

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

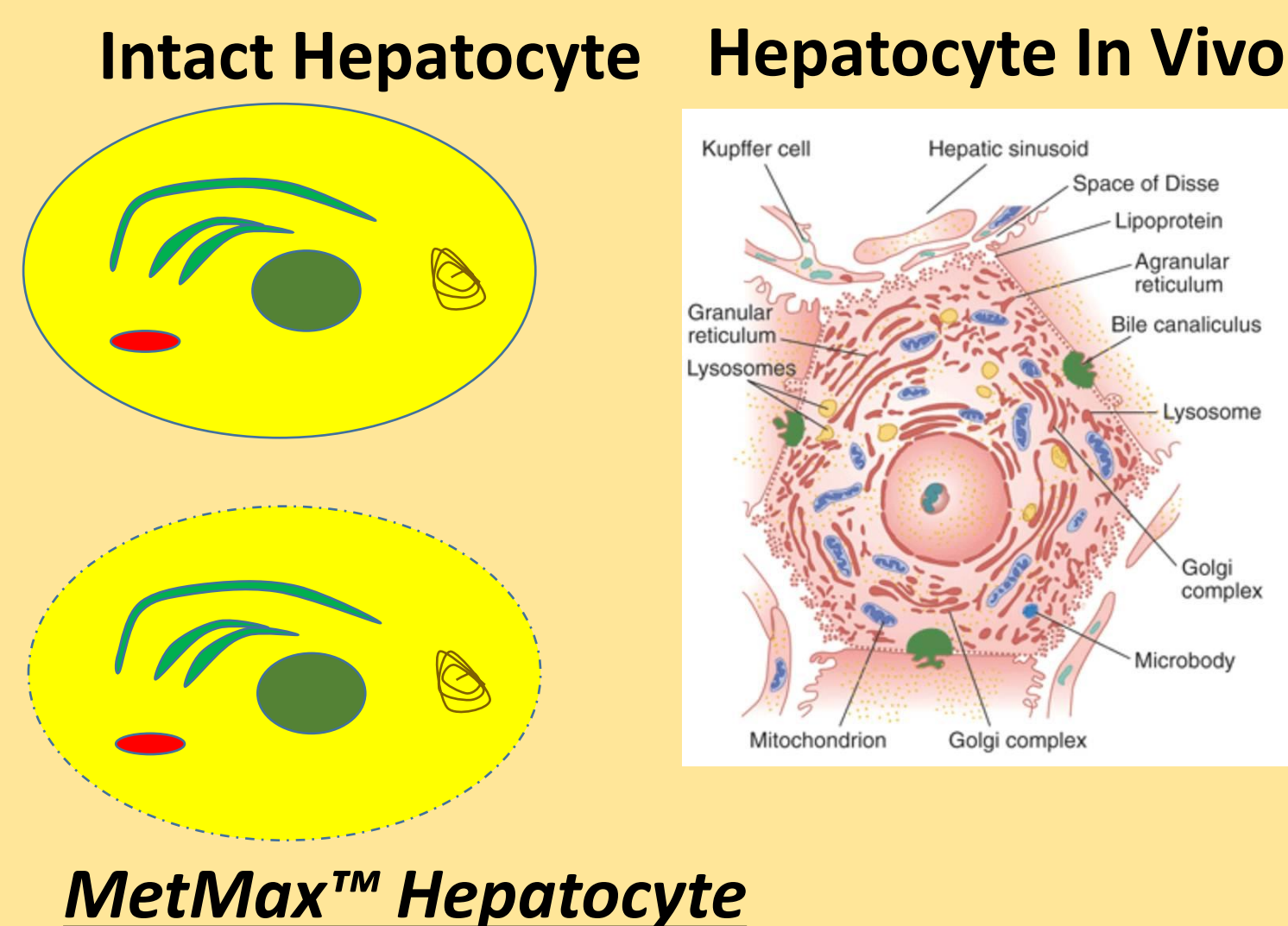
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Scientific Rationale

