

# MetMax™ Pooled Donor Human Hepatocytes (*Patent Pending*): A Novel In Vitro System for the Evaluation of Hepatic Drug Metabolism

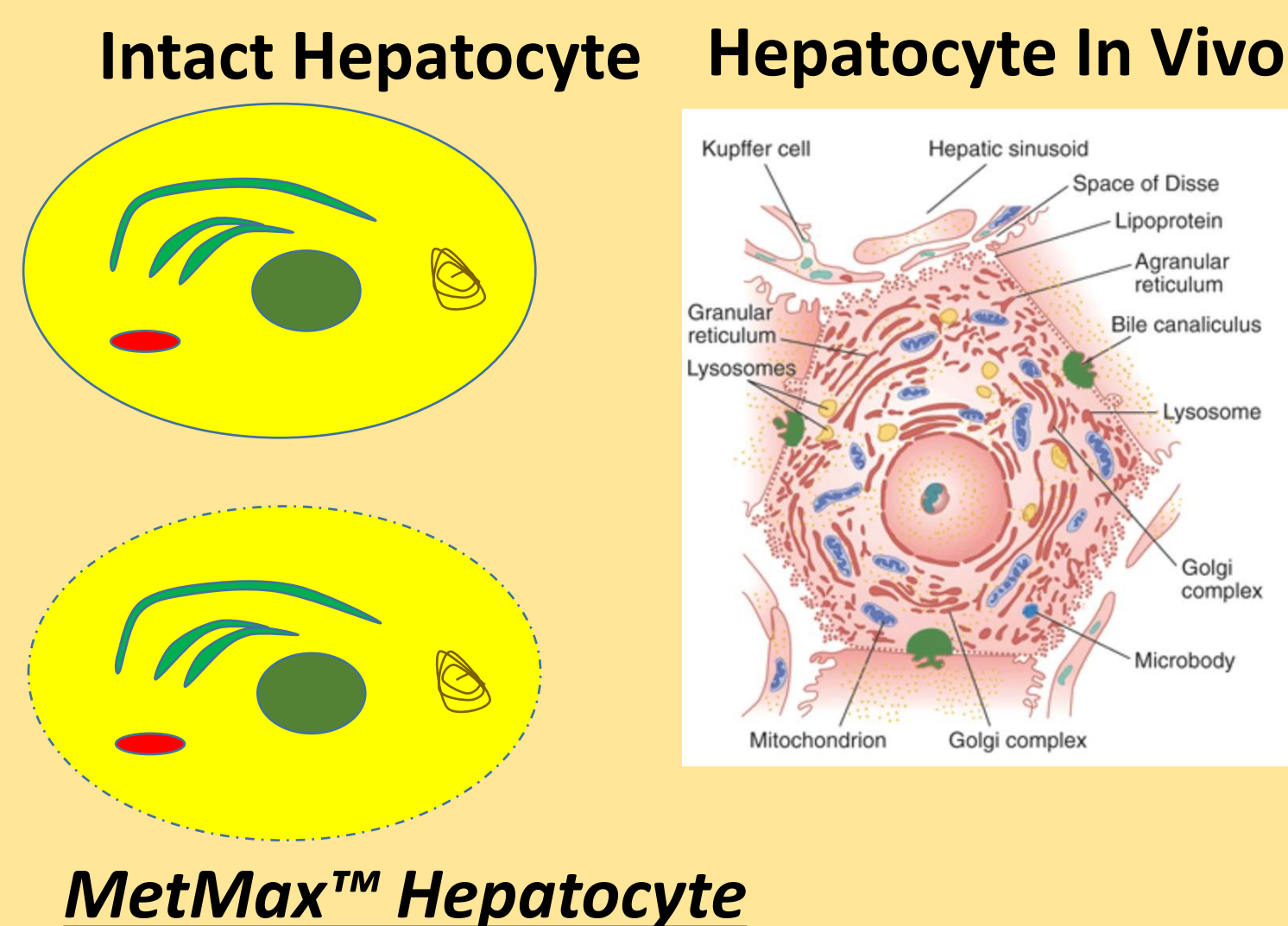
David Ho, Carol Loretz, Kirsten Amaral and Albert P. Li,  
In Vitro ADMET Laboratories Inc., Columbia, MD and Malden, MA

