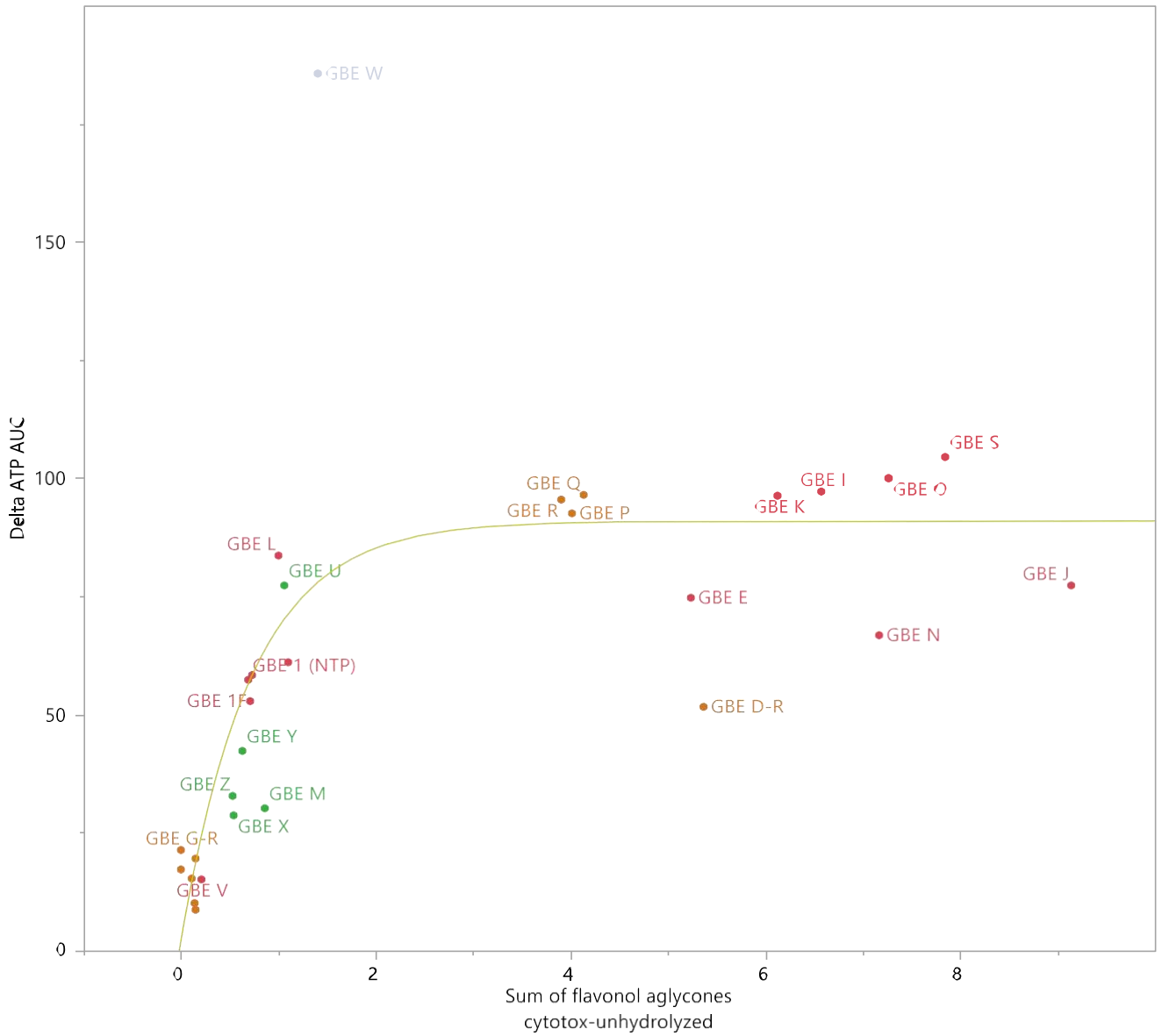


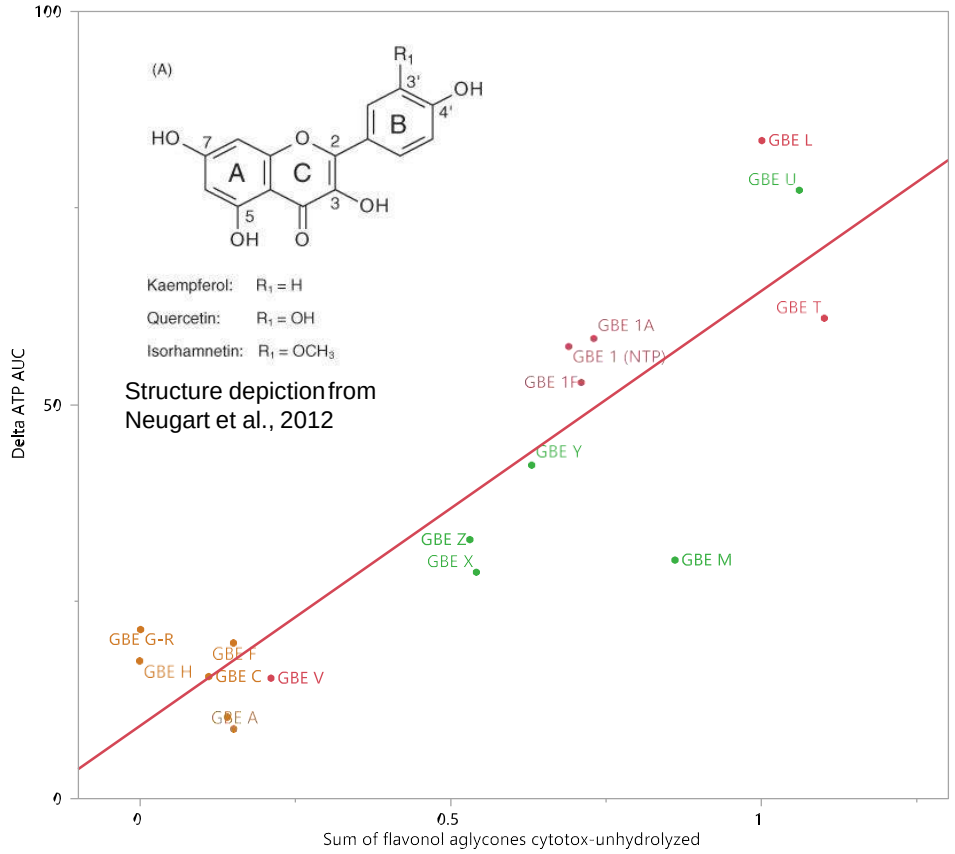
ATP Delta-AUC
Hierarchical Clustering