- 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

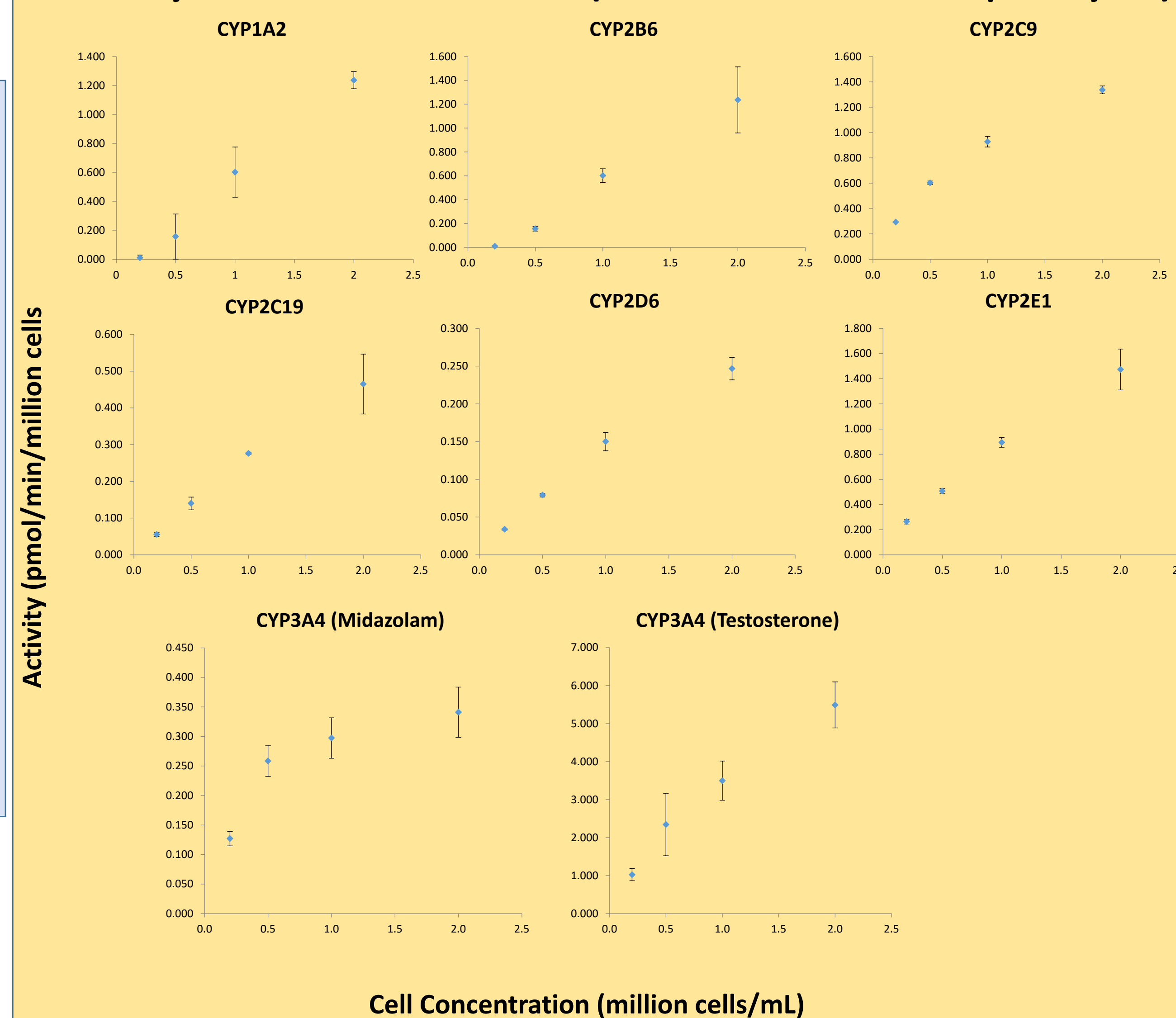
Cryopreserved Hepatocytes

Freezer to Incubation:
>30 minutes

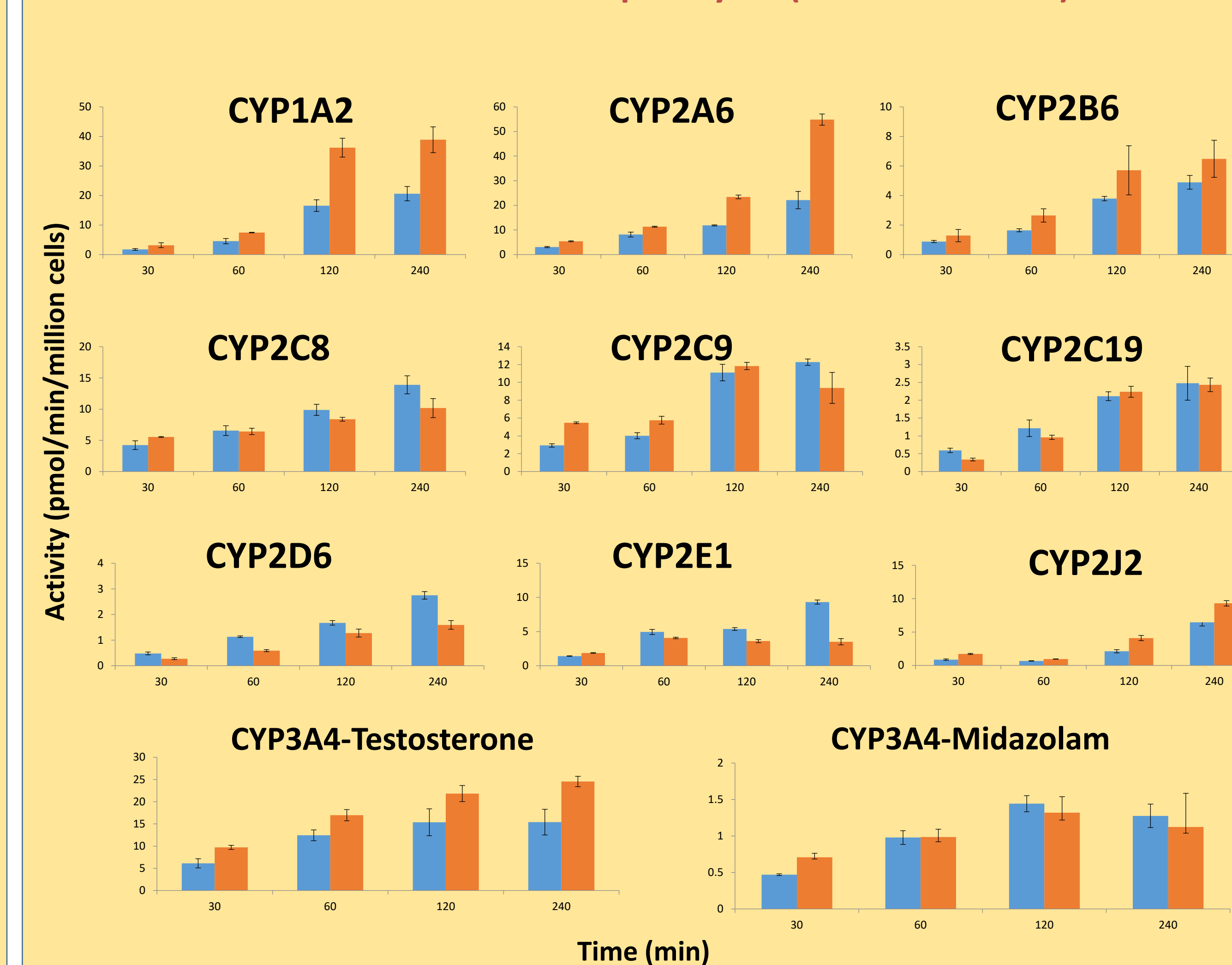
- Retrieve from LN2 Freezer
- Thaw in a 37 C water bath
- Add to recovery medium
- Centrifuge
- Microscopically quantify viability and cell number
- Adjust to 2X final cell density
- Add at equal volume to 2X test article
- Incubate

