XENOTECH
A BioIVT Company

Hepatotoxicity

Drug induced liver injury (DILI) or hepatotoxicity is one of the leading causes of adverse events during clinical trials, which often results in failures or clinical holds during the development of new drug candidates. DILI has historically plagued drug makers with withdrawals from the market (*e.g.* troglitazone, oral bromfenac and tienilic acid). Furthermore, hepatotoxicity discovered post-approval has limited the use of several effective drugs, ultimately costing the pharmaceutical industry billions. To date, several mechanisms of DILI have been well-characterized and have the potential to be tested in a non-clinical setting or with *in vitro* tools. However, DILI observed in a clinical setting is often idiosyncratic with undefined mechanism(s) of toxicity and low incidence rates, even in large populations. With these factors in mind, early discovery of hepatotoxicity is essential to screening new drug candidates but poses many inherent challenges.

XenoTech offers flexible *in vitro* hepatotoxicity studies tailored to meet the needs of our clients with a focus on pragmatic solutions through the careful design of *in vitro* studies with a 'built-for-purpose' framework. Our goal is to work with your project team to design and guide an intelligent approach to the evaluation of hepatotoxicity with knowledge of mechanisms, *in vitro* tools and the application of various endpoints combined to provide data suitable for decision making, whether during early-stage discovery or late-stage development. Our extensive knowledge of *in vitro* drug metabolism, transport and production of liver-based products (*e.g.* liver microsomes and hepatocytes) from over 20 years of research and contract studies further aids in the development of a pragmatic approach to evaluate *in vitro* hepatotoxicity. We offer a variety of *in vitro* ADME assays, in either subcellular fractions and/or cell-based systems, from screening for reactive metabolites to screening for multiple mechanisms of cytotoxicity in primary or immortalized cell lines. Please contact us to discuss our full range of capabilities suited to your specific project needs.

Hepatotoxicity Methodologies

Available test systems in multi-well plates (*e.g.* 96-well)

- Cultured primary human hepatocytes (fresh/cryopreserved, individual /pooled), pre-evaluated with cytotoxic and non-cytotoxic compounds
- Cultured hepatocytes from non-human species (mouse, rat, dog, monkey, etc)
- Immortalized human hepatocytes (*e.g.* HepG2 or other commercially available cell lines)

Note: Cytoxicity assays can be adapted to non-hepatic cell lines (primary or immortalized) based on project needs and availablilty of cell lines.

Example cytotoxicity endpoints (including, but not limited to):

- Evaluation of cell membrane integrity (*e.g.* by LDH [lactate dehydrogenase] release)
- Mitochondrial respiration via measurement of indirect and direct loss of ATP production (*e.g.* ATP or reduction of resazurin)
- Early or late apoptosis (*e.g.* caspase 3/7 or caspase 8/9 activities)
- Glutathione (GSH) content or ratio of reduced to oxidized (GSH:GSSG)
- qRT-PCR analysis of mRNA (single or multiple gene expression assays)
- Other endpoints or mechanisms based on specific project needs through collaborators or commercial sources (*e.g.* project specific gene arrays)

Note: Evaluation of these endpoints is often time-dependent following treatment with compound(s). Incorporation of multiple time-points into the assay design is recommended in some cases.

Example Cytotoxicity Data (continued on back)

Cytotoxicity was evaluated in cultured human hepatocytes with five compounds with various mechanisms of cytotoxicity

Digitonin	A steroidal saponin glycoside that permeabilizes the plasma membrane of mammalian cells in culture. The disruption of cell membranes results in a release of intracellular proteins which can be measured by the release of LDH enzyme.
Oligomycin	A macrolide (or a gram-positive antibiotic) that inhibits ATP synthase which is necessary for oxidative phosphorylation of ADP to ATP. The cytotoxic effects of oligomycin can be observed by the loss of ATP measured by the reduction of resazurin, an indirect marker of mitochondrial respiration.
Benzobromarone	A non-purine inhibitor of xanthine oxidase used for the treatment of gout in Europe was withdrawn from the market by its manufacturer in 2003. Benzobromarone is known to cause hepatocyte and mitochondrial toxicity. The cytotoxicity of benzbromarone can be observed by the loss of ATP measured directly or indirectly (reduction of resazurin).
Staurosporine	A natural product derived form bacteria with a bis-indole structure. Staurosporine acts biologically to inhibit protein kinases. Its involvement in the induction of apoptosis is featured by the activation of Caspase 3, an early marker of apoptosis. The activation of Caspase 3 in response to staurosporine treatment can be observed after only a few hours.
Flumazenil	A selective benzodiazepine antagonist used to counter the effects of long-acting benzodiazepines prescribed medically. Flumazenil is a non-hepatoxic drug with corresponding profiles both *in vitro* and *in vivo*. Flumazenil is often used in cultured human hepatocytes as a negative control for CYP enzyme induction.

