A Virtual Liver for Simulating Chemical-Induced Injury

John Wambaugh[1], John Jack[1], Chris Corton[2], and Imran Shah[1]

[1]National Center for Computational Toxicology (NCCT), US EPA, RTP, NC, USA.

[2]National Health and Environmental Effects Research Lab (NHEERL), US EPA, RTP, NC, USA.

research&development

Introduction

Humans are exposed to over 6,000 environmental chemicals. The liver is the primary organ for metabolism and often the first site of chemical-induced toxicity in animal testing. It remains difficult to translate these outcomes to humans due to uncertainties in extrapolating pathways from rodents to humans, and from *in vitro* to *in vivo*. The Virtual Liver (v-Liver™) is an *in silico* platform aimed at simulating clinically-relevant effects in the liver. The proof of concept is defined by 20 chemicals (VL-20) with ToxCast™ *in vitro* assay data that produce a range of chronic liver lesions in rodents. The VL-20 are high-production volume (HPV) chemicals including: pesticides, persistent toxic substances, and plasticizers.

Objectives

- Develop decision support tools to extrapolate from rodents to humans and *in vitro* to *in vivo*
- Create a knowledgebase (KB) of literature-derived information on pathways to tissue lesions for VL-20
- Simulate a virtual hepatic lobule to quantitatively evaluate tissue outcomes for VL-20

Timeline

2009 Prototype Tissue Simulator (*Draft manuscripts prepared)*

2010 Engage with EPA Program Offices on MOA analysis of hepatocarcinogens

Simulate Lesion Formation for for VL-20 using ToxCast™ data and predictions with ToxRefDB

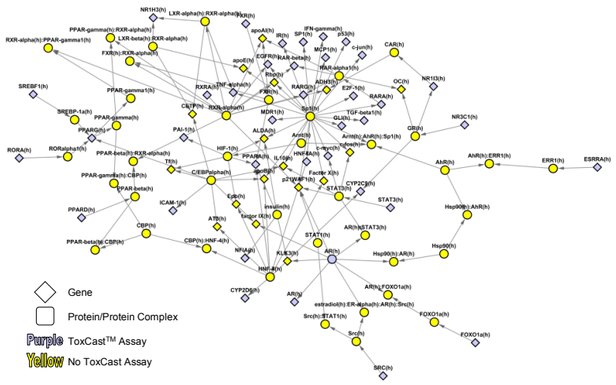
2011 Work with EPA Program Offices to evaluate quantitative effects of new chemicals

Use system to evaluate hepatic effects across human subpopulations / genetic variation

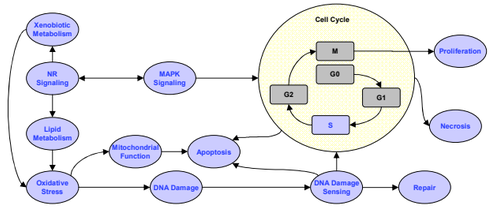
Molecular and Cellular Responses

VL-20 Chemicals ↔ Internal Dose ↔ NR-Mediated Pathways ↔ Cell State Changes ↔ Tissue Injury/ Repair ↔ Preneoplasia ↔ Neoplasia

Knowledgebased analysis of putative nuclear receptor (NR)-mediated molecular interactions. (Data on human NR activation for VL-20 from ToxCast™ shown in purple.)

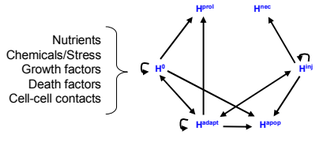


NR-mediated cellular changes. (Putative relationships between NR-activity to cellular processes using data from ToxCast™ (shown in purple.)



Probabilistic model of hepatocellular response to chemicals.

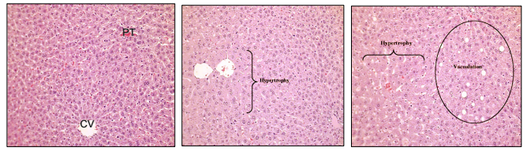
- Initially we are using an automaton model of cell response to microenviornment including: chemical (stress), nutrient, growth factors and cell-cell contacts.
- Based on histological observations define six hepatocellular states: normal, adapting, injured, necrotic, apoptotic or proliferating



Cellular Changes to Tissue Effects

VL-20 Chemicals ↔ Internal Dose ↔ NR-Mediated Pathways ↔ Cell State Changes ↔ Tissue Injury/ Repair ↔ Preneoplasia ↔ Neoplasia

Hepatic Microanatomy and Toxicity



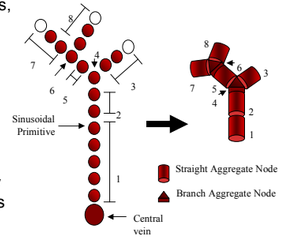
Cross section of classic lobule in Untreated female rat liver: blood flows from portal triads (PT) into the central vein (CV).

