A Model for Micro-Dosimetry in Virtual Liver Tissues

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Methods

I. Abstract

Motivation: Humans are potentially 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, but it remains difficult to translate these outcomes to humans. To address this issue the US Environmental Protection Agency is developing a Virtual Liver – v-Liver™ – in order to computationally simulate clinically-relevant effects in humans using *in vitro* data. As a proof of concept, the Virtual Liver Project is focusing on tissue lesions caused by 20 non-genotoxic rodent carcinogens including: pesticides, persistent toxic substances, and plasticizers. Currently, we are modeling a canonical hepatic lobule (~ 1 mm diameter) as a complex cellular system in which parenchymal and non-parenchymal cells are spatially organized according to lobular morphology around a network of vessels, called sinusoids. We assume that lesions arise from cellular alterations, which are caused by concentration-dependent perturbations in molecular pathways. Here we describe our initial approach for estimating the micro-anatomic distribution to hepatocytes of environmental chemicals in the lobule.

Approach: Organ-level concentrations are computed from environmental exposure via inhalation, ingestion, or dermal transport using physiologically-based pharmacokinetic (PBPK) models. Exact approaches for estimating cell-scale chemical concentrations within the liver are difficult because the sinusoidal structure is complex, highly permeable, and dynamic. To address this issue we propose a discrete, graph-based method for quantitatively approximating the transport of blood as it flows in from the portal triad, filters through the sinusoidal network, and ultimately leaves the lobule through the central vein. We represent lobular microanatomy as a graphical structure composed of two main node types: cell-nodes and vascular-nodes corresponding to regions through which blood can flow. The model of the lobule is synthesized geometrically using physiologic parameters in two steps: (i) layout the vascular-nodes as a connected vascular-graph along whose edges blood flows through the lobule, and then (ii) organize cell-nodes around the vascular-graph. We reduce the vascular-graph by aggregating vascular nodes into well-mixed sinusoids. Using the reduced sinusoidal-graph **G,** network flow algorithms, and mass balance we derive the concentration distribution across the hepatic lobule,

$$\frac{d\vec{C}^i}{dt} = \vec{E}\cdot\vec{C}^i - \vec{M}^i\cdot\vec{I}\cdot\vec{C}^i$$

where **E** is the adjacency matrix of **G** and vectors $\mathbf{C}^i(t)$ and $\mathbf{M}^i(t)$ are the concentration and aggregated metabolic clearance of compound *i* in each sinusoid.

II. Spatially Inhomogeneous Hepatic Lobule Model

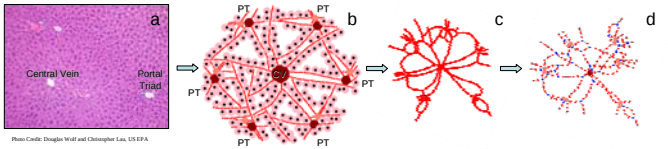


Photo Credit: Douglas Wolf and Christopher Lau, US EPA

• Our goal is to simulate a homeostatic liver lobule in which cell death, cell division, and changes in vasculature are all present.

• In the lobule veinous blood from the gut mixes with arterial blood as both bloods flow from the portal triad through the hepatic sinusoids and into the central vein **(a).**

•We model a single classical hepatic lobule **(b).** The morphology and function of this lobule is intended to represent all lobules within the liver.

• We use a syntheized lobule as a prelude to solving for flow in histomorphometric data.

• Flow through the hepatic sinusoids **(below)** responds to a dynamic boundary – we approximately solve for this flow.

Hepatic sinusoids flow into the central vein

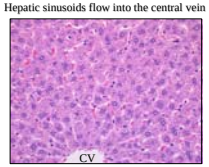


Photo Credit: Douglas Wolf and Christopher Lau, US EPA

• The sinusoids are represented by a series of sinusoidal primitives – small, well-mixed regions of sinusoidal space through which blood flows.

• We represent the spatial extent of the lobule via a connectivity graph **(c)** and solve using a network flow approach.

• Node types include hepatocytes, sinusoidal primitives, arterial and venous sources, and the central vein, while edges represent spatial proximity between the nodes.

• Interactions are restricted to occur across edges or flow through the sinusoidal spaces.

• The hepatocyte-hepatocyte and hepatocyte-sinusoid edges determine the inputs to the cell-state model within each hepatocyte.

• For computational efficiency, the connectivity graph is aggregated **(right)** into a simpler graph **(d).**

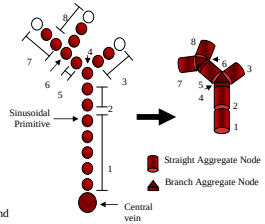
• The sinusoids primitives are aggregated into larger objects corresponding to well-mixed "straight" sections of sinusoids, "branch" sections where straights meet and mix, and "deadend" sections that reflect flow back into the rest of the lobule.

• Fluid dynamics requires modeling cell walls and using fluid dynamics to describe the flow through the lobule.

• We simply describe flow through the sinusoids using ODEs.