- 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

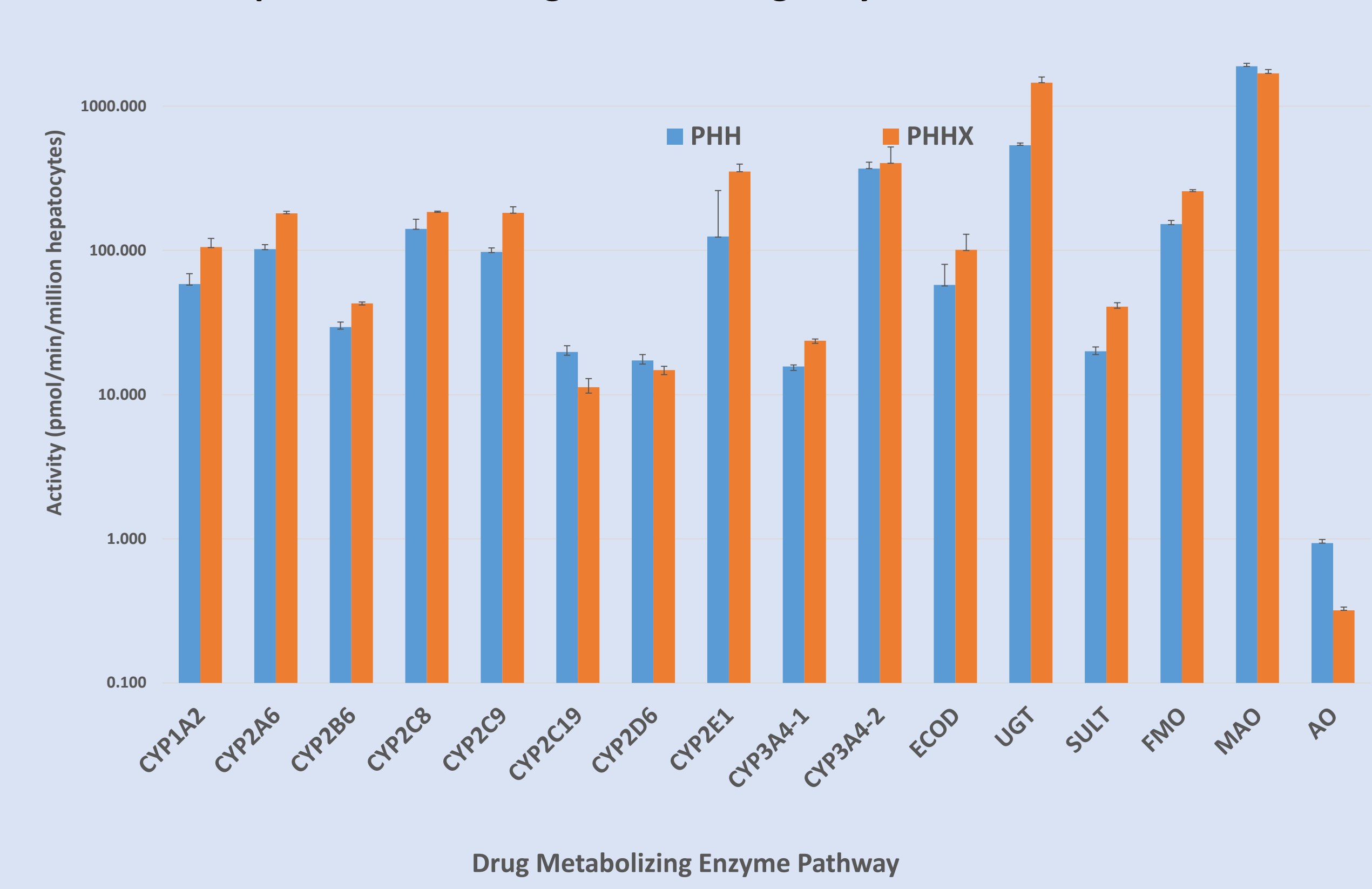
Activity vs Cell Concentration (MetMax™ Human Hepatocytes)



