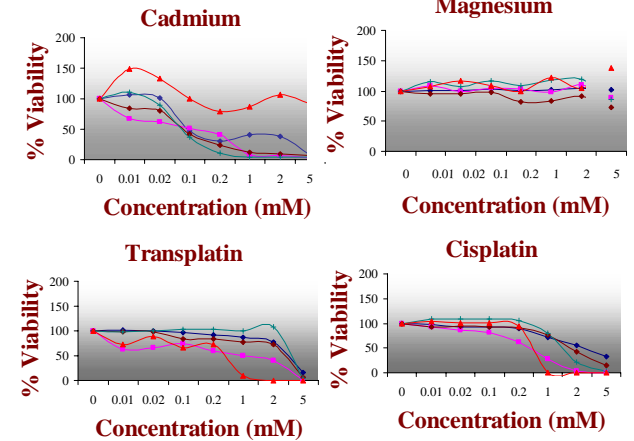




# High Throughput Screening for Drug Toxicity

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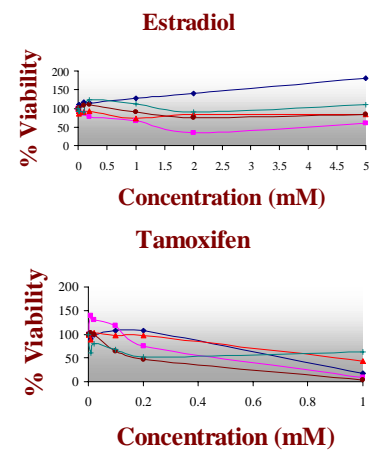
## Nephrotoxicity



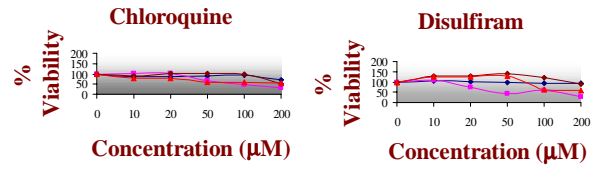
## Hepatotoxicity

Responses of Multiple Human Donors to Treatment with Drugs

Donor	EC50 Values (µM)			
	Estradiol	EE2	Tamoxifen	Rosiglitazone
105	102.6	105.0	89.1	87.1
108	207.3	69.9	105.0	105.3
109		52.7		128.6
111	122.0	35.0	55.7	88.8
113		97.6	91.4	132.2
114			82.7	88.1
118			142.8	233.8
119		84.5	90.7	191.4
122		90.2	71.9	124.4
129		37.9	92.3	196.4
130		29.6	77.8	111.7
133	82.5	76.7	81.5	125.3
59		94.6	92.6	101.7
61		35.5	63.7	62.6
62	155.1	73.4	63.6	73.1
66			95.1	146.2
70	118.9	35.9	82.0	95.3
71	104.7	85.7	83.4	103.2
75		40.8		
86	650.6	73.3	76.3	135.9
88			95.6	142.7
89	80.9		99.6	57.0
90	88.0	50.6	78.0	162.2
91			139.4	
95		32.3	64.9	138.8
CNV			93.2	133.8
DRL			78.1	103.8
EPA			65.2	98.9
ETR			85.6	117.9
EVY		64.5	69.3	105.8
GNG	148.9	70.6	85.3	105.4
HRK	187.8		115.2	124.5
KMI	146.5	76.2	101.3	103.7
MOF		39.1	57.8	123.3
MYO	97.9			
TVC	203.6	0.8	91.7	74.4



## Neurotoxicity

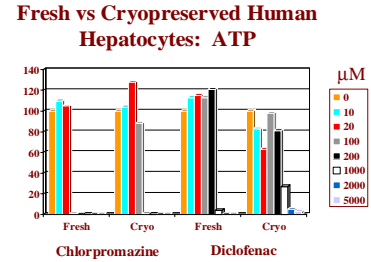


### Introduction

In our laboratory, assays are being developed for early screening of human target organ toxicity. Hepatotoxicity is studied using primary and previously cryopreserved human hepatocytes. Nephrotoxicity is studied using primary cells derived from human kidneys. Neurotoxicity is being studied using neuroblastoma cell lines. Several endpoints for toxicity are being evaluated, including cellular adenosine triphosphate (ATP) content, mitochondrial metabolism of (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (MTT), cellular reduced glutathione (GSH) levels, Neutral Red uptake, and Alamar Blue metabolism.

### Materials and Methods

- Cryopreserved human hepatocytes (Li et al., 1999) and renal cortical cells were isolated using previously published methods.
- Human neuroblastoma cells were purchased from American Type Culture Collection.
- All cells were incubated with compounds of known toxicity for 24 hours at 37 °C, 5% CO<sub>2</sub>.
- Cytotoxic effects were evaluated by measuring ATP production, reduction of MTT and Alamar Blue, and Neutral Red uptake.
- Glutathione depletion by toxic compounds was measured by incubation with o-phthalaldehyde.



### Legend

(Graphs)  
 Alamar Blue  
 ATP  
 GSH  
 MTT  
 Neutral Red

(Table)  
 EC50 >500 µM  
 EC50 100-500 µM  
 EC50 <100 µM

### Conclusions

- High throughput *in vitro* toxicology assays have been developed for hepatotoxicity, nephrotoxicity and neurotoxicity.
- Hepatotoxicity studies using cryopreserved human hepatocytes allow the detection of human-specific effects.
- An *in vitro* model for phase I clinical trial has been developed using cryopreserved human hepatocytes from multiple donors, yielding data illustrating individual differences in response to hepatotoxicants.
- Primary human kidney tubule cells have been established and cytotoxicity assays developed. This assay may allow the evaluation of human-specific toxicity
- Neuroblastoma cells are currently used for the evaluation of neurotoxicity. The relevance of this model may be hampered by the species of origin (rats) and the transformed status of the cells.

### References

Li, A. P.; Lu, C.; Brent, J. A.; Pham, C.; Fackett, A.; Ruegg, C. E.; and Silber, P. M. Cryopreserved human hepatocytes: characterization of drug-metabolizing enzyme activities and applications in higher throughput screening assays for hepatotoxicity, metabolic stability, and drug-drug interaction potential. *Chem. Biol. Interact.* 1999, 121, 17-35.