cyprotex AN EVOTEC COMPANY

In vitro **Toxicology**

pH3 and pH2AX Genotoxicity

Background Information

'Aneugenic compounds induced either an increase or a decrease in p-H3 depending on their mode of action. Clastogens induced γH2AX, and cytotoxic compounds generated a marked decrease in these two biomarkers.'

[1]Khoury L, Zalko D and Audebert M (2016) *Arch Toxicol* **90(8)**; 1983-1985

- High Content Screening uses automated fluorescence microscopy to measure indicators of cellular health and quantify biomarkers.
- Two key classes of genotoxic agents are clastogens and aneugens. Clastogens directly damage DNA resulting in double strand DNA breaks. Aneugens produce numerical chromosome aberrations (aneuploidy), the result of "lagging" chromosomes.
- Phospho-histone 3 (pH3) is a marker of mitosis and cell cycle arrest in G2/M phase, anuegens have been shown to increase pH3 levels[1]. Phospho-histone 2AX (pH2AX) is a marker for double strand DNA breaks, indicating direct DNA damage caused by clastogens and some aneugens.
- HepG2 cells were selected due to their wild type p53 status, shown to be important in accurate genotoxicity prediction[2].

Protocol

Cell Line
HepG2 cells

Time Point
24 hr

HCS Analysis Platform
Cellomics ArrayScan® VTI (Thermo Scientific)

Metabolising System
With or without S9 fraction

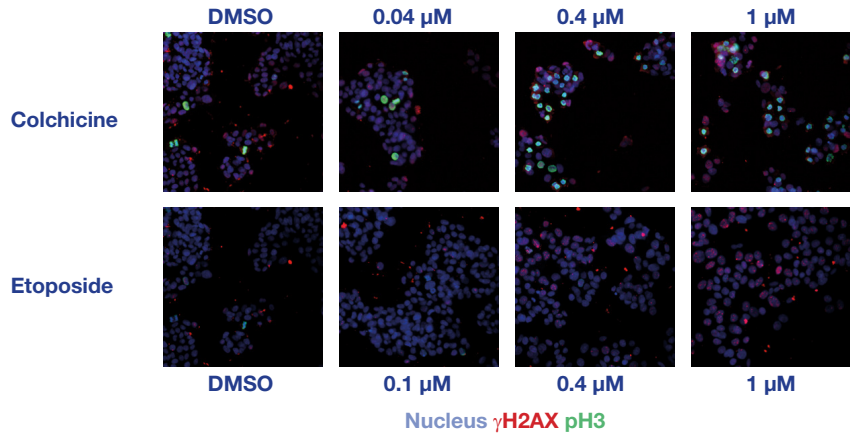
Test Article Requirements
5-10 mg solid or equivalent solution depending on top concentration required (200x to maintain 0.5% vehicle)

Assay Controls
Chlorpromazine and colchicine (+S9)
Colchicine and methyl methanesulfonate (-S9)

Endpoints
Cell count
Nuclear size
DNA structure
pH3 level
pH2AX level
Genotoxicity category

Figure 1

Representative HCS images of pH3 and pH2AX immunocytochemistry.



HepG2 cells were treated with colchicine and etoposide for 24 hrs prior to staining for pH3 (green) and pH2AX (red). Hoechst 33342 (blue) provides a cell count and identifies changes in DNA structure associated with cytotoxicity. Colchicine, an aneugen, demonstrated a dose dependent increase in pH3 staining. The clastogen etoposide shows an increase in γH2AX in a dose dependent fashion.

Table 1

pH3 and pH2AX data for a set of 15 compounds incubated over 24 hr with HepG2 cell in the presence and absence of S9

A.

+ pH2AX	- pH3 = Clastogen
	+ pH3 = Aneugen
- pH2AX	- pH3 = Cytotoxic/- ve
	+ pH3 = Cell cycle inhibitor/Aneugen
Threshold 1.5	

B.

		-S9				+S9				Mechanism	
		pH2AX		pH3		pH2AX		pH3			
Compound	**Mechanism**	**MEC**	**Max response**	**MEC**	**Max response**	**MEC**	**Max response**	**MEC**	**Max response**	**-S9**	**+S9**
vinblastine	Aneugen	NR	NR	<0.004	2.28	0.0193	1.6	<0.004	2	Cell cycle inh/Aneugen	Aneugen
colchicine		0.022	1.47	0.0112	4.2	0.0592	1.39	0.00889	1.93	Cell cycle inh/Aneugen	Cell cycle inh/Aneugen
paclitaxel		0.325	1.3	0.006	3.91	0.189	1.41	0.0253	3.95	Cell cycle inh/Aneugen	Aneugen
carbendazim		NR	NR	1.56	2.74	0.0272	3.23	0.0136	1.34	Cell cycle inh/Aneugen	Clastogen
griseofulvin		17.2	1.41	4.54	3.2	26.1	1.74	8.81	2.68	Cell cycle inh/Aneugen	Aneugen
methyl methanesulfonate	Clastogen	124	2.53	NR	NR	41.1	3.49	264	1.34	Clastogen	Clastogen
etoposide		0.141	1.63	NR	NR	0.288	1.61	NR	NR	Clastogen	Clastogen
4-nitroquinoline N-oxide		0.277	6.18	1.76	-0.553	0.727	6.97	NR	NR	Clastogen	Clastogen
chlorambucil		2.12	3.05	NR	NR	4.15	7.01	185	1.31	Clastogen	Clastogen
cyclophosphamide		NR	NR	NR	NR	17.6	1.86	166	1.2	Cytotoxic/- ve*	Clastogen
araC		0.029	3.95	NR	NR	0.0272	3.23	0.0245	1.32	Clastogen	Clastogen
7, 12-dimethylbenz[a]anthracene		<0.08	1.57	NR	NR	1.55	1.81	NR	NR	Clastogen	Clastogen
chlorpromazine	Cytotoxic	NR	NR	NR	NR	NR	NR	NR	NR	Cytotoxic/- ve	Cytotoxic/- ve
CCCP		1.22	1.43	NR	NR	6.66	1.3	7.01	1.33	Cytotoxic/- ve	Cytotoxic/- ve
staurosporine		0.179	1.27	<0.012	-0.257	2.73	1.27	0.203	-0.655	Cytotoxic/- ve	Cytotoxic/- ve

HepG2 cells were incubated with a panel of compounds in the presence or absence of S9 fraction for 24 hours then stained for pH3 and pH2AX. The threshold for a positive response was set at 1.5 fold greater than vehicle controls. Any data point where over 50% cell loss had occurred were excluded for all endpoints. Compounds were categorised based on both endpoints (table 1A).

In table 1B, mechanisms were predicted according to the criteria set out in table 1A, green boxes show correctly predicted compounds. Orange boxes are above the 1.5 threshold, blue boxes are below the 1.5 threshold. * Cyclophosphamide is metabolically activated.

References

[1] Khoury L *et al*., (2016) *Arch Toxicol* **90(8)**; 1983-1995
[2] Kumari R *et al*., (2014) *Mole Cell Oncol* **1(13)**; DOI: 10.4161/23723548.2014.969653