MetMax™ Human Hepatocytes (MMHH) were prepared from a pool of 10 (5 male and 5 female) human donors using proprietary procedures (patent pending). The use of a multiple donor pool eliminates results unique to an individual, allowing results to be representative of the human population.

**Evaluation of metabolic capacity:** MMHH were incubated with various drug metabolizing enzyme-selective substrates for 30 minutes followed by LC/MS-MS quantification of metabolites.

**Metabolic Activation Assay for Protocixants:** HEK-293 cells, a commonly used cell line for in vitro cytotoxicity assays, were supplied to us by US EPA. The cells were maintained in T-75 flasks as log growing cells in DMEM containing 10% fetal bovine serum. On the day of experimentation, the cells were trypsinized and resuspended in the maintenance medium. Cell concentration was quantified via hemocytometer counting. Cell concentration was adjusted to 400,000 cells per mL. The cell suspension was plated into 384-well white cell culture plates with transparent bottom at a cell density of 4000 cells/well (10 uL of 400,000 cells/ml) and incubated overnight in a cell culture incubator with a humidified atmosphere of 5% carbon dioxide and 95% air. An addition-only high throughput procedure was used for the cytotoxicity assay. There were three treatment conditions: 1. HEK 293 cells without metabolic activation; 2. HEK 293 cells with metabolic activation; and 3. HEK 293 cells with inactivated metabolic activation. To group 1, 10 uL of hepatocyte medium but without MetMax™ human hepatocytes was added. To group 2, 10 uL of MetMax™ human hepatocytes was added. To group 3, 10 uL of inactive MetMax™ human hepatocytes (inactivated by heating for 15 minutes in boiling water) was added. Treatment was initiated by an addition of 10 uL of test articles at 1X of the final desired concentration. The final additional of test articles, the plates were returned to the cell culture incubator where they were incubated for treatment duration of 24 hrs followed by quantification of cell viability.

**GSH-Rescue Assay:** The MMHH/HEK293 GSH-Rescue assay is performed as described above in the presence and absence of exogenous GSH. Cell viability was determined at the end of the treatment duration using cellular ATP as endpoint quantified by luminescence using the Cell titer Glo (Promega) kit as per manufacturer’s instructions, and the PerkinElmer Victor3 Multimode Plate Reader. Results are presented as Relative Viability calculated using the following equation:

$$\text{Relative Viability} = \frac{\text{Luminescence (treatment)}}{\text{Luminescence (solvent control)}} \times 100$$

**Summary**

Two protocixants, acetaminophen and cyclophosphamide were evaluated for their cytotoxicity in HEK-293 cells in the presence and absence of MetMax™ Human Hepatocytes.

- The cytotoxicity of all four protocixants was increased by MetMax Human Hepatocytes.
- Metabolic activated cytotoxicity of the protocixants by MetMax™ Human Hepatocytes was attenuated by exogenous GSH, suggesting that the cytotoxicity was a result of the exposure of the HEK293 cells to reactive metabolites.

**Conclusions**

- MetMax™ Human Hepatocytes represent an effective exogenous metabolic activation system for the provision of human-specific drug metabolizing enzymatic activities for the evaluation of chemical toxicity in cell based in vitro cytotoxicity assays.
- In drug development, the assay can be used to identify drugs that are metabolically activated.
- The GSH-Rescue Assay may be useful in the identification of chemicals that are metabolized to cytotoxic reactive metabolites.
- In drug development, the assay can be used to identify drugs that are metabolized to cytotoxic reactive metabolites.

Drugs that are metabolically activated may have the following complications in drug development:

- Safety findings in preclinical animal species may not accurately reflect human effects due to species differences in drug metabolism.
- Individual differences in drug metabolism capacity in human populations may lead to individual differences in drug toxicity such as idiosyncratic drug toxicity (<5 per 100,000 patients).
- Idiosyncratic drug toxicity that, due to its low incidence, cannot be readily detected in Phase I, II and III clinical trials due to the limited number of patients.
- A large majority of drugs with idiosyncratic hepatotoxicity are known to form cytotoxic reactive metabolites.

There is a need to identify drug candidates with the potential to form cytotoxic reactive metabolites so that they can be excluded from further development to avoid the development of a drug with idiosyncratic drug toxicity.

**A Need for an Assay to Identify Drugs/Drug Candidates that Can Be Metabolized to Cytotoxic Reactive Metabolites**

**A Need for an Exogenous Activating System for the Evaluation of Xenobiotic Toxicity**

- Drug metabolism is a key determinant of drug toxicity. Drug metabolism may increase toxicity via the formation of metabolites with higher toxic potential than the parent drugs (metabolic activation), or lower toxicity due to the biotransformation of toxic drugs to less toxic metabolites (detoxification).
- In vitro hepatocytotoxic evaluation using primary hepatocytes allows the detection of metabolism-dependent toxicity due to the inherent drug metabolizing enzyme activities.
- Cytotoxicity assays using non-hepatic target cells (e.g. cardiomyocytes for cardiotoxicity) which do not have drug metabolizing enzyme activities may not be relevant to toxicity observed in vivo.
- While the liver post-mitochondrial supernatant (liver S9) has been used successfully as a metabolic activating system for in vitro genotoxicity assays, it is not applicable for in vitro cytotoxicity assays due to inherent cytotoxicity of the S9 cofactor mixture.

*We report here on a novel exogenous metabolic system, the MetMax™ human hepatocytes (MMHH), as a potentially useful system for the incorporation of hepatic metabolism in cell based in vitro toxicity assays.*