United States Environmental Protection Agency

v-Tissues 2009

The EU-US Workshop on Virtual Tissues

~April 21-24, EPA, Research Triangle Park, North Carolina, USA

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This work has been reviewed by EPA and approved for presentation but does not necessarily reflect Agency views.

Outline

- "Virtual Tissues"
- Applications to Environmental Chemicals
  - Liver: Hepatotoxicity
  - Embryo: Eye Development
- Other Applications: modeling disease/therapeutic intervention in other organs
- Challenges
- Workshop on v-Tissues 2009

Virtual Tissues

Models of organs / tissues

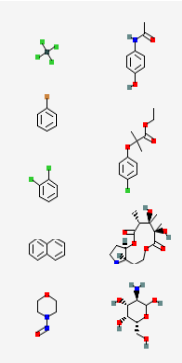
Application focus:

- Clinical outcomes: disease progression, therapeutic intervention, chemical-induced toxicity, etc.
- Translation: E.g. *in vitro* to *in vivo,* rodents to humans
- Others ?

Biological scope

- Histopathology gold-standard for disease
- *Cell behaviour* key to normal / abnormal states
- Molecular pathways → cellular phenotypes → tissue outcomes

EPA: Chemical Risk Assessment



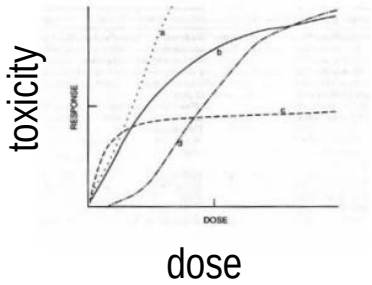
What chemicals are we exposed to?

Are the chemicals toxic?

Where do they cause Toxicity ?

What are the mechanisms of toxicity?

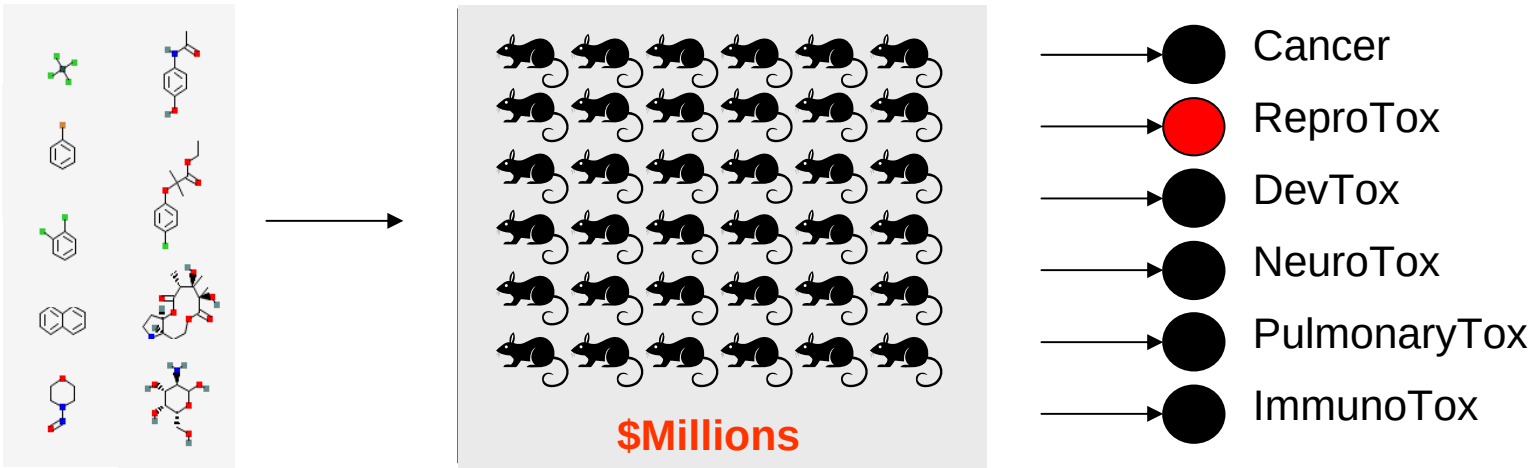
Who is susceptible?



At what dose is toxicity observed?

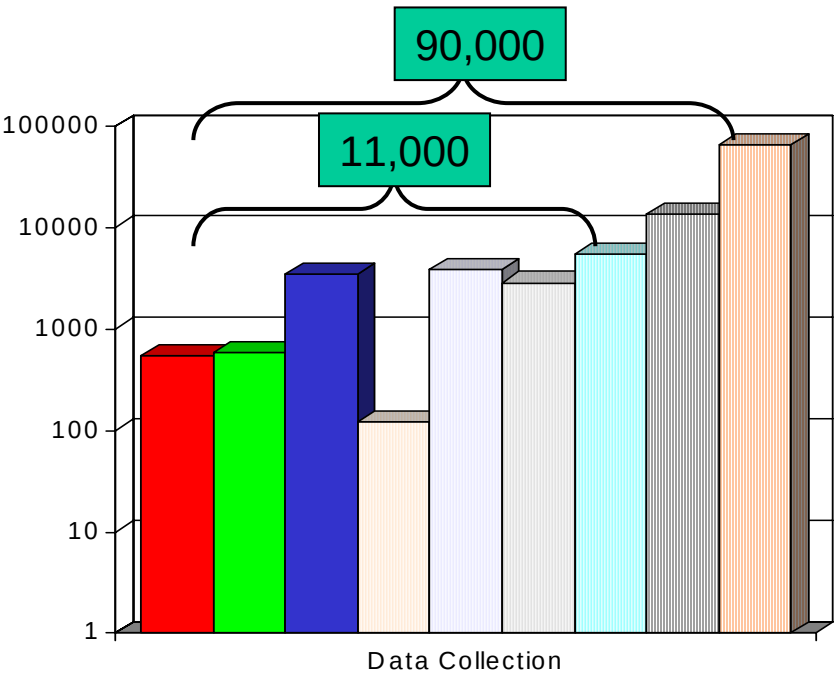
Current Approach for Toxicity Testing

in vivo testing

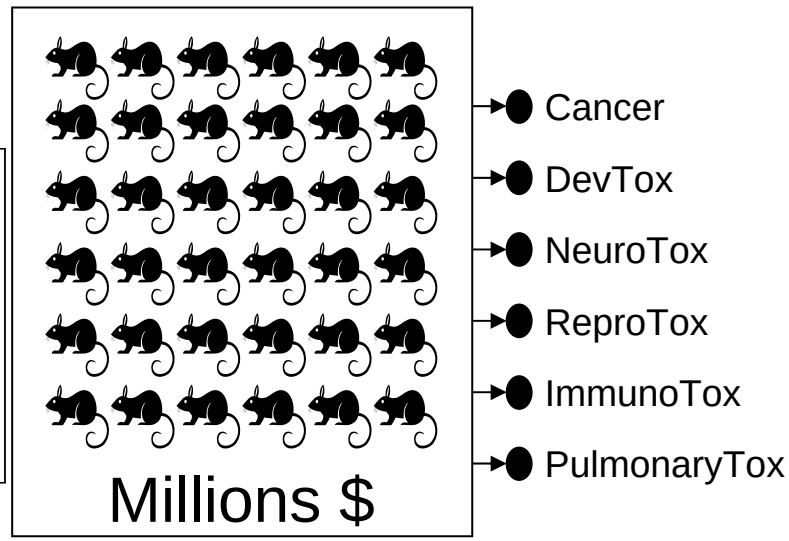


Putting Numbers on the Problem

Too Many Chemicals



Too High a Cost



…and not enough data.

