Chemical Selection via *in vitro-in vivo* Correlation of ToxCast and ToxRefDB Data for the Virtual Liver Project

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Introduction

The Virtual Liver Project (v-Liver™) is a US EPA effort to simulate the function of the human liver with sufficient accuracy to predict how environmental exposure to xenobiotic compounds will perturb homeostasis.

The better we understand the liver, the better we will understand the toxicity of many chemicals. Most metabolism occurs in the liver, including the first-pass of most everything that enters through the digestive tract before it moves on to the rest of the body.

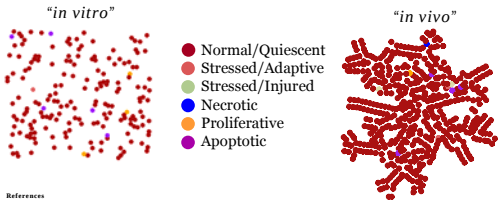
Our aim is to use an agent-based approach in which each liver cell is represented by an independent realization of a dynamic model of cellular function, i.e. an agents, that changes state in response to chemical and inter-cellular signals. Chemical-specific *in vitro* assay results can be used to determine the inputs to the intra-cellular and chemical distribution models.

These simulated cells are arranged in both *in vitro* and *in vivo* configurations to allow inference of *in vivo* consequences of cell-based assay results.

To both characterize the range of chemical attributes that must be captured and evaluate the usefulness of predictions made by the model, we are first selecting chemicals with a range of permutations of known mouse and/or rat *in vivo* tumorigenicity. As a proof of concept, we would like to predict potential non-genotoxic lesion progression for mouse, rat, and human biochemistries.

Our analysis was performed on the freely available statistics computing platform R [R Development Core Team, 2009].

Hepatocyte agents determine state in response to chemical and cellular environment



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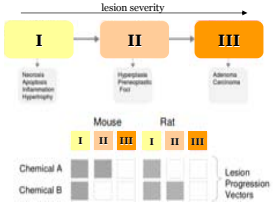
in vivo Carcinogenicity Data: ToxRefDB

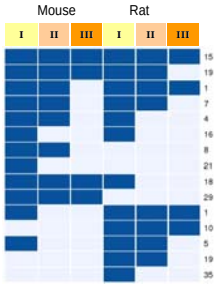
Histopathology observations were obtained from Toxicology Reference Database [Martin et al. 2009], a public relational database of *in vivo* toxicology data, primarily for pesticide active ingredients.

For 208 chemicals there are data on mouse and rat tissue changes following chronic (2 year) studies.

We have quantified lesion progression using three broad categories:



The dimensionality of the 2^6 lesion progression vectors was reduced by the assumption that if a more advanced lesion was observed in a species that prior lesion stages were also present, whether or not they were reported.



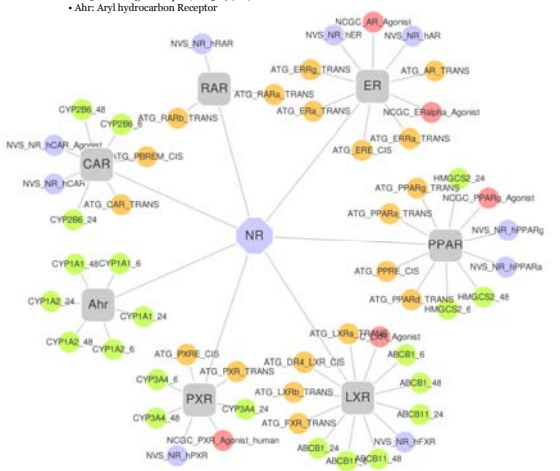
Generally, more compounds were mouse tumorigens than rat tumorigens. 15 of the 208 compounds caused tumors in both species.

in vitro Activity Data: ToxCast

The ToxCast dataset [Dix et al., 2007] includes high-throughput and high-content screening *in vitro* assays across multiple technology platforms for 309 environmental chemicals

We analyzed the results of 64 assays including CYP's and target genes which characterize the activity of ten human proteins in the nuclear receptor superfamily.:

- NR1B (RAR): Retinoic acid receptor-like, NR1B1 (RARα) and NR1B2 (RARβ)
- NR1C (PPAR): Peroxisome proliferator-activated receptor-like, NR1C1 (PPARα), NR1C2 (PPARβ/δ) and NR1C3 (PPARγ)
- NR1H (LXR): Liver X receptor-like, NR1H3 (LXRα), NR1H2 (LXRβ), and farnesoid X receptor, NR1H4 (FXR)
- NR1I: Vitamin D receptor-like, constitutive androstane receptor, NR1I3 (CAR), and pregnane X receptor, NR1I2 (PXR)
- NR3A: Estrogen receptors, NR3A1 (ERα)
- NR3B: Estrogen related receptors, NR3B1 (ERRα) and NR3B3 (ERRγ)
- NR3C: Androgen receptor, NR3C4 (AR)
- Ahr: Aryl hydrocarbon Receptor



To ensure that the v-Liver simulation responds to a range of assay results and endpoints, we will select compounds representing the following categories:

- Chemicals that caused tumors in rats and mice which were active for most ToxCast assays considered
- Mouse-specific tumor-causing compounds
- Rat-specific tumor-causing compounds
- Non-tumor causing compounds with clean activity profiles

Selecting Chemicals to Evaluate *in silico* Predictions

There were 180 environmental chemicals with both *in vivo* and *in vitro* data: