

## BD Gentest<sup>SM</sup> Contract Research Services

Utilize our ADME experts to conduct your toxicity experiments and deliver results in a timely manner.

# Toxicity Services

## INTRODUCTION

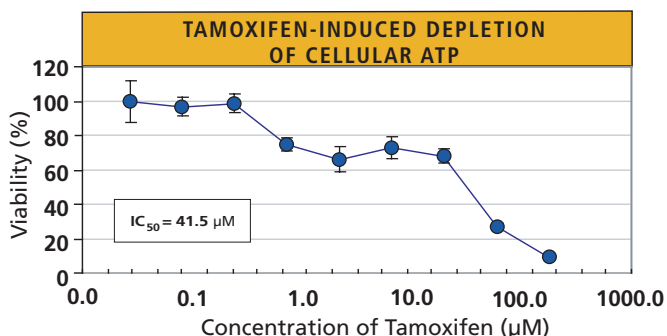
Toxicity is a critical area of concern for drug candidates. Compounds may exhibit intrinsic toxicity or must undergo metabolic activation by cytochrome P450 or other enzymes to manifest toxicity.

BD Biosciences combines its' expertise and state of the art toxicity detection technologies to help identify better drugs faster. We offer testing methods that capitalize on our extensive experience working with hepatocytes and recombinant cytochrome P450 enzymes.

## HEPATOCTE TOXICITY

Hepatotoxicity is a major cause of drug candidate failure, both pre- and post-market launch. The following assays for testing the potential of drug candidate-induced hepatocytes toxicity are available: 1) MTT reduction, 2) lactate dehydrogenase (LDH), and 3) Cellular ATP content. All tests are conducted using hepatocytes, the "gold standard" for analyzing hepatocyte toxicity *in vitro*. These tests serve as independent means to determine cytotoxicity with cell viability as the end-point.

### TAMOXIFEN-INDUCED DEPLETION OF CELLULAR ATP

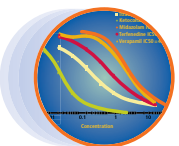
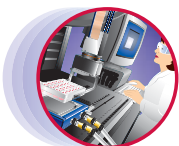


*BD Gentest<sup>SM</sup> Cryopreserved Human Hepatocytes were thawed and purified using the BD Gentest Cryopreserved Hepatocyte Purification Kit (Cat. No. 454500). Cells were resuspended in MEM and incubated with tamoxifen over a four-hour time period. The concentration of cellular ATP was determined using a chemiluminescent assay (Perkin Elmer). The data are the mean  $\pm$  standard deviation of four wells.*