Computational Toxicology

"...to integrate modern computing and information technology with molecular biology to improve Agency prioritization of data requirements and risk assessment of chemicals"

www.epa.gov/ncct

Future of Toxicity Testing

in vitro testing *in silico* analysis

HTS
-omics

Bioinformatics/
Machine Learning

EPAs Contribution: The ToxCast Research Program

Future of Toxicity Testing

POLICYFORUM

TOXICOLOGY

Transforming Environmental Health Protection

We propose a shift from primarily in vivo animal studies to in vitro assays, in vivo assays with lower organisms, and computational modeling for toxicity assessments.

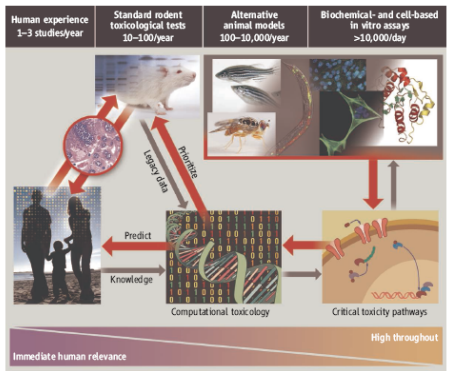
Francis S. Collins,*† George M. Gray,‡* John R. Bucher§*

In 2005, the U.S. Environmental Protection Agency (EPA), with support from the U.S. National Toxicology Program (NTP), funded a project at the National Research Council (NRC) to develop a long-range vision for toxicity testing and a strategic plan for implementing that vision. Both agencies wanted future toxicity testing and assessment paradigms to meet evolving regulatory needs. Challenges include the large numbers of substances that need to be tested and how to incorporate recent advances in molecular toxicology, computational sciences, and information technology; to rely increasingly on human as opposed to animal data; and to offer increased efficiency in design and costs (*1*–*5*). In response, the NRC Committee on Toxicity Testing and Assessment of Environmental Agents produced two reports that reviewed current toxicity testing, identified key issues, and developed a vision and implementation strategy to create a major shift in the assessment of chemical hazard and risk (*6*, *7*). Although the NRC reports have laid out a solid theoretical rationale, comprehensive and rigorously gathered data (and comparisons with historical animal data) will determine whether the hypothesized improvements will be realized in practice. For this purpose, NTP, EPA, and the National Institutes of Health Chemical Genomics Center (NCGC) (organizations with expertise in experimental toxicology, computational toxicology, and high-throughput technologies, respectively) have established a collaborative research program.

EPA, NCGC, and NTP Joint Activities

In 2004, the NTP released its vision and roadmap for the 21st century (*1*), which established initiatives to integrate high-throughput screening (HTS) and other automated screening assays into its testing program. In 2005, the EPA established the National Center for Computational Toxicology (NCCT). Through these initiatives, NTP and EPA, with the NCGC, are promoting the evolution of toxicology from a predominantly observational science at the level of disease-specific models in vivo to a predominantly predictive science focused on broad inclusion of target-specific, mechanism-based, biological observations in vitro (*1*, *4*) (see figure, below).

Toxicity pathways. In vitro and in vivo tools are being used to identify cellular responses after chemical exposure expected to result in adverse health effects (*7*). HTS methods are a primary means of discovery for drug development, and screening of >100,000 compounds per day is routine (*8*). However, drug-discovery HTS methods traditionally test compounds at one concentration, usually between 2 and 10 μM, and tolerate high false-negative rates. In contrast, in the EPA, NCGC, and NTP combined effort, all compounds are tested at as many as 15 concentrations, generally ranging from ~5 nM to ~100 μM, to generate a concentration-response curve (*9*). This approach is highly reproducible, produces significantly lower false-positive and false-negative rates than the traditional HTS methods (*9*), and facilitates multiassay comparisons. Finally, an informatics platform has been built to compare results among HTS screens; this is being expanded to allow comparisons with historical toxicologic NTP and EPA data (http://ncgc.nih.gov/pub/openhts). HTS data collected by EPA and NTP, as well as by the NCGC and other Molecular Libraries Initiative centers (http://mli.nih.gov/), are being made publicly available through Web-based databases [e.g., PubChem (http://pubchem.ncbi.nlm.nih.gov)]. In addition,



Transforming toxicology. The studies we propose will test whether high-throughput and computational toxicology approaches can yield data predictive of results from animal toxicity studies, will allow prioritization of chemicals for further testing, and can assist in prediction of risk to humans.

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*The views expressed here are those of the individual authors and do not necessarily reflect the views and policies of their respective agencies.

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THE NATIONAL ACADEMIES

REPORT IN BRIEF

July 2007

Toxicity Testing in the 21st Century: A Vision and a Strategy

Advances in molecular biology, biotechnology, and other fields are paving the way for major improvements in how scientists evaluate the health risks posed by potentially toxic chemicals found at low levels in the environment. These advances would make toxicity testing quicker, less expensive, and more directly relevant to human exposures. They could also reduce the need for animal testing by substituting more laboratory tests based on human cells. This National Research Council report creates a far-reaching vision for the future of toxicity testing.

Toxicity tests on laboratory animals are conducted to evaluate chemicals—including medicines, food additives, and industrial, consumer, and agricultural chemicals—for their potential to cause cancer, birth defects, and other adverse health effects. Information from toxicity testing serves as an important part of the basis for public health and regulatory decisions concerning toxic chemicals. Current test methods were developed incrementally over the past 50 to 60 years and are conducted using laboratory animals, such as rats and mice. Using the results of animal tests to predict human health effects involves a number of assumptions and extrapolations that remain controversial. Test animals are often exposed to higher doses than would be expected for typical human exposures, requiring assumptions about effects at lower doses or exposures. Test animals are typically observed for overt signs of adverse health effects, which provide little information about biological changes leading to such health effects. Often controversial uncertainty factors must be applied to account for differences between test animals and humans. Finally, use of animals in testing is expensive and time consuming, and it sometimes raises ethical issues.