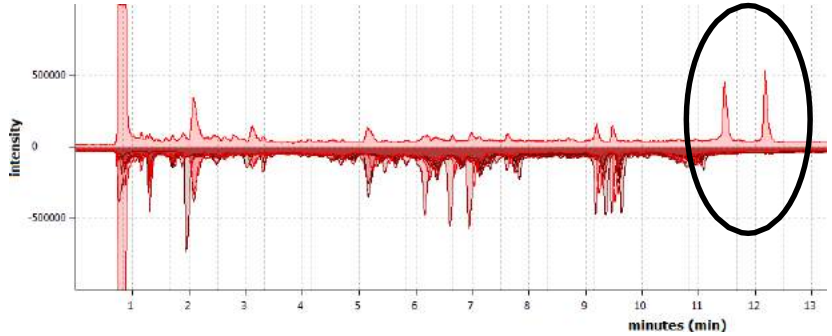


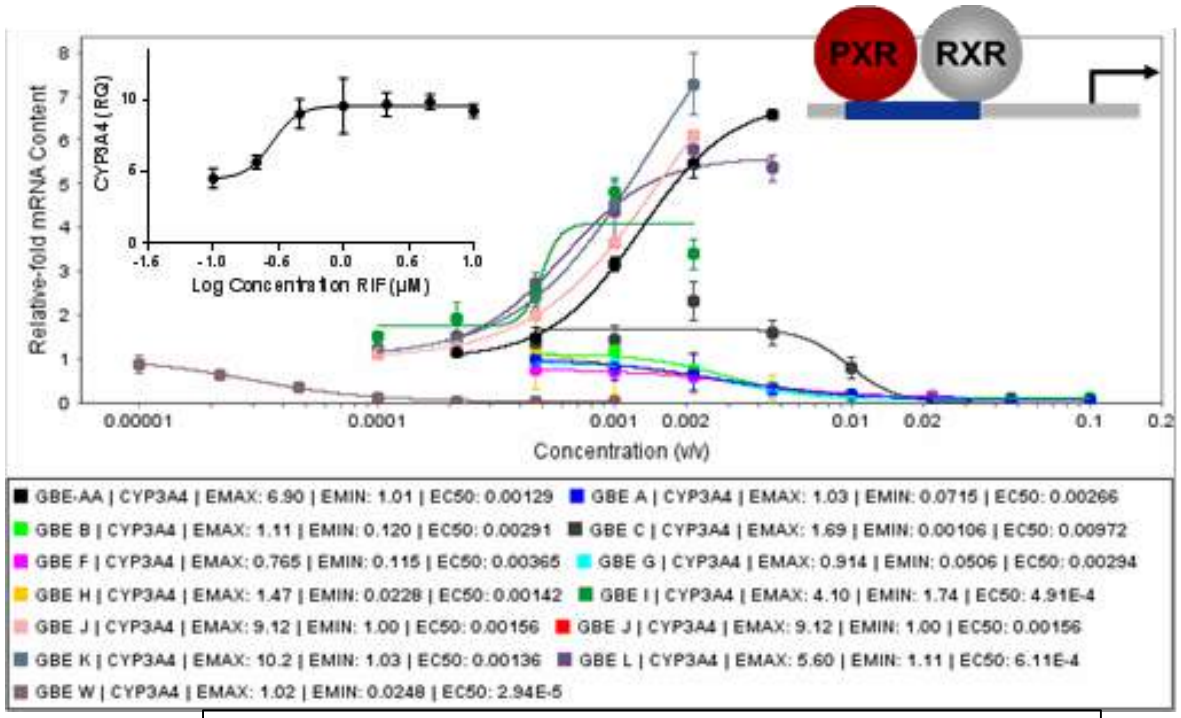


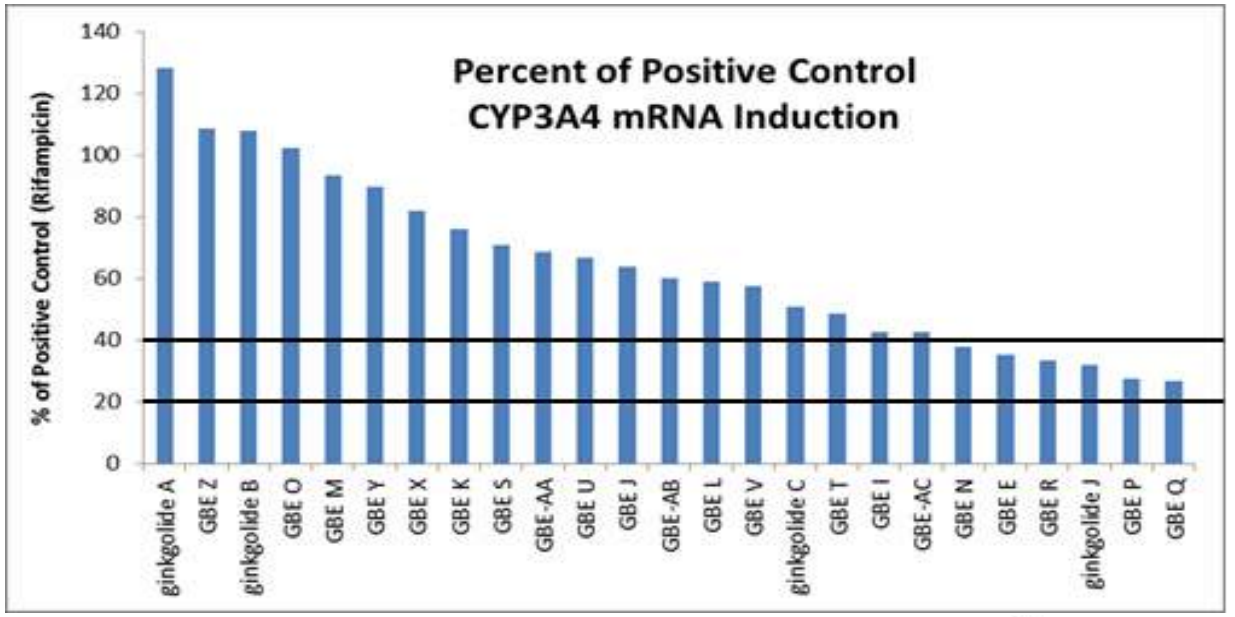
Linking GBE Constituents to Cytotoxicity via ATP Depletion in Primary Cultures of Human Hepatocytes