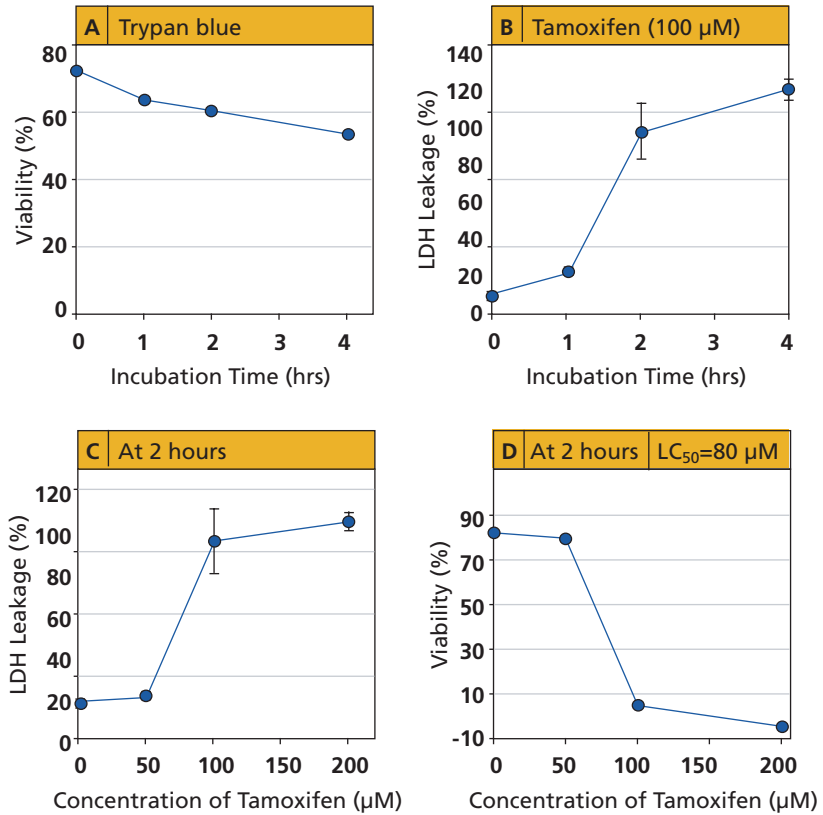


## EXPERIMENTAL OUTLINE

- 1 Determine study design based on client needs
- 2 Prepare the desired test systems; short-term incubation (hepatocyte suspensions) or long-term incubation (primary cultures)
- 3 Incubate cells with test articles over desired time periods
- 4 Determine the toxicity potential of test article using appropriate endpoints
- 5 Expert data analysis is provided in a signed, detailed report from one of our Study Directors

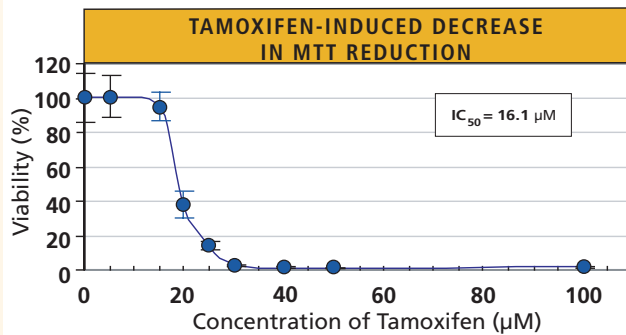


### TAMOXIFEN-INDUCED LEAKAGE OF LACTATE DEHYDROGENASE (LDH)



*BD Gentest™ Cryopreserved Hepatocytes were thawed and purified. The cells were then incubated with tamoxifen in a 24-well plate. The activity of LDH in medium was determined at different period of incubation. The data are the mean ± standard deviation of three wells. A: Control viability determined by trypan blue; B: Time-response; C: Concentration- response; D: Cell viability and LC<sub>50</sub> calculated from concentration-response.*

### TAMOXIFEN-INDUCED DECREASE IN MTT REDUCTION



*Primary cultured BD Gentest™ Human Hepatocytes were incubated with tamoxifen in a 96-well plate for three days. The reduction of MTT in hepatocytes was determined. The data are the mean ± standard deviation of five wells.*

In these tests, the concentration of drug candidate needed to cause 50% cell toxicity/death to human hepatocytes is determined. The number and spacing of drug concentrations are flexible for these tests. BD Biosciences also offers testing using hepatocytes from other preclinical species.

## TOXICITY TESTING IN A HUMAN CELL LINE ENGINEERED TO EXPRESS P450

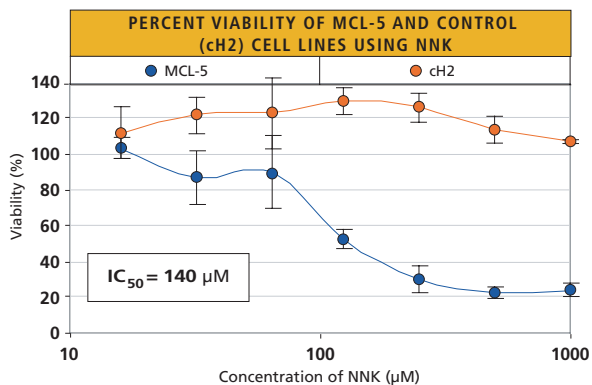
The BD Gentest™ Metabo-Tox Assay Kit is designed to assess P450-mediated toxicity. BD Biosciences uses its' MCL-5 cell line that expresses five human P450 enzymes (CYP 1A1, 1A2, 3A4, 2A6, and 2E1) and epoxide hydrolase in our toxicity assays. Using this line, BD Biosciences can create a toxicity assay with consistent results over time. This is achieved because this cell line avoids the variations in P450 expression that occur when using human hepatocytes.<sup>2</sup> The human P450s incorporated into the MCL-5 cell line are the major P450s implicated in creating toxic metabolites. The MCL-5 assay can differentiate between parent and metabolite toxicity in one assay by comparing IC<sub>50</sub> values with the control cell line cH2, which does not contain the transfected genes. BD Biosciences measures oxygen respiration as an indicator of cell viability,<sup>3</sup> although other assays such as measurement of cellular ATP content are available. The number and spacing of drug concentration is flexible.

