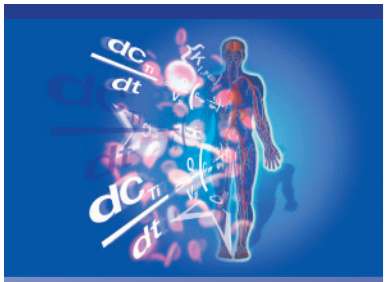
cyprotex AN EVOTEC COMPANY

*In vitro* **Toxicology**

# High Content Toxicology: CellCiphr® Premier

## Background Information



'The rapid expansion of HCS technology throughout the pharmaceutical industry and academic research centers validates the usefulness of the information-rich screening approach.'

[2]Zanella F, Lorens JB and Link W (2010) *Trends Biotechnology* **28(5)**; 237-245

- Hepatotoxicity is one of the main reasons for drug withdrawals, accounting for 37% of all therapeutics taken off the market between 1994 and 2006.[1]
- CellCiphr® Premier combines an extended panel of toxicologically-relevant endpoints with Cyprotex's CellCiphr® classifier to provide one of the most reliable clinical hepatotoxicity predictors available (sensitivity = 70% and specificity = 100%).
- Both HepG2 (replicating cells) and primary rat hepatocytes (metabolically competent cells) are investigated at multiple time points (extending from 24 hrs to 5 days to identify early and late stage toxic effects).
- The data can be integrated with actual or predicted $C_{max}$ to correct for exposure-related effects.
- The extended panel of end points allows CellCiphr® Premier to offer enhanced predictivity for hepatotoxicity risk over the standard CellCiphr® panels.

### Protocol

**Instrument**
Cellomics ArrayScan® VTI (Thermo Scientific)

**Analysis Method**
High Content Screening with CellCiphr® Classifier System

**Cell Types**
HepG2 (replicating) and primary rat hepatocytes (metabolically competent)

**Toxicity Markers**
Extended panel of toxicity markers including:

Apoptosis
Cell cycle arrest
Cell loss
Cytoskeletal disruption
DNA fragmentation and damage response
Glutathione depletion
Mitochondrial function
Mitosis marker
Nuclear size
Oxidative stress
Phospholipidosis
Reactive oxygen species
Steatosis
Stress kinase activation

**Test Article Concentration**
10 point dose response curve in duplicate

**Data Delivery**
CellCiphr® toxicity report including;
$AC_{50}$
Safety ranking
Safety alert
Heat maps (normalized to $C_{max}$ if available)

To find out more contact **enquiries@cyprotex.com**

## **CellCiphr® Premier is one of the most reliable** clinical hepatotoxicity predictors available.

**Figure 1a**
Reactive Oxygen Species (ROS) Assay. Rat hepatocytes were stained with Hoechst and CM-$H_2$DCFDA after 4 hour incubation with vehicle (DMSO) or Compound 1, which induces ROS. CM-$H_2$DCFDA enters the cell and reacts with ROS to create a fluorescent signal. ROS levels were determined using the ArrayScan® VTI.

**Figure 1b**
Glutathione (GSH) Assay. Rat hepatocytes were stained with Hoescht and monochlorobimane after 18 hour incubation with vehicle (DMSO) or Compound 2, which depletes GSH. Monocholorobimane reacts with cellular GSH. GSH levels were determined in the cytoplasm of the cells using the ArrayScan® VTI.