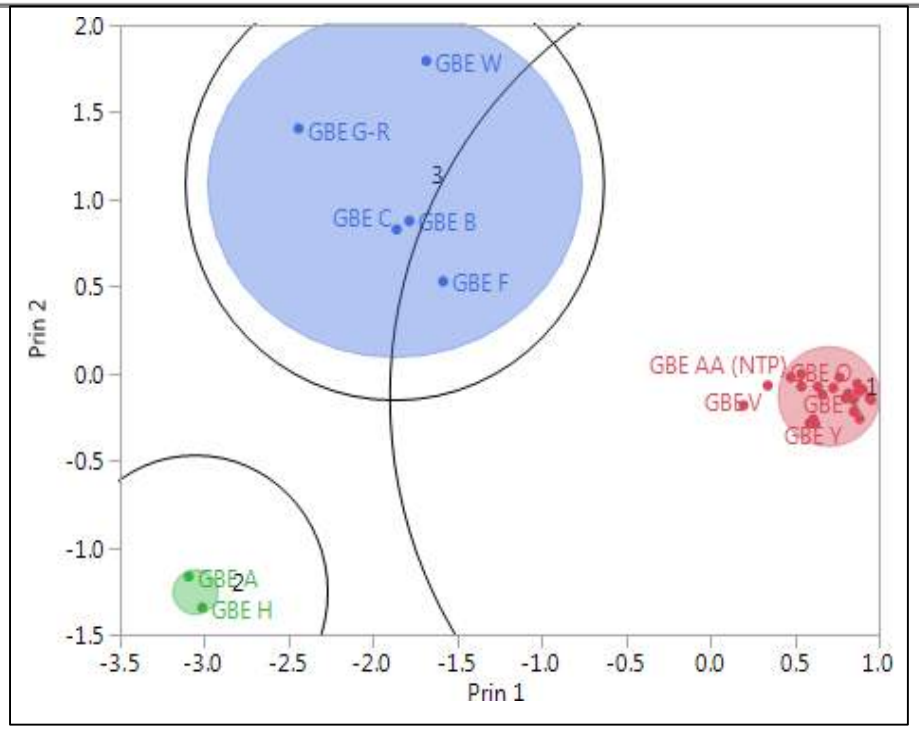




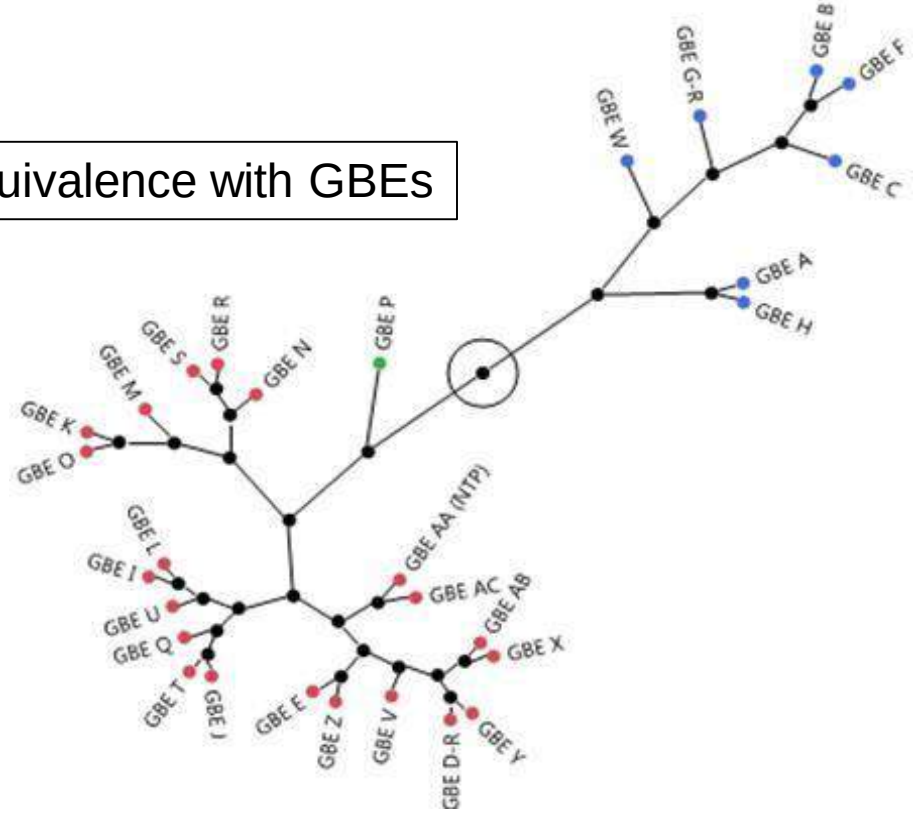








Phytoequivalence with GBEs



Hengstler JG et al., *Drug Metab. Reviews*. (2000) v.32(1), 81-118

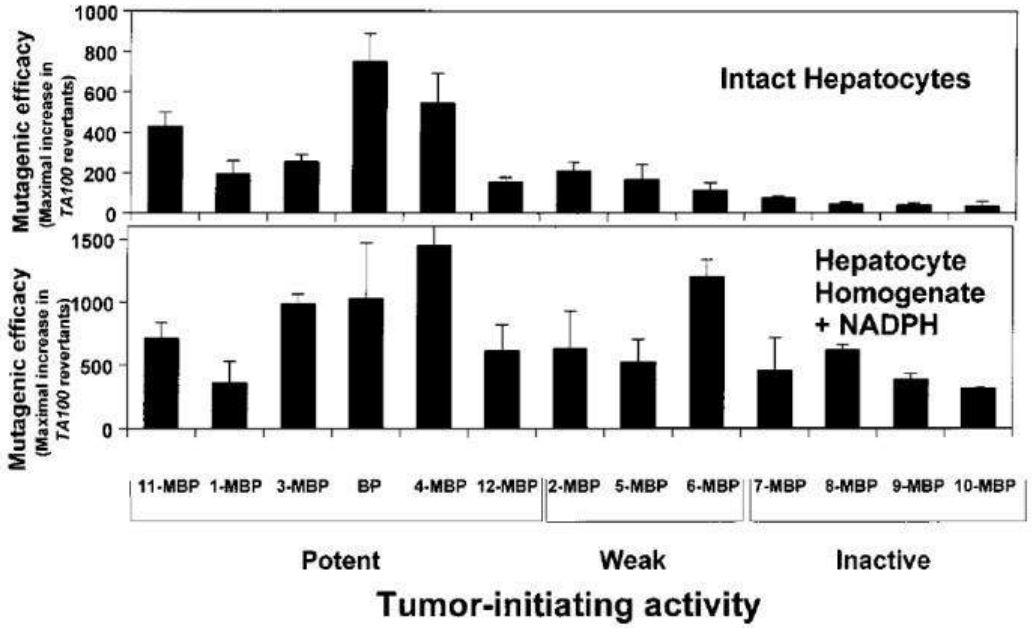


FIG. 1. Mutagenicity of benzo[*a*]pyrene (BP) and the 12 monomethylbenzo[*a*]-pyrenes (MBP) using intact hepatocytes and NADPH-fortified hepatocyte homogenate as metabolizing systems. (From Ref. 1.)

Modeling Metabolically-dependent Liver Toxicity

- Intact hepatocytes better distinguish potent tumor initiators from non-carcinogens (Ames)