Today, toxicological evaluation of chemicals is poised to take advantage of the on-going revolution in biology and biotechnology. This revolution is making it increasingly possible to study the effects of chemicals using cells, cellular components, and tissues—preferably of human origin—rather than whole animals. These powerful new approaches should help to address a number of challenges facing the

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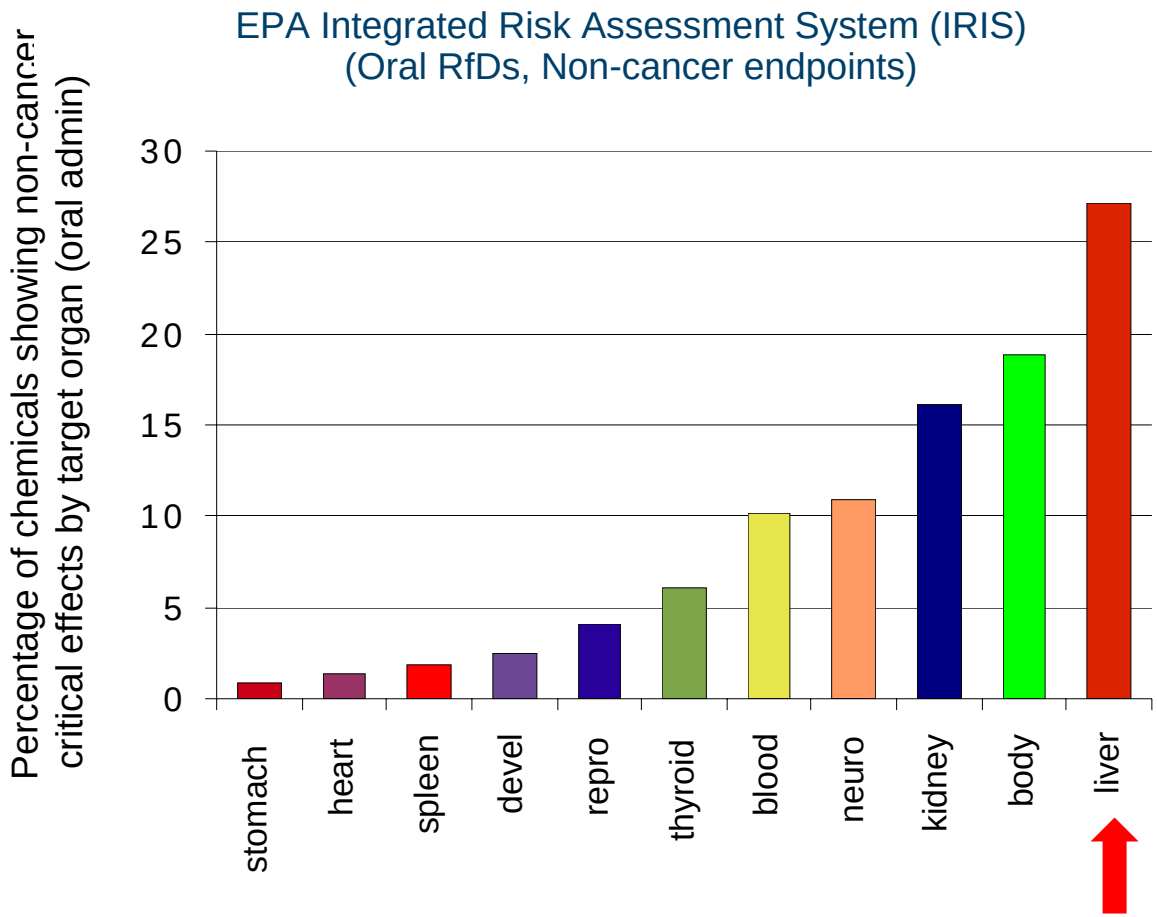
Modeling Toxicologic Processes

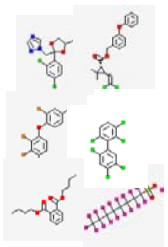
- Computational modeling & simulation of key aspects of biology that are difficult to analyze empirically
- Knowledgebases to integrate data with models in liver biology (v-Liver™) and in fetal development (v-Embryo™)
- Multi-scale/level models to simulate key events during chemical toxicity (e.g., liver toxicity, birth defects)
- Goal: Eludicate ‘toxicity pathways’ through which chemical perturbations at a molecular level invokes dose-dependent tissue damage

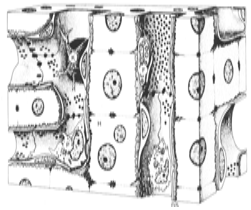
Why Liver ?

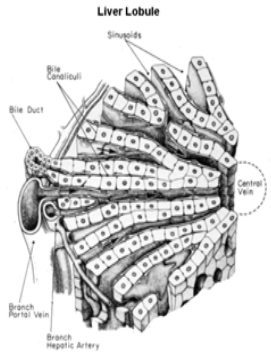

EPA Integrated Risk Assessment System (IRIS)
(Oral RfDs, Non-cancer endpoints)
Percentage of chemicals showing non-cancer critical effects by target organ (oral admin)
30
25
20
15
10
5
0
stomach
heart
spleen
devel
repro
thyroid
blood
neuro
kidney
body
liver

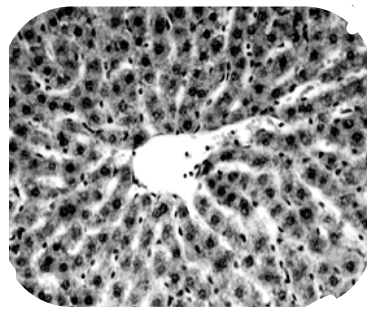
Virtual Tissues: Simulation of Dose-dependent Lesions

Environmental Chemicals → *Molecular Pathways* → *Cell Responses* → *Cellular Organization* → *Tissues Alteration*