• Even large systems of ODEs can be rapidly solved using numerical approaches.

• We believe this approach is tractable for simulating sub-chronic and chronic exposure scenarios while preserving mass-balance.



III. A Simple Cell State Model

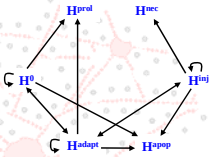
• We currently assume a simple probabilistic state-transition model for hepatocellular behaviour

• This allows rapid simulation of microdosimetry for different lobular architectures

• We represent hepatocytes in one of seven possible states: normal, adapting, injured, necrotic, apoptotic or proliferating based on histologic observations

• Only biologically relevant state transitions are allowed

• State transition probabilities are a function of the current state and inputs from the microenvironment



IV. Multiple Scales of Exposure

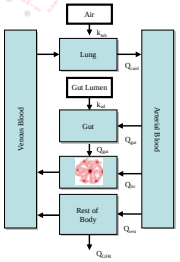
• We make use of a simple physiologically-based pharmacokinetic (PBPK) model structure (**right**) to find the concentration of compound(s) in the blood flowing into the liver as a function of environmental exposure.

• A PBPK model consists of a system of ordinary differential equations for the concentration of a compound or compounds in different tissues.

• Typically some key tissues are treated as separate compartments for which a tissue-specific concentration is calculated, while other tissues are modeled using aggregate compartments.

• The equations are parameterized by subject- or species-specific physiologic parameters such as cardiac output and tissue volumes as well as compound-specific parameters such as diffusion/transport rates and tissue-specific plasma to tissue "partition coefficients" corresponding to the assumption of a rapidly-established ratio of concentration of compound stored in the tissue relative to that of the plasma flowing through the tissue.

• Because we are developing a chemical-independent approach we have for convenience assumed that all chemical-specific parameters are equal to one.

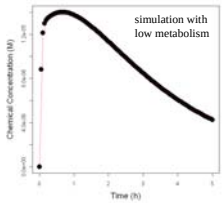


V. Results

• We simulated an oral exposure of 1 mMol total (equivalent to 3 mg/kg BW for a 200 MW compound) with an intrinsic hepatic clearance due to metabolism of either 0.1 or 1.0 mL/min/mg liver. The average results over an ensemble of ten randomly generated lobules are presented.

• For a single dose the number of molecules at higher concentrations is sufficiently large **(1)** to be comfortable using ODEs (instead of stochastic aproach, *e.g.* Gillespie) for flow.

• The average concentration in the lobule **(below)** behaves very similarly to a well-mixed PBPK compartment (**dashed line**) when metabolism is low.



• We assumed that individual hepatocytes are more likely to transition into adaptive, injured or apoptotic/necrotic states when the local xenobiotic concentration exceeds an assumed, "IC50" threshold.

• As expected, death of hepatocytes slows the overall clearance of the compound **(2).**

• Variability in concentration (**2 inset)** indicate that the particular geometry of the surroundings influences the concentrations received by a hepatocyte.

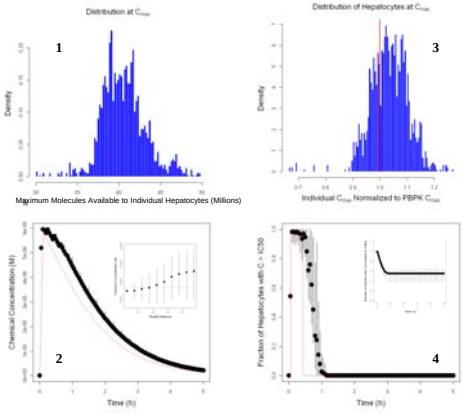
• Figure **3** shows the distribution of maximum concentration experienced by the hepatocytes.

•The width of the distribution is proportional to the metabolic clearance rate.

• A well-mixed compartment (**4 - dashed line**) would predict a square wave, all-or-nothing response.

• The number of hepatocytes (**4 inset)** does not recover on the time scales simulated.

Simulation with Fast metabolism and Cytotoxicity



VI. Summary

• The morphology of the simulated hepatic lobule alone did not explain typically observed zonal gradients in chemical concentrations or hepatocellular response.

• We observed variability in predicted concentration due to the action of hepatocytes, *e.g.*. metabolism, and not geometry alone.

• The graphical model can be augmented to include additional vasculatures (e.g. bile ductules and lymphatics) without major alogrithmic changes or increases in computational cost.

• In contrast to computationally-intensive spatially-continuous approaches such as fluid dynamics, this graph-theoretic approach has sacrificed little physiologic detail but gained a great deal in terms of computational efficiency, allowing us to focus on cellular physiology.

• More complex models of hepatocellular and non-parenchymal cell behavior are needed to adequately represent homeostatic liver function, and this will require extensive molecular and cellular data.

• We are calibrating and evaluating this framework to simulate chemical-induced hepatic injury using data from *in vitro* high content screening (HCS) assays, and histomorphometric data from fixed liver tissues.