- Short $t_{1/2}$ limits utility of PHH suspensions, lower activities & longevities limit SC-PHHs
- How "much" xenobiotic metabolism competence is sufficient?
- What about neonatal/developmental susceptibilities

Benchmarking Metabolic 'Competence'

- **Specific Activity Assays** of major human drug metabolizing enzymes
- **Metabolic Clearance Assays** with high, moderate, & low turnover compounds
- **Metabolite Profiling Assays** with orbitrap LC-MS (HRAM)

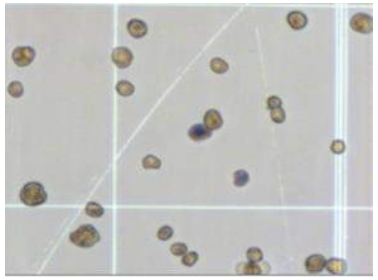
TABLE 5
Separation of Database into Low, Intermediate, and High Clearance Chemicals

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

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

Metabolic Competence

Suspension PHHs isolated from human liver



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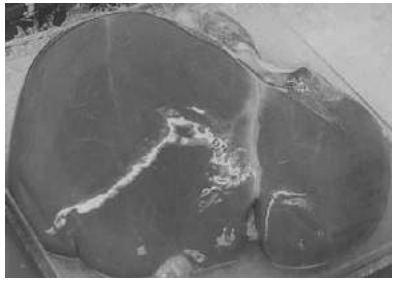
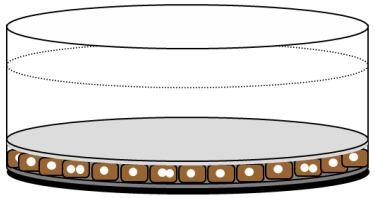


SC-PHHs



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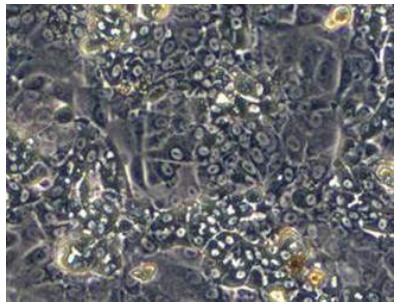
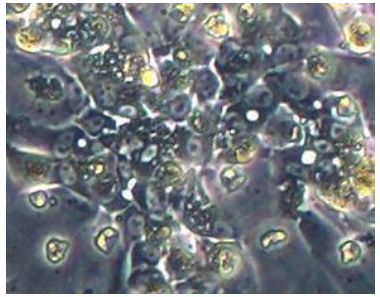
2D Differentiated HepaRG



