



Use of Cellular Systems Biology to Evaluate the ToxCast™ Phase I Data in Two Liver Cell Models

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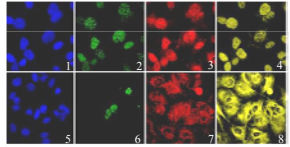
US EPA ToxCast Data Summit Meeting

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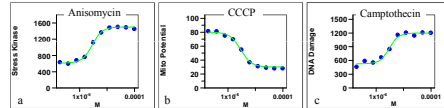
Introduction

- Cellular System Biology uses highly multiplexed cell-based assays with relevant cell types and toxicity biomarkers that represent the cellular system response



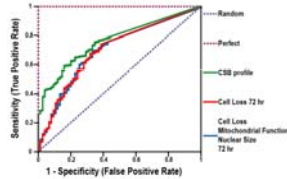
- Cell Number
- Stress Pathway Activation
- Mitochondria Function
- Oxidative Stress
- Nuclear Area
- DNA damage
- Cell Cycle
- Cytoskeleton Integrity

- End points are evaluated over 3 time points (acute, early & late exposures) across a 10-point dose response curve in a 384-well format

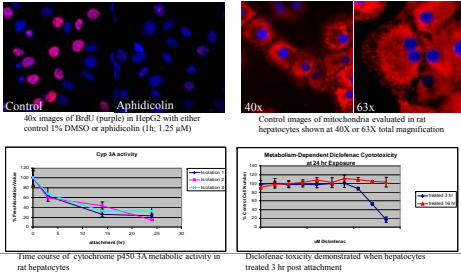


Representative 10-point dose response curves in HepG2 cells for c-JUN-P (a), mitochondria Ψ (b), or p-53 activation (c) following 1 hr exposures

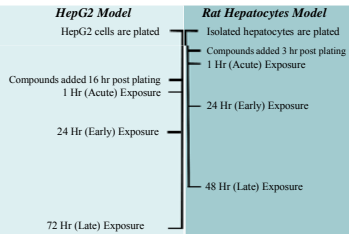
- Use of Cellular Systems Biology improves toxicological predictivity



- Characteristics of dividing HepG2 (~50% BrdU+) and metabolically-competent rodent hepatocyte liver models used to evaluate the EPA ToxCast 320 test set



Methods

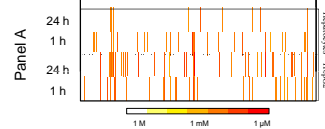


Experimental flow chart for HepG2 and rat hepatocyte assays

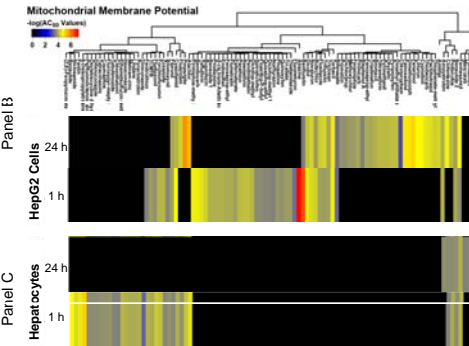
HepG2	Rat Hepatocytes
Cell Loss (CL)	Cell Loss (CL)
Nuclear Size (NS)	Nuclear Size (NS)
DNA Damage (DDA)	DNA Damage (DA)
Mitochondria Potential (MP)	Mitochondria Potential (MP)
Mitochondria Mass (MM)	Apoptosis (AP)
Cytoskeleton (CCA)	DNA Fragmentation (DF)
Microtubule Stability (MS)	Steatosis (ST)
Mitotic arrest (MA)	Lysosomal Mass (LM)
Oxidative Stress (OS)	
Stress Kinase (SK)	

Toxicity biomarkers evaluated in the HepG2 and rat hepatocyte assays with abbreviations in parenthesis

Mitochondrial Membrane Potential Effects in Both Models

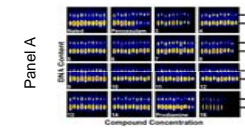


Panel A. Decreased membrane mitochondria Ψ observed following acute exposures in both models. Pesticides had less effect on rat hepatocytes following 24 hour exposure (Early) compared to HepG2 cells.