## Scientific Rationale

- Cryopreserved human hepatocytes possess complete hepatic drug metabolizing enzymes and are generally regarded as the Gold Standard for in vitro human drug metabolism studies
- The use of hepatocytes is challenged by the fragility of the cells, the need for liquid nitrogen storage and the laborious cell preparation procedures
- Many investigators therefore prefer to use cell free systems such as S9 and microsomes in spite of the incomplete drug metabolizing enzyme pathways
- The MetMax™ human hepatocytes - permeabilized cofactor-supplemented hepatocytes - possess the advantages of the hepatocytes (complete hepatic drug metabolism pathways) and S9/microsomes (robustness and operational efficiency)**

## MetMax™: Cofactor-Supplemented Permeabilized Hepatocytes

MetMax™ Advantage: Complete Drug Metabolizing Pathways (as with Hepatocytes) and Simplified Use Procedures (as with HLM)



## Comparison of MetMax™ Hepatocytes to Intact Hepatocytes, Microsomes and S9

Organelles	MetMax™	Intact Hepatocytes	Microsomes	S9	Organelles	MetMax™	Intact Hepatocytes	Microsomes	S9
Endoplasmic Reticulum	😊	😊	😊	😊	Storage	-80 C	LN2	-80 C	-80 C
Cytosol	😊	😊		😊	Centrifugation	No	Yes	No	No
Mitochondria	😊	😊			Microscopic Examination	No	Yes	No	No
Lysosomes	😊	😊			Cell Counting	No	Yes	No	No
Golgi	😊	😊			Cofactor Addition	No	No	Yes	Yes
Plasma Membranes	😊	😊			Thaw and Use	Yes	No	No	No
Nucleus	😊	😊							

## Drug Metabolizing Enzyme Activities

## Materials & Methods

### MetMax™ Hepatocytes

Freezer to Incubation:  
**<5 minutes**

- Retrieve from -80 C freezer
- Thaw in a 37 C water bath
- Add equal volume to 2X test article
- Incubate