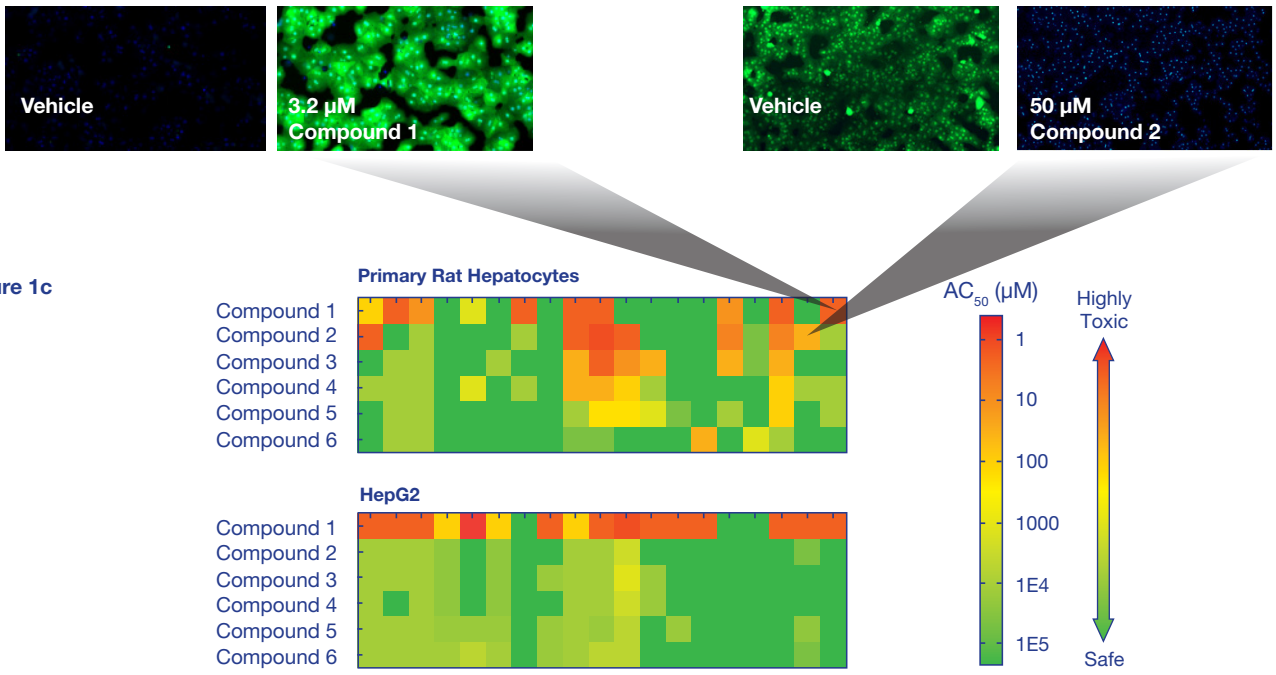



**Figure 1c**

Representative heat maps for CellCiphr® Premier. The heatmaps demonstrate the pattern of endpoint response for the six compounds in a set. The $AC_{50}$ of the endpoint response is shown using a colour scale from red (highly toxic) to green (safe) with the bar at the right showing the full range of the $AC_{50}$ scale. The heatmaps demonstrate various biomarkers at different time points giving rise to a "toxicity risk fingerprint" for each compound. These data can be used to determine the mechanistic pathways causing the toxic response and the time course over which it occurs. The $AC_{50}$ data from the different endpoints, for example GSH and ROS activity, are represented by specific boxes on the heatmaps, as demonstrated by the grey connectors that link to the detailed images shown in Figures 1a and 1b.

**Table 1**
Sensitivity and Specificity of CellCiphr® Premier to Predict Human Hepatotoxicity.

| | Sensitivity | Specificity |
|---|---|---|
| HIAT Method[3] | 35% | 100% |
| CellCiphr® Premier | 70% | 100% |

A blind set of 47 compounds were screened in CellCiphr® Premier to establish the sensitivity and specificity of the model. Data generated were compared with existing data from the HIAT method. Mechanistic endpoints were normalised for $C_{max}$ to predict toxicity relative to exposure. The compounds chosen were known to be poorly sensitive (i.e.: they were mainly false negatives) in the HIAT method. In summary, the CellCiphr® Premier method shows greatly increased sensitivity over the HIAT method for this set of compounds.


**References**

1 Dykens JA and Will Y (2007) *Drug Discovery Today* **12**; 777-785
2 Zanella F *et al.*, (2010) *Trends Biotechnol* **28(5)**; 237-245
3 Xu JJ *et al.*, (2008) *Toxicol Sci* **105(1)**; 97-105