### SUMMARY OF IC<sub>50</sub> VALUES FOR METABOLISM-DEPENDENT TOXINS USING THE BD GENTEST™ MCL-5 METABO-TOX ASSAY

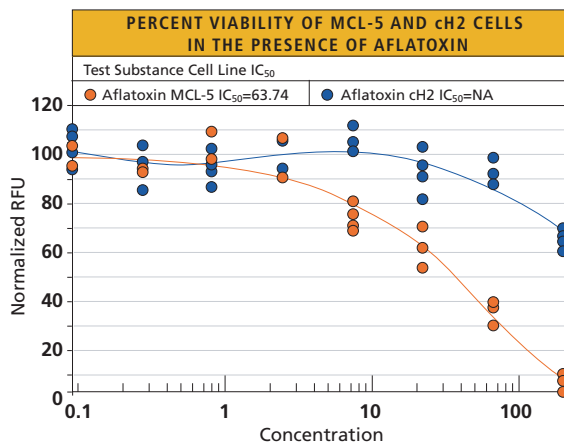
Test Substrate	cH2 cells	MCL-5 cells	Major P450s
Benzopyrene	8% toxicity > 20 µM	1.6 µM	1A1, 3A4, 1B1
Aflatoxin	20% toxicity > 200 µM*	48 µM	3A4, 1A2, 1A1
NNK	no toxicity > 1000 µM	140 µM	2A6, 2E1, 2D6
Dibenzopyrene	10% toxicity > 200 µM*	2.3 µM	1A1, 3A4, 1A2
Acetaminophen (50 µM BSO pretreated)	2.9 mM	1.2 mM	2E1, 3A4, 1A2
PhIP	111 µM	21 µM	1A1
Cyclophosphamide	5.5 mM	2.8 mM	2B6, 3A4

\*Data are obtained from cHOL cells which are the parent cell to the cH2 cells.

NNK, 4-(methylnitrosoamino)-1-(3-pyridinyl)-1-butanone;  
PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine.



The IC<sub>50</sub> values calculated for MCL-5 cells by linear interpolation was determined to be 140 µM NNK. The cH2 cells demonstrated no toxicity up to 1,000 µM.



The IC<sub>50</sub> value was generated using the BD Gentest™ MPMIADMET Software Program and a 4-parameter fit model. The cH2 cells demonstrated 20% inhibition at 200 nM aflatoxin after 72 hours of exposure.

## CONTACT INFORMATION

For more information, contact Technical Service at:

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Your local BD Gentest™ Products and Services Sales Specialist will provide you with initial study and price information. Your project will be assigned to a Study Director who will coordinate and tailor your Toxicity Study to your total satisfaction. A finished detailed report will be provided.

### References

1. Tirmerstein M.A., et al. Glutathione depletion and the production of reactive oxygen species in isolated hepatocyte suspensions. *Chemico-Biological Interactions*. **127**:201 (2000).
2. Crespi, C.L., et al. A metabolically competent human cell line expressing five cDNAs encoding procarcinogen-activating enzymes: application to mutagenicity testing. *Chem. Res. Toxicol.* **4**:566 (1991).
3. Wodnicka, N., et al., Novel fluorescent technology platform for high throughput cytotoxicity and proliferation assays. *J. Biomol. Screen.* **5**:141 (2000).

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