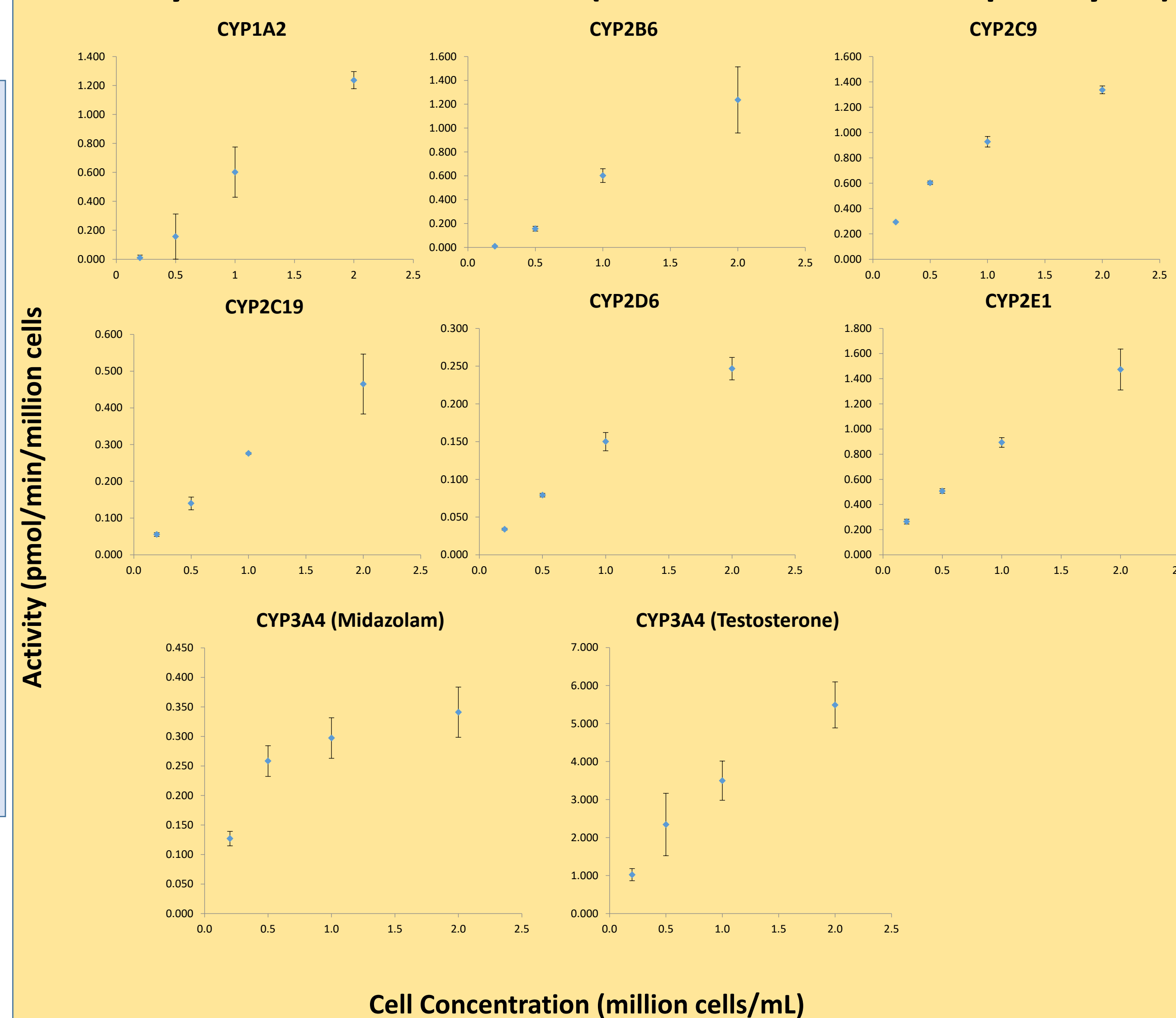
### Cryopreserved Hepatocytes

Freezer to Incubation:  
**>30 minutes**

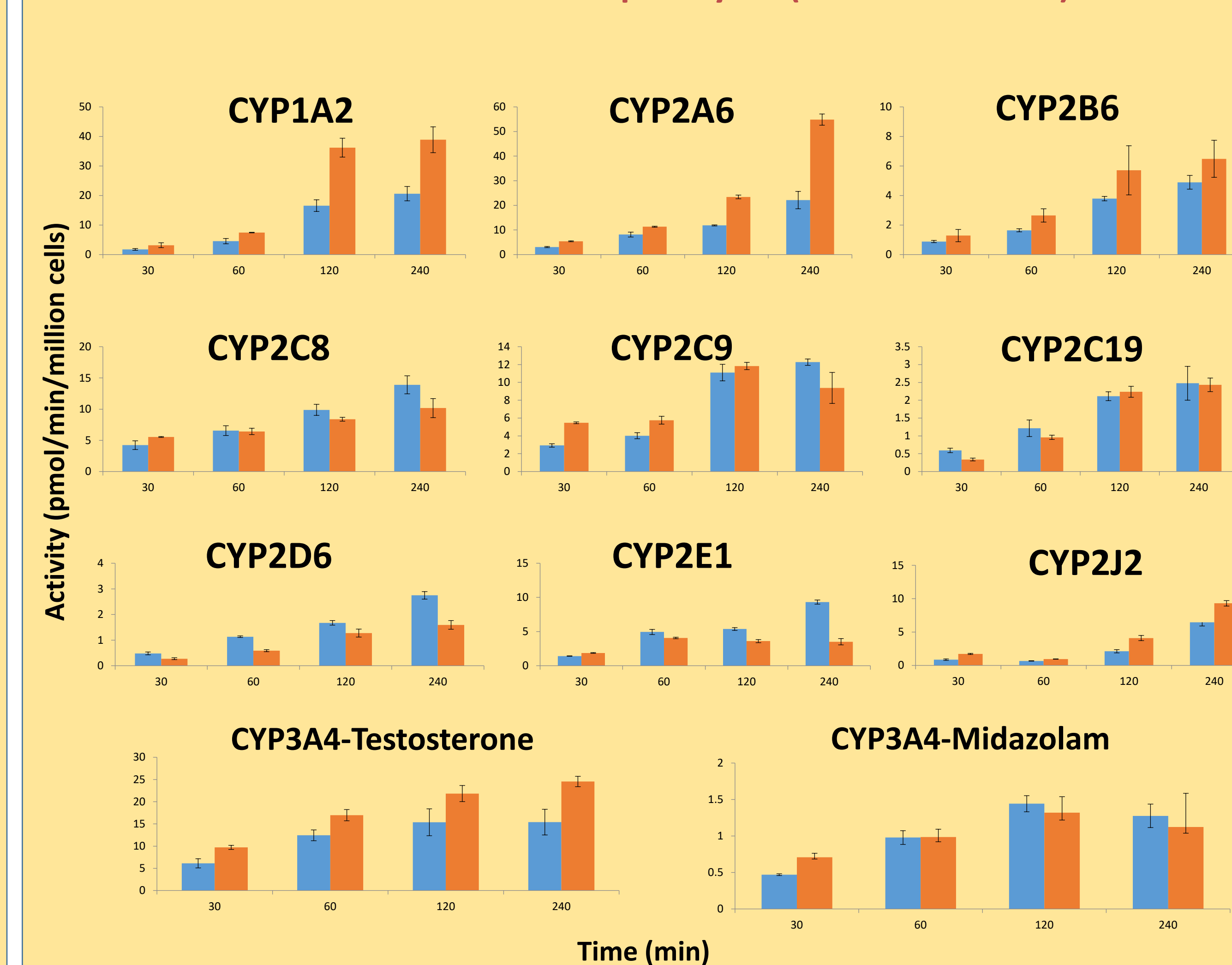
- Retrieve from LN2 Freezer
- Thaw in a 37 C water bath
- Add to recovery medium
- Centrifuge
- Microscopically quantify viability and cell number
- Adjust to 2X final cell density
- Add at equal volume to 2X test article
- Incubate

- MetMax™ Pooled Donor Human Hepatocytes were prepared from IVAL Pooled Donor Cryopreserved Human Hepatocytes using proprietary procedures
- Drug metabolizing enzyme activities of the MetMax™ and intact human hepatocytes were determined by incubation with pathway-selective substrates followed by LC/MS-MS quantification of metabolite formation