Tissue Context: Hepatic Lobule

Heterogeneous structure

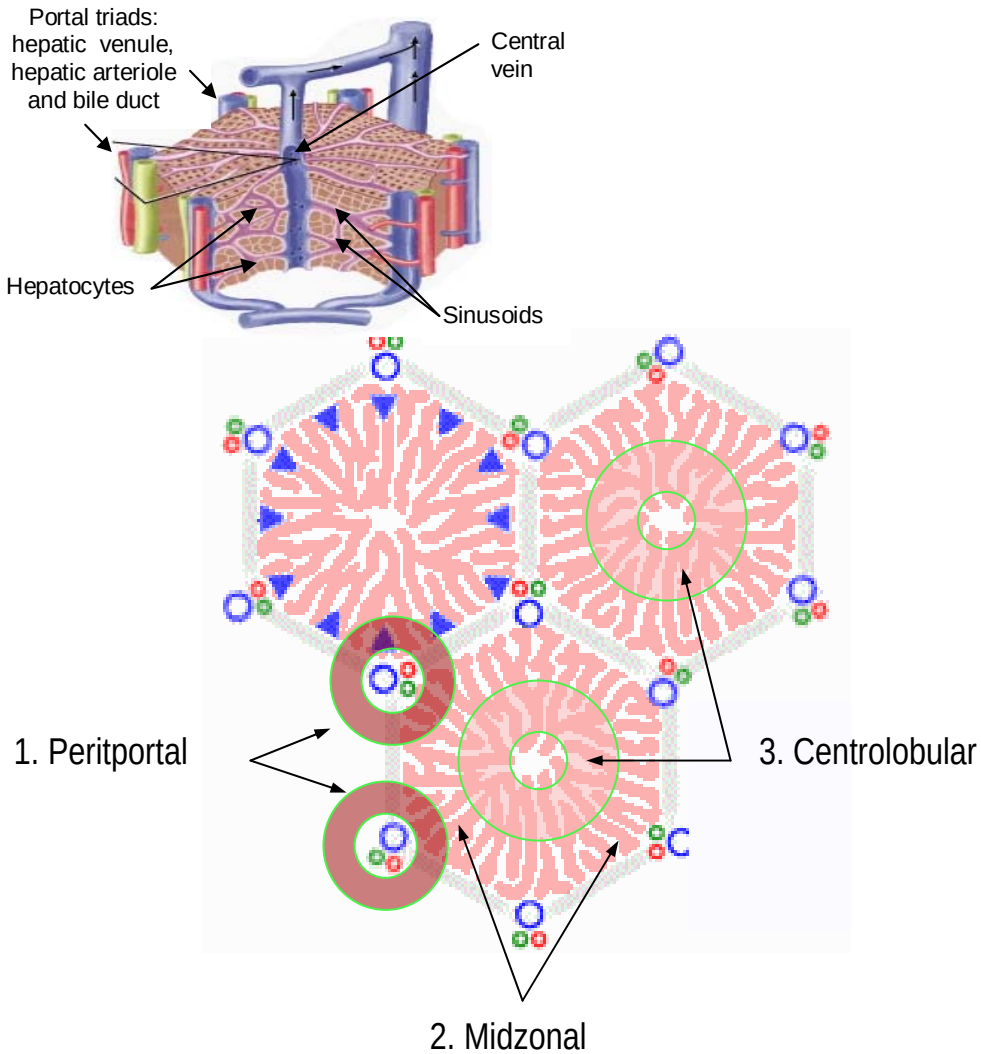
5 Cell types organized in a network around sinusoids

-Adaptation to gradients=> zones

-Zones are functionally different

-Injury can be zonal

Agent	*Necrosis*		
	1	*2*	*3*
Acetminophen	-	-	+
$Fe_2 (SO_4)_3$	+	-	-
Beryllium	-	+	-
Aflatoxins	+	-	+