Images from Rockett et al. (2006)

Chemicals distribute through sinusoidal network. In female rats orally treated with 2500 ppm propiconazole for 12 weeks, hepatomegaly and centrilobular hypertrophy were observed.

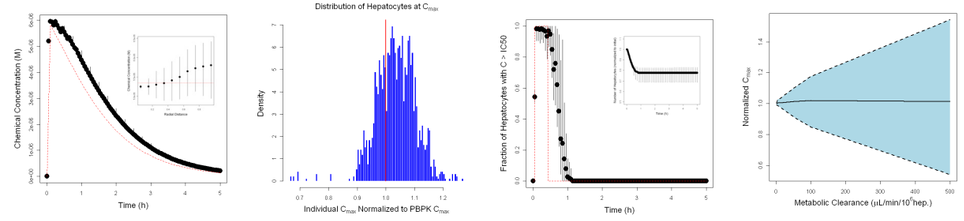
Tissue changes include pablobular hypertrophy and cytoplasmic vacuolation for female rats orally exposed 1800 ppm triadimefon for 12 weeks.

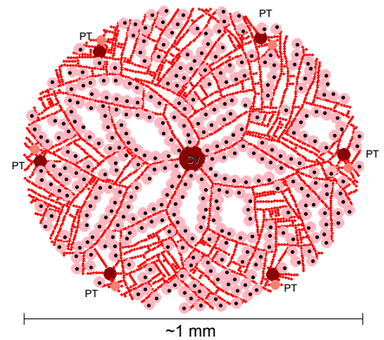
- Developing an agent-based spatial model of a single classical hepatic lobule in 2D
- Hepatic parenchymal and non-parenchymal cells represented as agents arranged according to lobular morphology.
- Agent responses and states defined by probabilistic hepatocellular model
- Sinusoidal network represented graphically by sinusoidal primitives, arterial and venous sources, and the central vein
- Flow through the sinusoids modeled by ordinary differential equations (ODEs) – efficient for large systems vs. PDEs
- Graphical model allows flexibility for dealing with histologic changes over doses and times



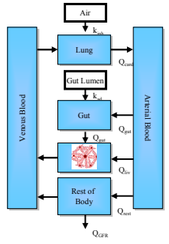
Integration with *in vitro* Data (e.g. ToxCast™)

- Variability in predicted local concentration due to the action of hepatocytes, e.g.. metabolism, and not geometry alone.
- *in* vitro testing can determine thresholds for endpoints such as cytotoxicity under controlled dosing conditions – we use our model to determine local conditions for individual hepatocytes

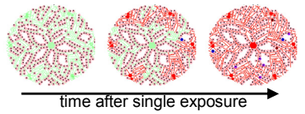




Relating Environmental Exposure to Local Cellular Environment for Risk Assessment



- We make use of a simple physiologically-based pharmacokinetic (PBPK) model structure to find the concentration of compound(s) in the blood flowing into the liver as a function of environmental exposure.
- A PBPK model consists of a system of ODEs for the concentration of a compound or compounds in different tissues.



in this simulation exposure to compound enhances cytotoxicity above a threshold

Impact and Outcomes

Simulation can enhance prioritization efforts by providing context for HTS data

Modeling liver homeostasis will provide better insights into the dose response relationship for environmentally-relevant concentrations

Organizing and modeling hepatic biology can improve identification of toxic modes of action for risk assessors in NCEA and outside ORD

The flexibility of an *in silico* approach allows for interspecies extrapolation and novel simulated experiments

Ultimately the v-Liver™ should reduce animal testing

Future Directions

Estimate tissue dosimetry for VL-20 to effectively utilize *in vitro* assay data from ToxCast

Link NR-mediated gene-networks for VL-20 to key cellular pathways involved in their MOA. Encode these pathways in Agent-Based model. Calibrate and Evaluate using *in vitro* assays

Enhance agent-based model to consider relevant parenchymal and non-parenchymal cell interactions to model homeostatic liver function. Calibrate and Evaluate using *in vivo* histomorphometry data

Augment sinusoidal network to include additional vasculatures (e.g. bile ductules and lymphatics)

References

JC Rockett, MG Narotsky, KE Thompson, , I Thillainadarajah, CR Blystone, AK Goetz, H Ren, DS Best, RN Murrell, HP Nichols, JE Schmid, DC Wolf and DJ Dix, “Effect of conazole fungicides on reproductive development in the female rat,” Reproductive Toxicology 22, 647-658 (2006).

JF Wambaugh and I Shah, “A Microdosimetry Model for Virtual Liver Simulation,” *in preparation*.