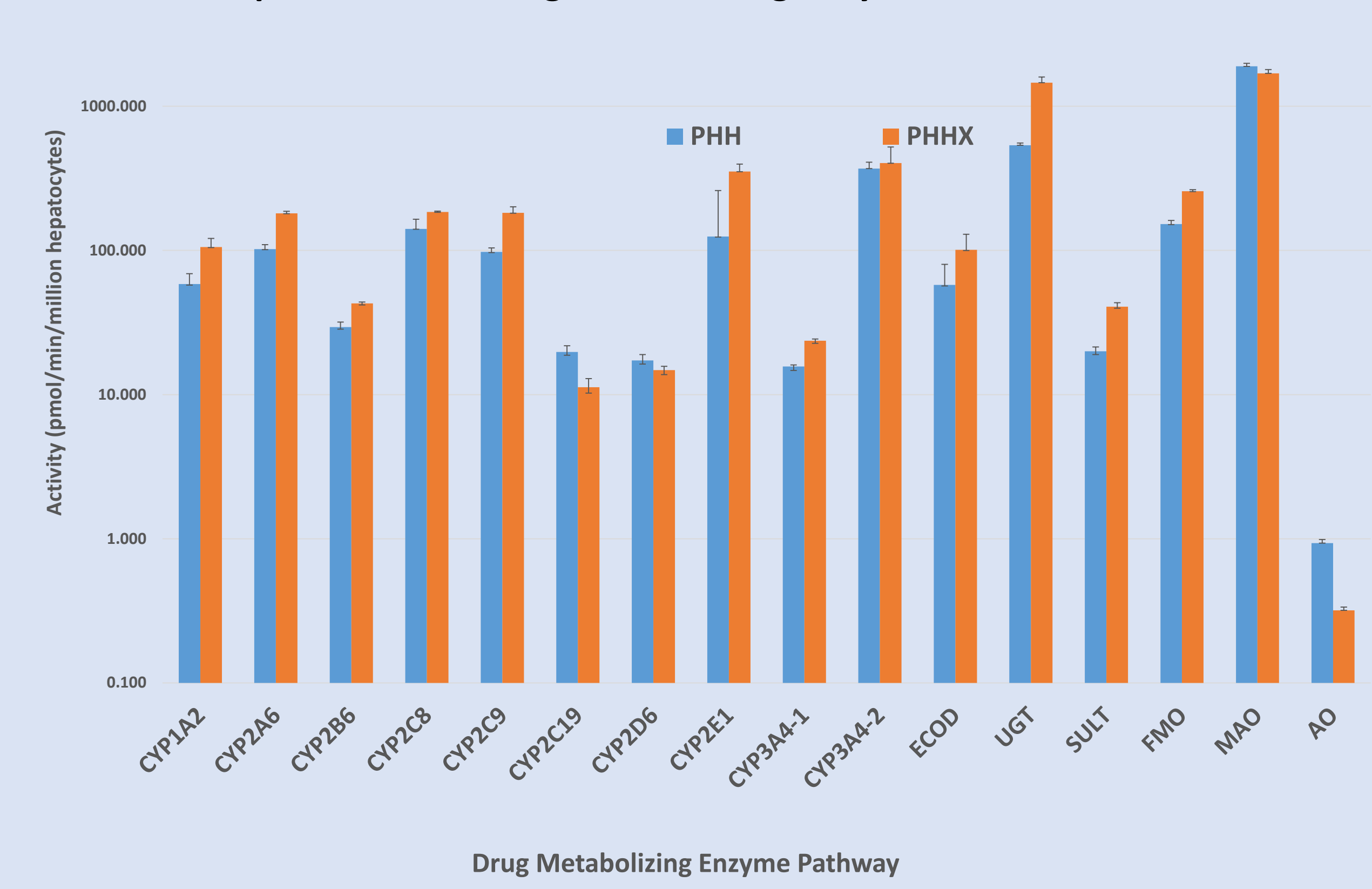
### Activity vs Cell Concentration (MetMax™ Human Hepatocytes)