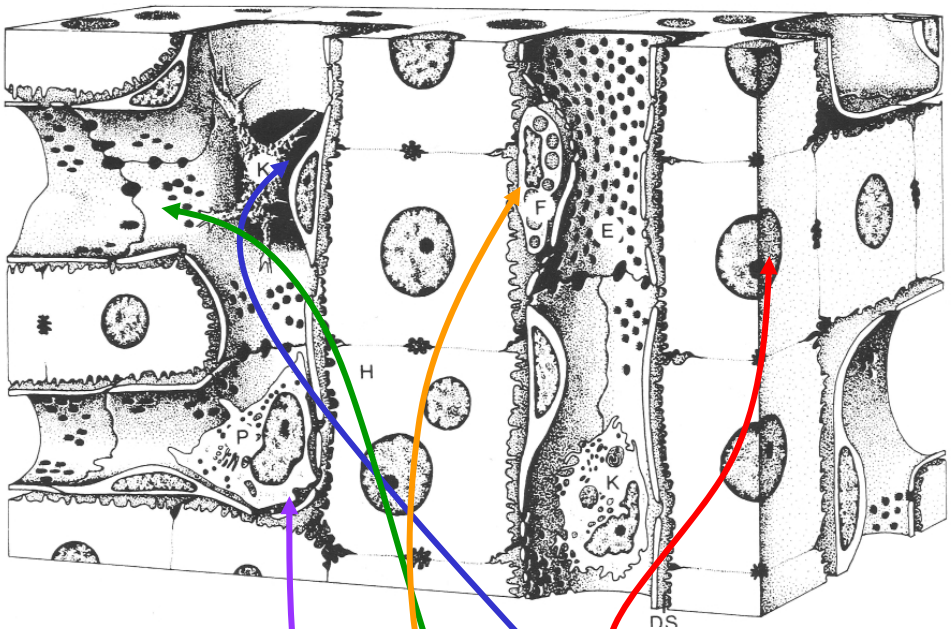


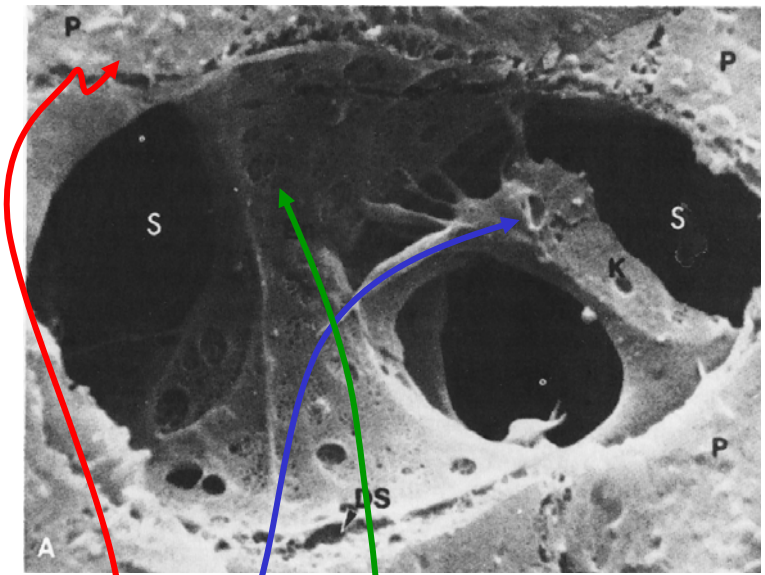
Zimmerman 1978

Meeks, Harrison & Bull, 2000 | jhu.edu

Cellular Organization

Liver section





X15,000

Hepatocytes

Kupffer cells

Endothelial cells

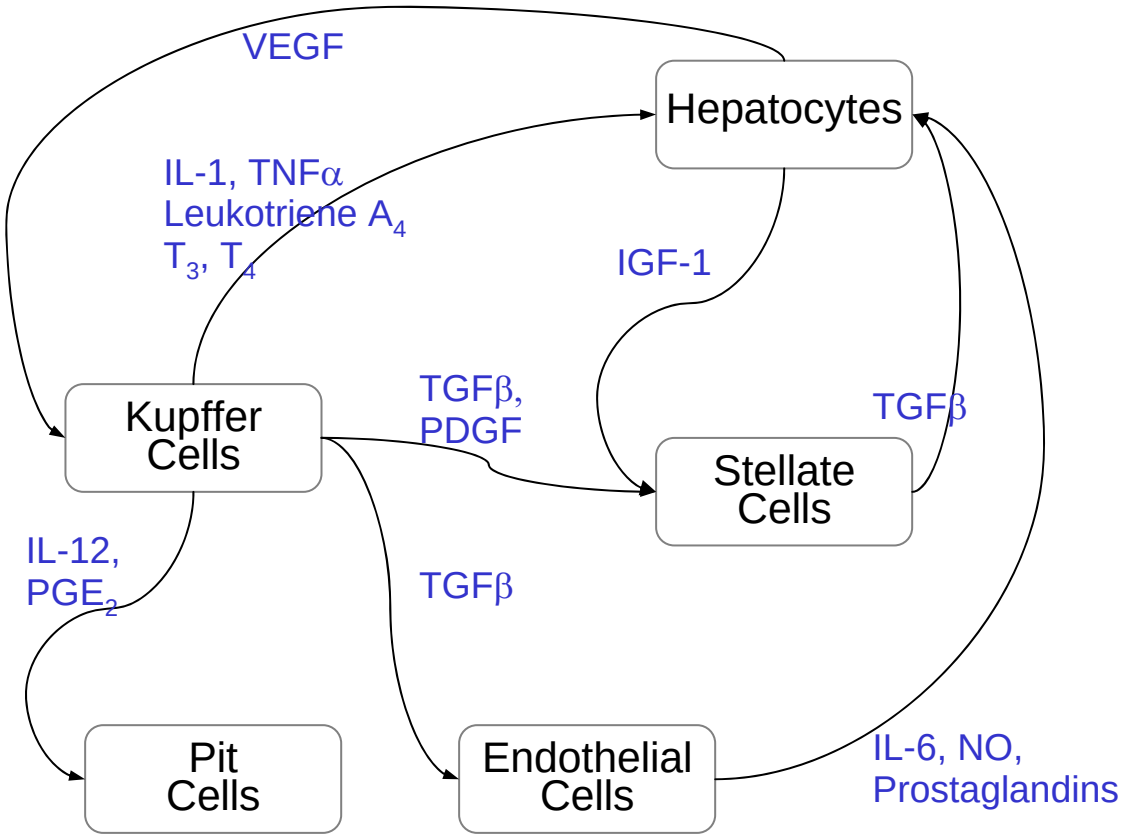
Stellate cells

Pitt cells

Sasses, *et. al.* 1992

Meeks, Harrison & Bull, 2000

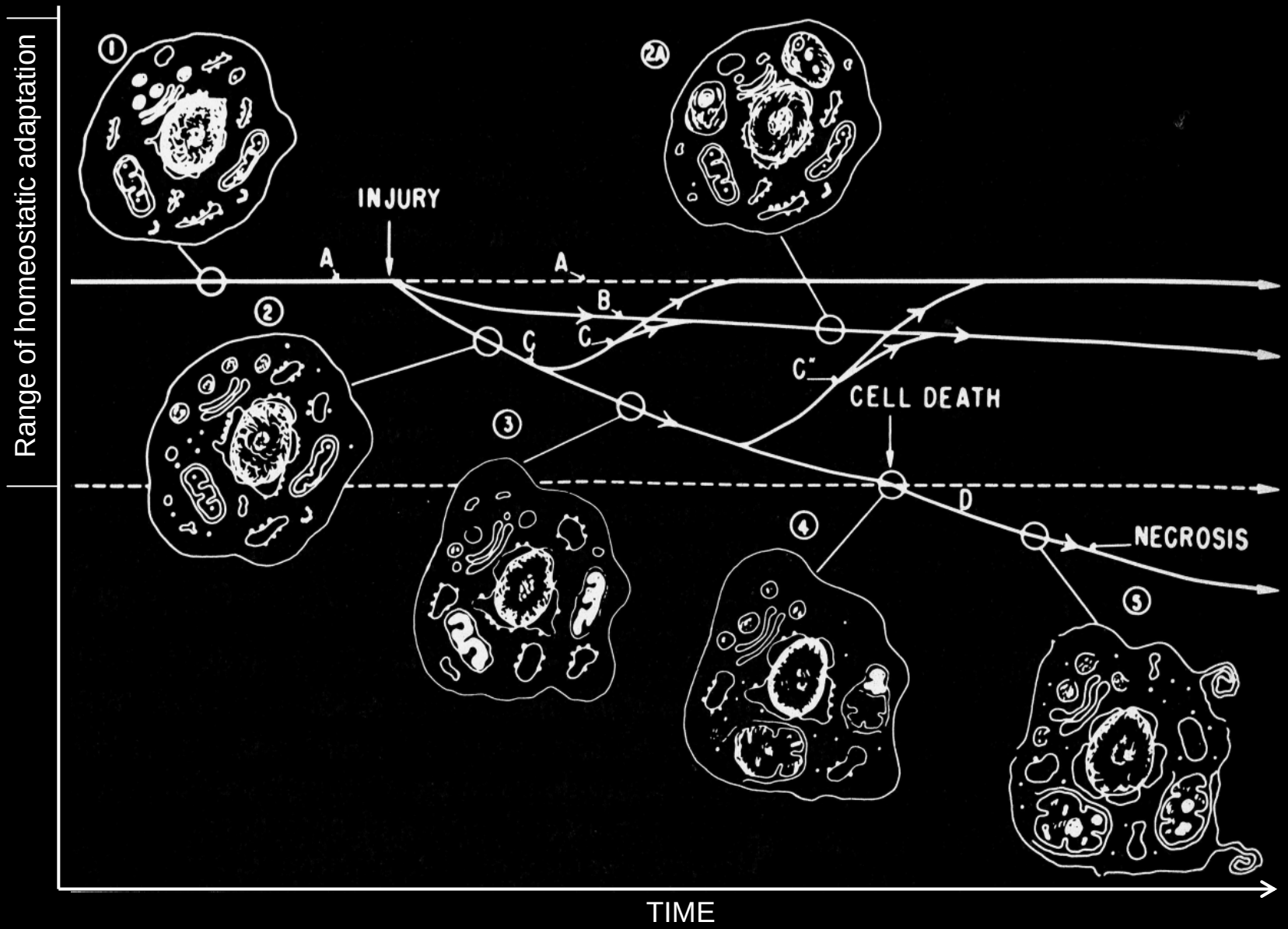
Complex Cell-Cell Interactions



In vitro culture conditions

Zimmerman 1978
Meeks, Harrison & Bull, 2000 | jhu.edu

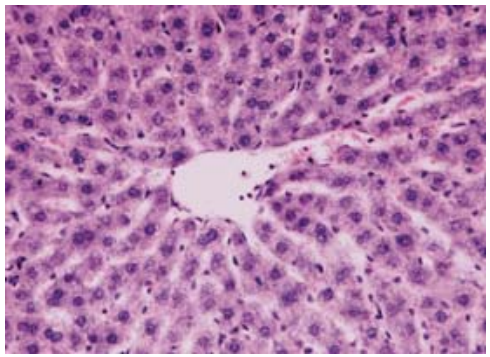
Injury Result of Dynamic Processes



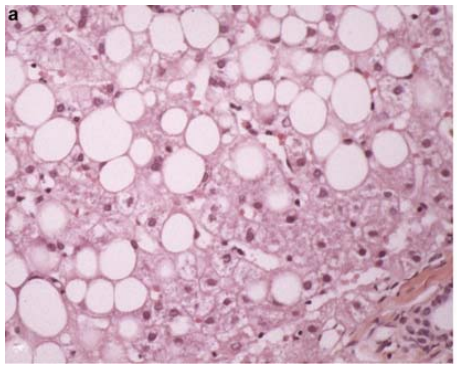
Trump, McDowell & Arstila, 1980

Tissue Change Due to Cell Alteration

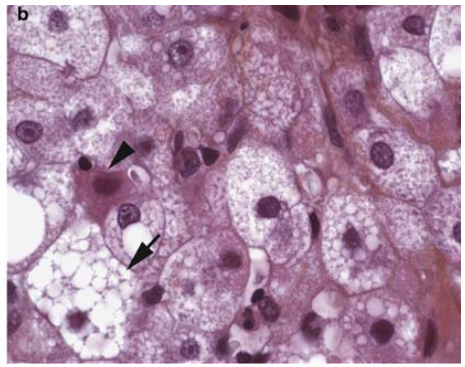
Swelling