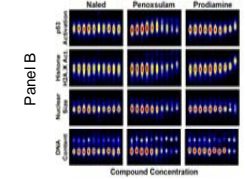


Pesticide cluster relationships based on mitochondria membrane potential AC50 values in HepG2 cells (Panel B) and hepatocytes (Panel C). In both panels, a time-dependent change in pesticide effects is observed, indicating the value in evaluating multiple time points

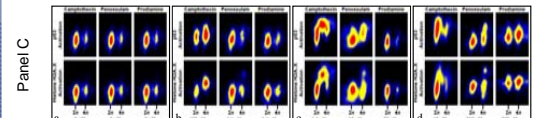
Toxicity Mode of Action Evaluation in a Subset of the Pesticide Test Set



Panel A. The DNA content distributions for 16 pesticides are shown as a matrix of distribution maps at 24 h. Penoxsulam and proflumicarb induced a loss of S-phase cells as well as an increase in the population of 4n cells relative to the untreated cell population, consistent with the induction of both a G1 and G2 cell cycle.



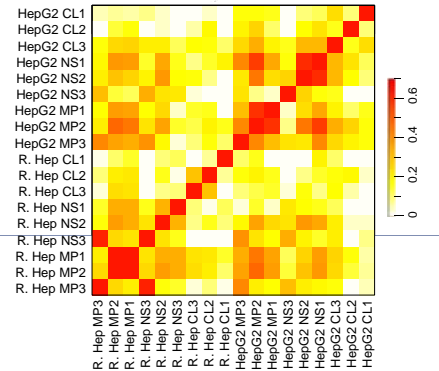
Panel B. Distribution maps of the p53, histone H2A.X, and nuclear size responses at 24 h. Penoxsulam and proflumicarb showed distinct concentration-dependent effects on all three biomarker features. High concentrations of proflumicarb induced a relatively homogeneous activation of histone H2A.X whereas high concentrations of penoxsulam induced a much more heterogeneous increase in this biomarker feature.



Panel C. Cell maps reveal molecular interrelationships that underlie the toxic response of cells. a-d. Camptothecin induced a G2 cell cycle block at low concentrations (b, dominant 4n peak) and a G1 cell cycle block at higher concentrations (d, dominant 2n peak). Penoxsulam and proflumicarb showed aspects of G1 and G2 cell cycle blocking activity dependent on compound concentration. Penoxsulam and proflumicarb induced moderate increases in histone H2A.X and p53 activation that was maximal at 200 μ M.

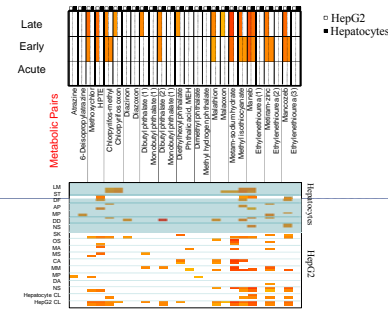
■ Low Cell Population Density
■ Medium Cell Population Density
■ High Cell Population Density

Correlation of Common Endpoints Between Cell Models



Correlation of endpoints common between the HepG2 and rat hepatocyte (R. Hep) models following acute (1), early (2) and late (3) exposures.

Differential Activity Observed Between Parent/Metabolite Pairs in HepG2 cells and Rat Hepatocytes



In general, parent compounds demonstrate greater toxicity in HepG2 cells than hepatocytes (ex. Metam-sodium hydrate > methyl isothiocyanate) possibly due to decreased metabolic ability or conjugation potential.

Summary & Conclusions

- Cell model comparison indicated differential toxicity susceptibilities
 - Dividing HepG2 cell model enables detection of pesticide activities affecting cell cycle regulation.
 - Primary rat hepatocytes model provided information on the response of a metabolically active cellular system.
- Effects of pesticides on mitochondrial membrane potential
 - The metabolic activity of primary hepatocytes resulted in diminished sensitivity to pesticide activity
 - Both model systems showed time-dependent effects demonstrating the need for multiple time point evaluation
- Dissecting and comparing cell population responses to pesticide action
 - Cell populations exhibit unique heterogeneous responses
 - Complex mechanistic interrelationships can be dissected using population analysis of multiplexed assays
- Results demonstrate the advantages of a cellular systems approach in the ranking of unknown compound toxicity outcomes.

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