### Time Course Human Hepatocytes (PHH9001--Blue) vs. MetMax™ Human Hepatocytes (PHHX8001--Red)

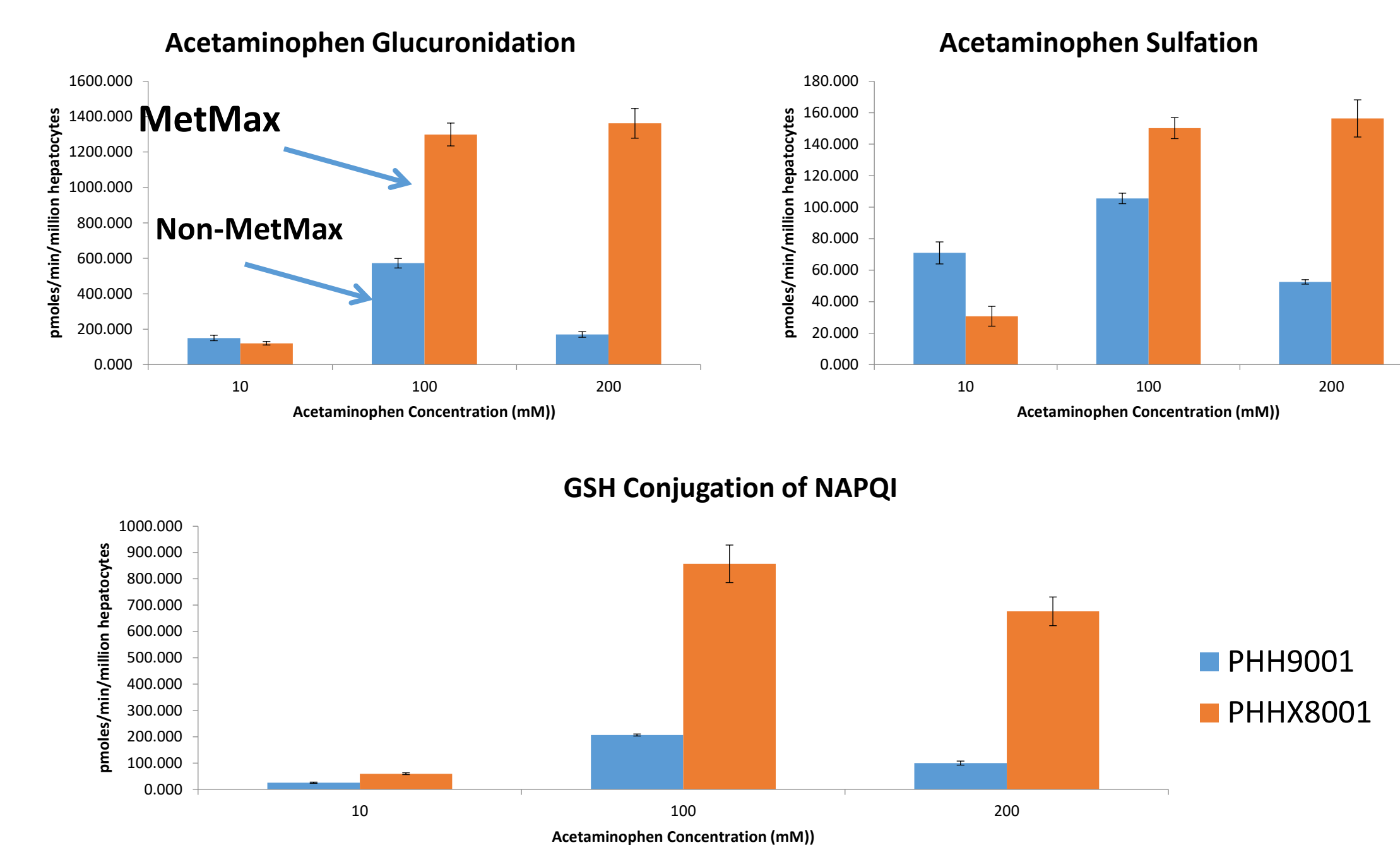


### Intact (PHH) Vs MetMax™ (PHHX) Pooled Donor Human Hepatocytes: Comparison of 16 Drug Metabolizing Enzyme-Selective Substrates



### MetMax™ Human Hepatocytes are superior to intact Human Hepatocytes in the evaluation of metabolite profiling and identification at high/cytotoxic drug concentrations:

Acetaminophen Metabolism at Nontoxic (10 mM) and Cytotoxic (100 and 200 mM) Concentrations



## Conclusion

MetMax™ Human Hepatocytes represent a desirable in vitro experimental system:

- Advantage over intact hepatocytes
  - Storage at -80 C (not LN2)
  - Thaw and use (no centrifugation, viability determination, cell counting)
  - Can be used with high/cytotoxic drug concentrations
- Advantage over S9 and microsomes
  - Complete drug metabolizing enzyme pathways

MetMax™ Human Hepatocytes should be compatible with robotic system for high throughput applications such as metabolic stability screening and P450 inhibition assays