1101 W Cambridge Cir Dr | Kansas City, KS 66103 | *p* (877) 588-7530 / +913.438.7450 | *f* (913) 227-7100 | xenotech.com | info@xenotechllc.com

Figure 1. Time-dependent response of LDH release from primary human hepatocytes

Cultures of primary human hepatocytes were treated with digitonin, oligomycin, benzbromarone or flumazenil for 24 or 48 hours followed by the measurement of LDH enzyme released into cell culture medium. Digitonin (A), a positive control for membrane perturbation, caused a concentration- and time-dependent increase in LDH release. Oligomycin (B) and benzbromarone (C) disrupt ATP production whereby leading to membrane disruption as a secondary effect. Flumazenil (D) caused little to no cytotoxicity as evidenced by the lack of LDH enzyme release from cultured human hepatocytes.

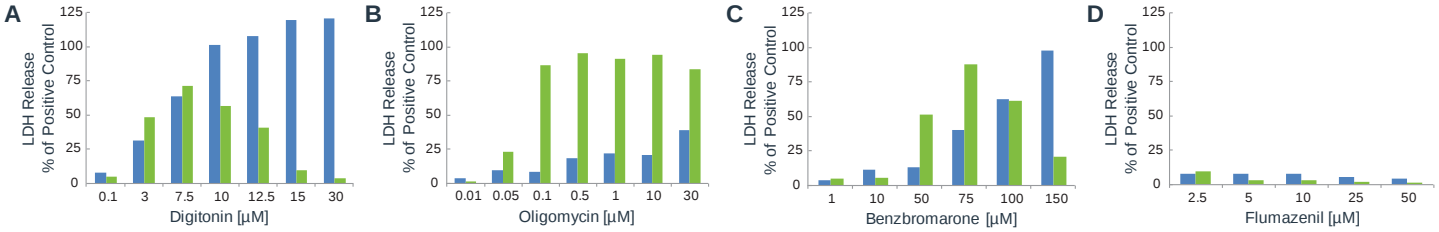

Figure 2: Time-dependent effect on mitochondrial respiration and ATP loss as measured by the reduction of resazurin

Cultures of primary human hepatocytes were treated with digitonin, oligomycin, benzbromarone or flumazenil for 24 or 48 hours followed by the measurement of the reduction of resazurin, an indirect marker of mitochondrial respiration. Digitonin (A), a positive control for membrane perturbation, caused a concentration- and time-dependent decrease in mitochondrial respiration with potency values similar to those observed for membrane disruption (LDH release). Oligomycin (B) and benzbromarone (C) both disrupt ATP production thus causing time- and concentration-dependent decreases in mitochondrial energy production as primary effect. Flumazenil (D) caused little to no cytotoxicity as evidenced by the lack of response in cultured human hepatocytes.

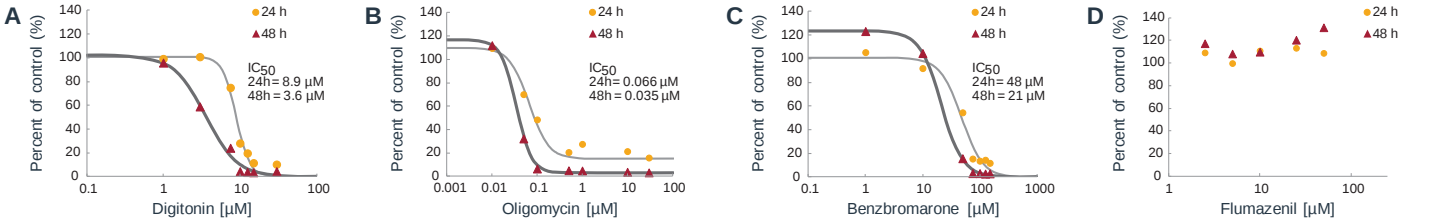

Figure 3: Concentration-dependent impairment of ATP production as measured by direct assessment of ATP levels

Cultures of primary human hepatocytes were treated with oligomycin, benzbromarone or flumazenil for 24 hours followed by the measurement of the reduction of resazurin, an indirect marker of mitochondrial respiration. Oligomycin (A) and benzbromarone (B) both disrupt ATP production thus causing concentration-dependent decreases in mitochondrial energy production as primary effect. Similar IC50 values between direct and indirect ATP measurements were observed for these compounds. Flumazenil (C) caused little to no cytotoxicity as evidenced by the lack of response in cultured human hepatocytes.

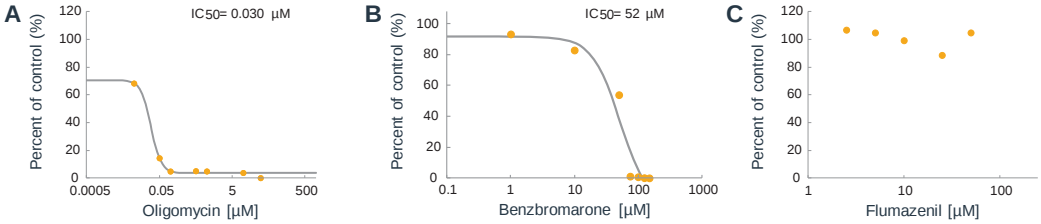

Figure 4: Concentration-dependent response of the early apoptosis marker caspase-3/7 by staurosporine

Cultures of primary human hepatocytes were treated with staurosporine for 6 hours followed by the measurement of caspase-3 and -7 activities, indirect markers of the early initiation of apoptosis. Staurosporine, a positive control for caspase-3/4 activation, caused potent and concentration- dependent increase in caspase activation.