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

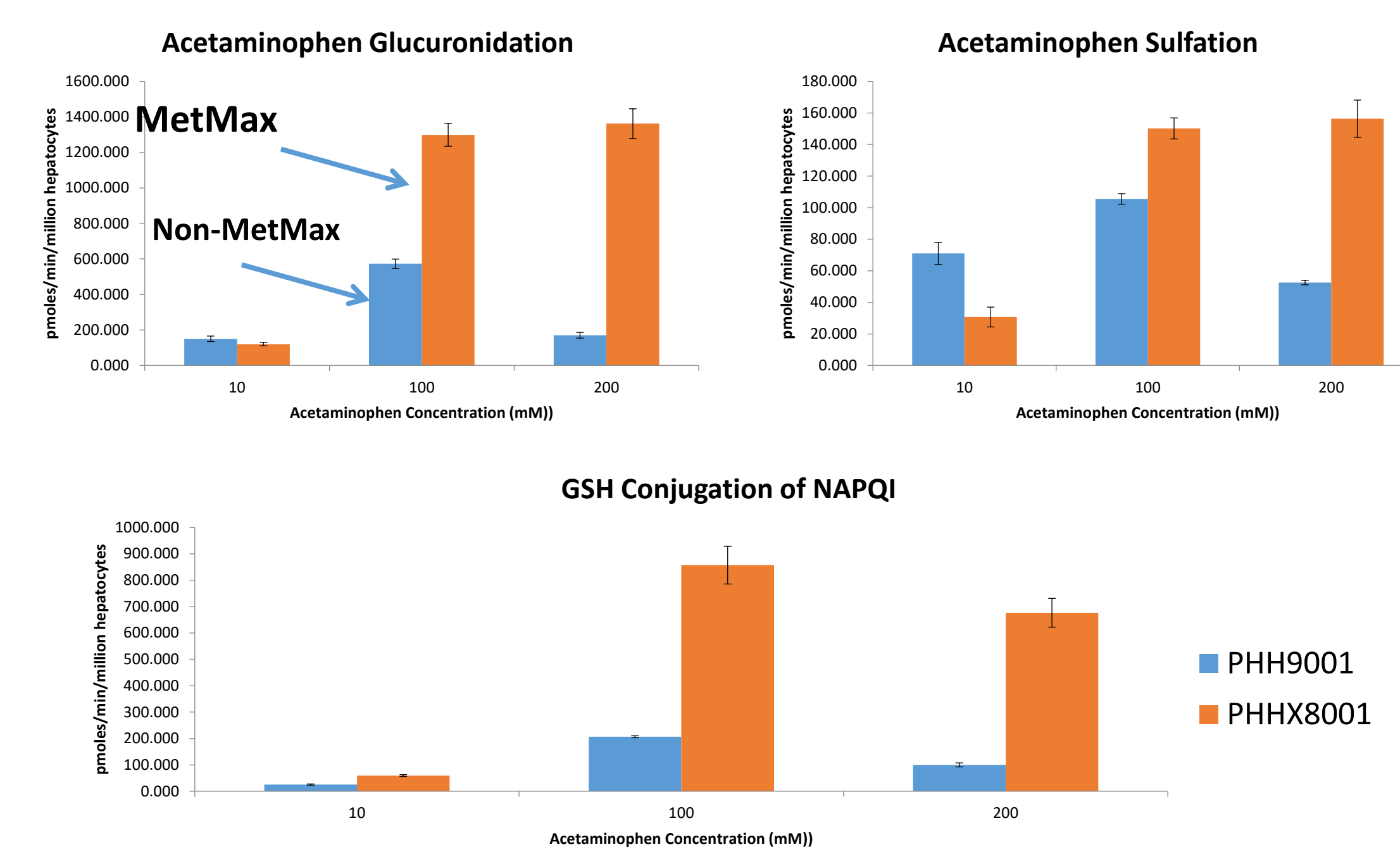


Intact (PHH) Vs MetMax™ (PHHX) Pooled Donor Human Hepatocytes: Comparison of 16 Drug Metabolizing Enzyme-Selective Substrates



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