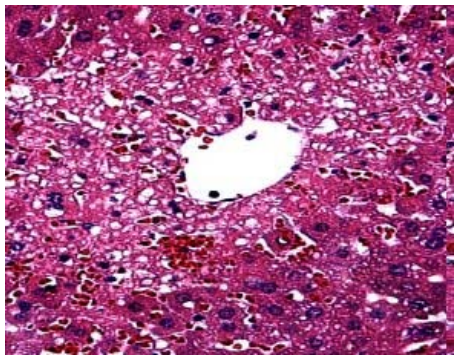
Steatosis, Macrovesicular



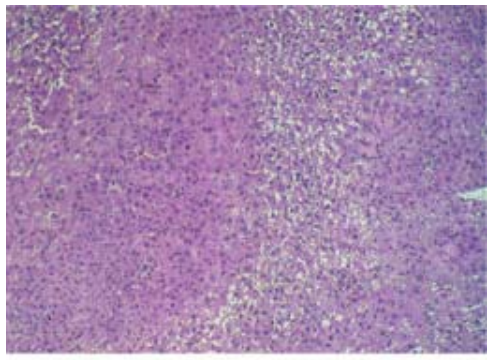
Steatosis, Microvesicular



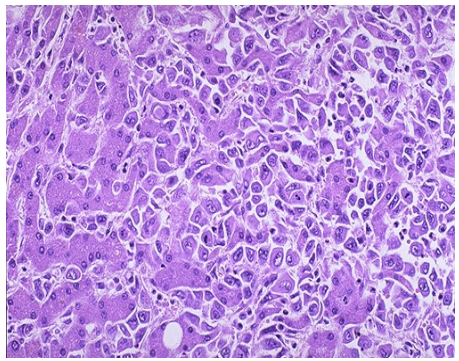
Necrosis



Hyperplasia



Carcinoma



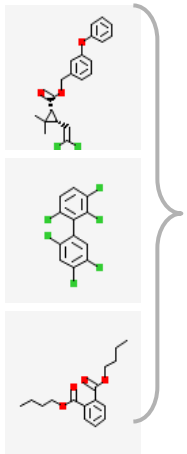
Fallsehr, *et. al.* World J Gastroenterol 2005 March 7;11(9):1303-1306
Jean-Paul Duong Van Huyen, *et. al. Modern Pathology (2006) 19, 1277–1288.0*
Uskoković-Marković, *et. al. J Pharm Pharmaceut Sci 10(3):340-349, 2007*
library.med.utah.edu/WebPath

v-Liver™ - Project Overview

United States Environmental Protection Agency

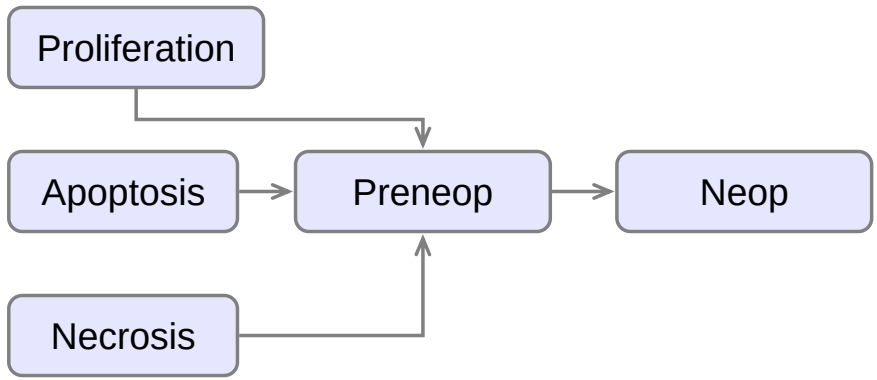
I. Molecule→Cell Response

II. Cell →Tissue Change



Hepatocyte

Molecular Processes



PoC: Nuclear receptor-mediated liver cancer

Short-term (1-2 y)

