Dose response from high throughput gene expression studies and the influence of time and cell line on inferred mode of action by ontologic enrichment.

### Introduction

Gene expression with ontologic enrichment and connectivity mapping tools is widely used to infer modes of action (MOA) for therapeutic drugs. Despite progress in high-throughput (HT) genomic systems, strategies suitable for identifying industrial chemical MOA are needed. The L1000 is a HT genomics platform that measures 1000 landmark genes to computationally predict expression across an equivalent whole human genome array. We used the L1000 system with visualization tools to assess predicted gene expression changes and ontologic enrichment for 8 agrochemicals at 9 concentrations (0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10 μM) across 5 cell lines (Table 1 & 2) at 6 and 24 hr after treatment. Genes significant for a monotonic dose response (log likelihood ratio test with permutations) were used for analyses.

### Results

Differential gene expression levels varied significantly across cell lines and times, with no one cell line consistently responsive for any chemical at both times. A broad assessment of the 9 chemicals showed that A549 and HT29 cells (at 6 hr) and HepaRG and A673 cells (at 24 hr) were typically the most responsive. However, at 24hrs, the greatest number of gene expression changes were seen with A673 cells. These too demonstrated significant enrichment for immune processes but there was an absence of the cell cycle and mitotic process signals seen earlier.

Comparison of HepaRG and A673 cell responses (at 24hr) for Fenbuconazole, a fungicide associated with liver effects, showed enrichment of common parent pathways (DNA repair, cell signaling) in both cell types, but several child categories of these were identified only in HepaRgs. Fenbuconazole exposure is linked with increased liver weight, hepatocellular hypertrophy and proliferation, and clinical chemistry changes (changes in cholesterol and triglyceride levels, and increases in serum activity of hepatic enzymes) were observed in rat, dog and mouse exposures. This fungicide has also been seen to induce mouse liver cytochrome P450 activity in a manner similar to phenobarbital. Active ToxCast assays for Fenbuconazole are associated with cell cycle pathways, cell proliferation and cell death.

Both A673 and HepaRG cells exposed to Fenbuconazole detected significant up-regulation in numerous cytochrome P450 genes (12 with A673 cells, and 25 with HepaRG cells), resulting in a strong enrichment signal for general metabolism pathways. However, only HepaRG cells showed wide ranging enrichment in cell cycle and mitotic processes and cell signaling processes consistent with cell proliferation and hypertrophy.

### Conclusions

- The L1000 platform has promise for HT genomic testing in human cells at reasonable costs after better validation efforts.
- Differential expression response varied widely across cell lines and time points. While L1000-based methods worked for predicting the expression responses, our sampling of compounds and time points in this poster remains limited.
- Where response was robust for multiple cell lines at a given time point, differences were seen between cell lines in ontologic enrichment. Future efforts with this platform might benefit from using compounds with more complete data bases on modes of action, including previous studies that include other gene expression data and toxicological studies.
- These findings suggest selection of appropriate time points and the use of multiple cell models should be considered in HT genomics strategies designed to inform chemical MOA.

### References


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