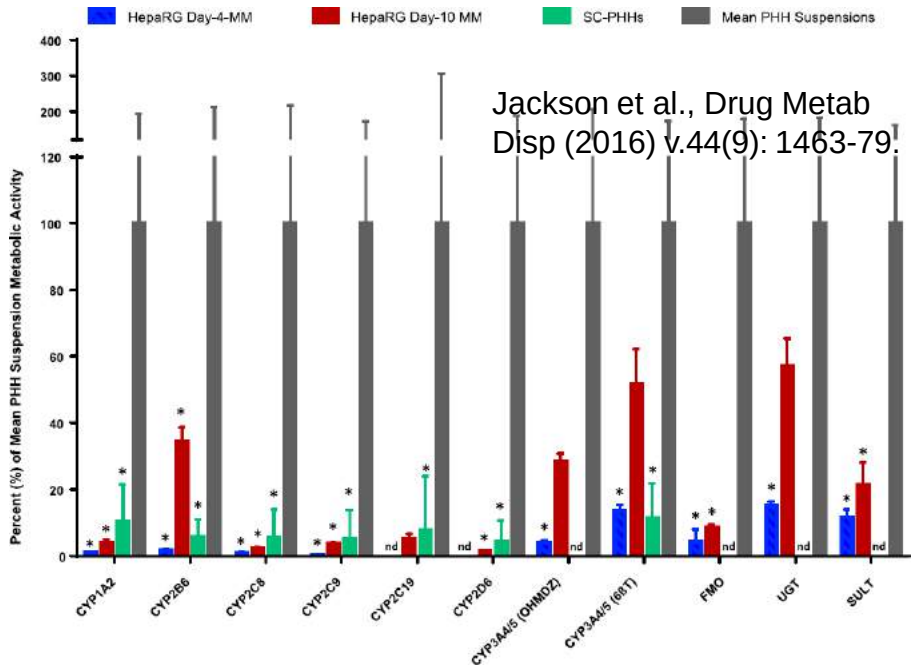
Day-4 HepaRG



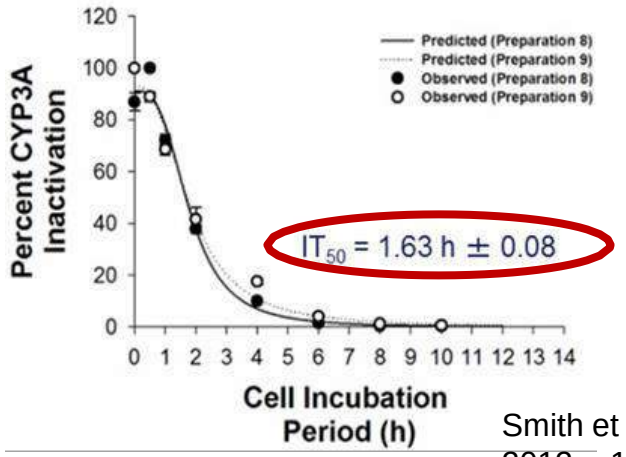
stem cells, transformed cell lines

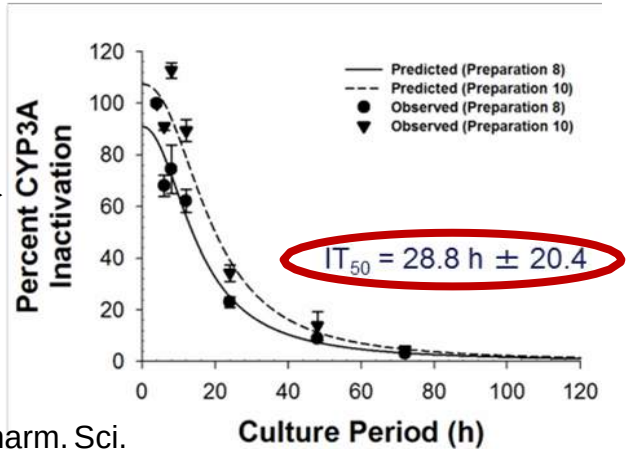
Defining Xenobiotic Metabolism "Competence"

How much is needed, and over what time frame?



Jackson et al., Drug Metab Disp (2016) v.44(9): 1463-79.





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

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We look forward to hearing from you!

Kind regards,

The PREDiCT Team

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