KB development and data acquisition

Cell-level model development

Tissue-level model prototype

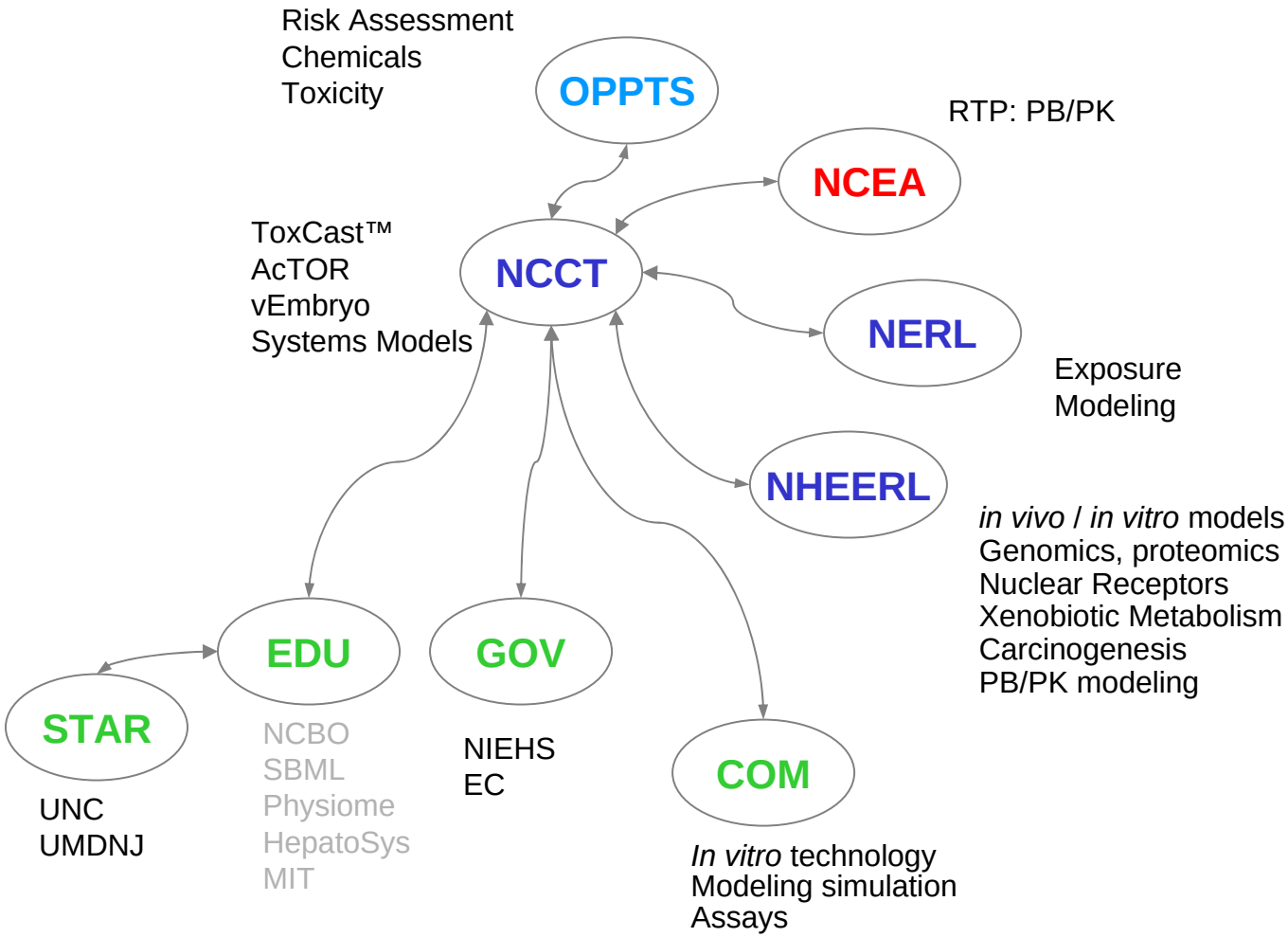
Long-term (3-5 y)

Expand mechanistic detail in models

Integrate Cell-level and tissue-level models

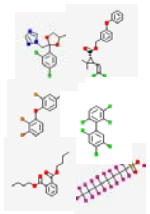
Evaluate against new chemicals

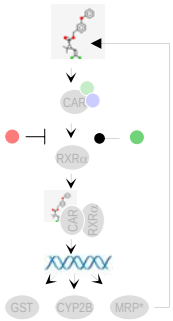
Multi-disciplinary Team: Cross-EPA/ORD & External

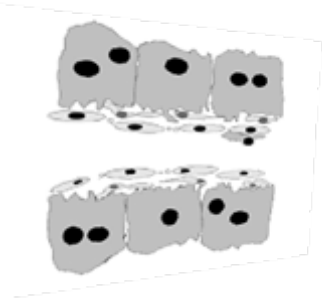


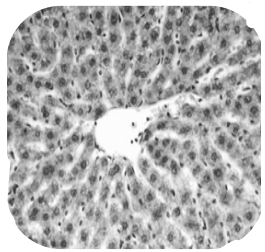
Overall Approach

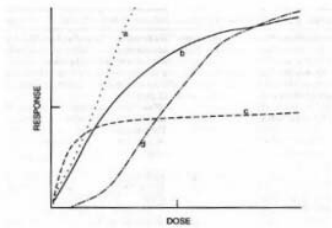
Environmental Chemicals → Molecular Sensing → Cellular Signaling → Tissue Responses

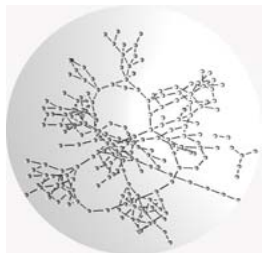


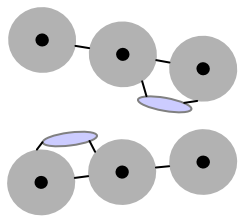


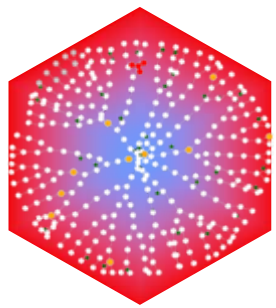






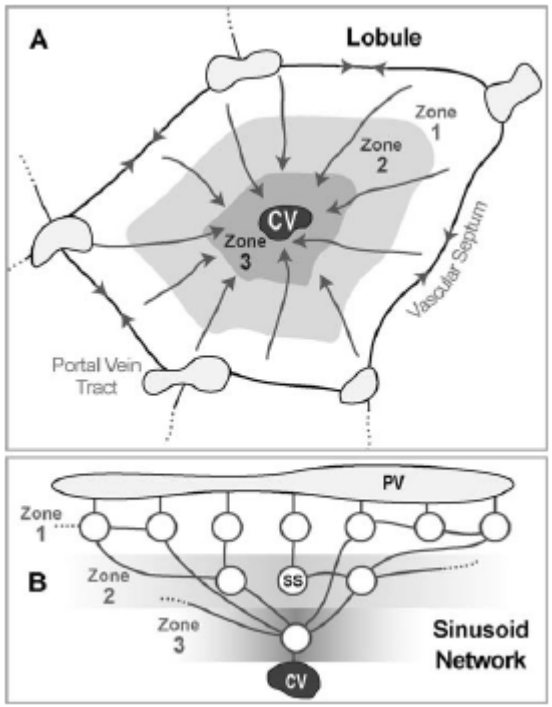


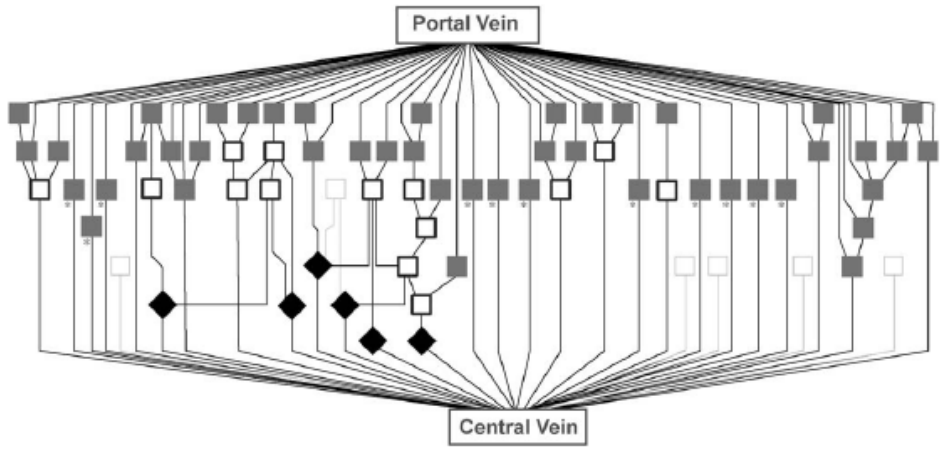


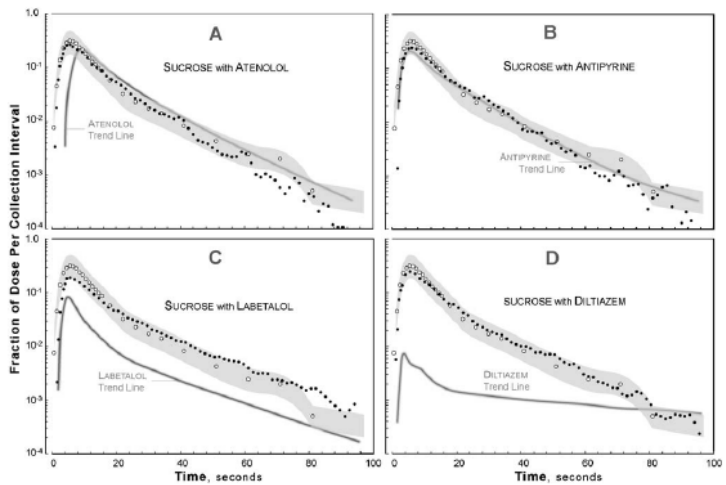


Knowledgebase Toxicity Pathways → Molecular Networks → Cellular Networks → Virtual Tissues → Dose-Response

Agent-Based Liver PK Modeling

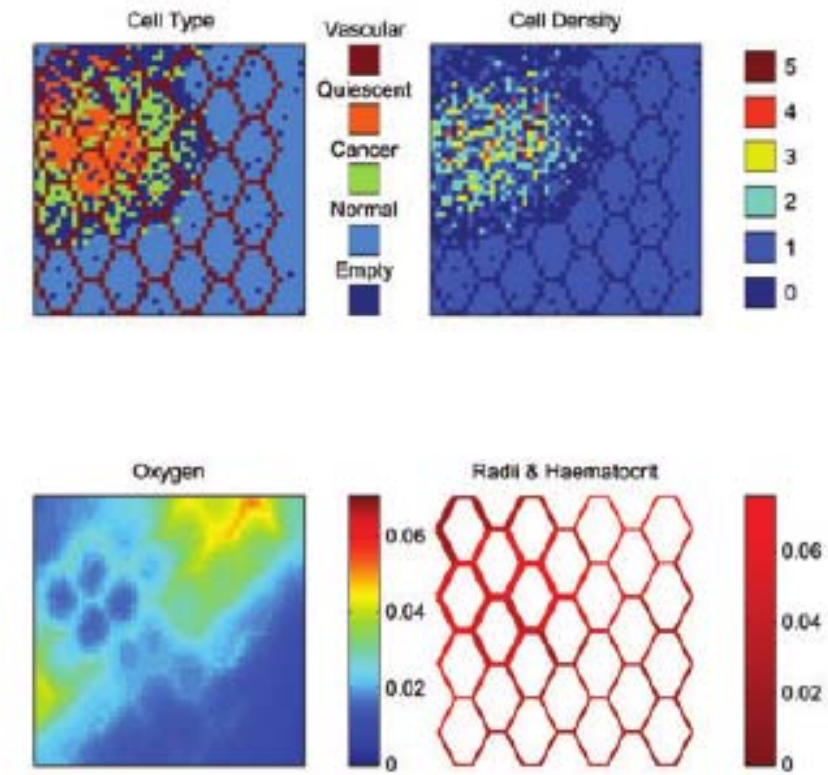


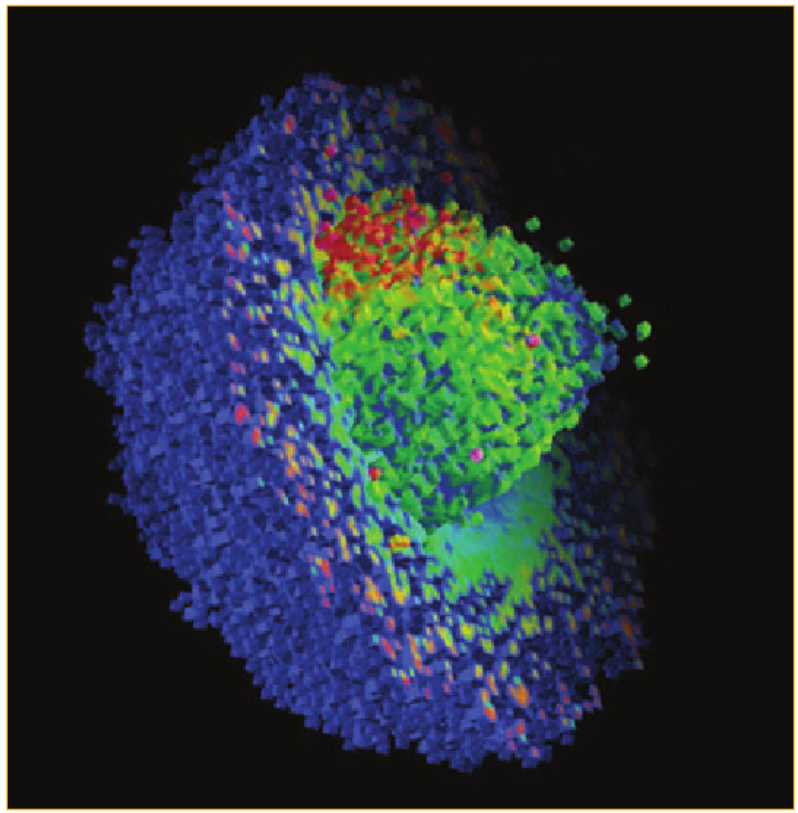




Yan and Hunt, 2007

Agent-Based Cancer Modeling





Anderson et al. Caell. 2006 Dec 1;127(5):905-15.

Alexander Anderson

Virtual Tissues: Challenges

- Biology: levels & linkage between levels
  - Molecular events and processes
  - Cellular events and processes
  - Tissue events and processes
- Representation qualitative information: events and processes
- Representing quantitative information:
- Simulating dynamics
- Experimental approaches for gathering data

v-Tissues 2009

- Focused meeting on specific topics of broader interest to the community
- Follow-up discussion at MSM meeting in August
- Organize workshop on Virtual Tissues April 20~23 , 2009 in Research Triangle Park, NC
- Auspices of EPA & EU-US Joint Task Force on Biotechnology