“Fit-For-Purpose” Population Study Designs to Address Specific Hypotheses

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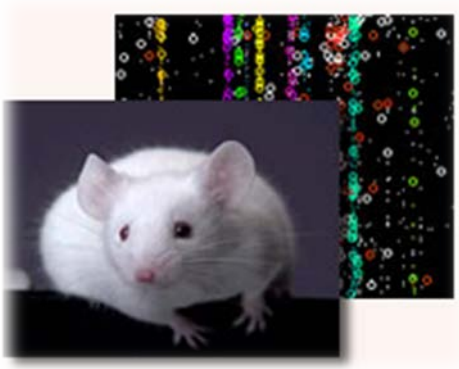

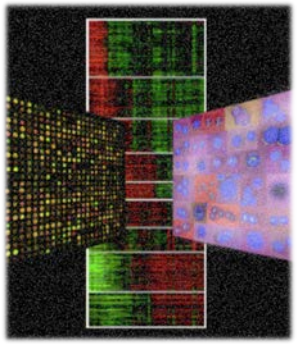

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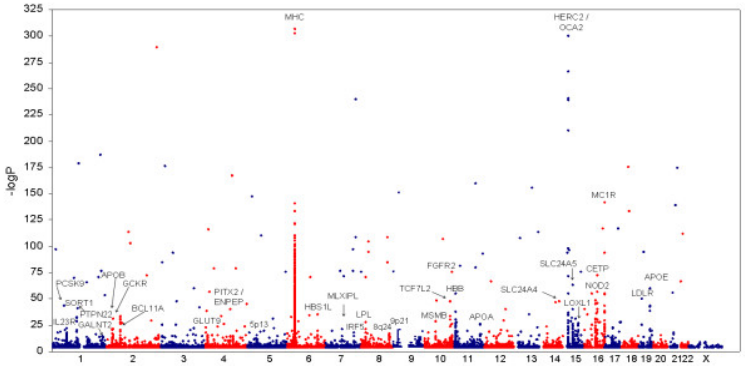
ADVANCES IN SEQUENCING ENABLE POPULATION-WIDE STUDIES

Epidemiological studies:
hundreds of individuals are now routinely genotyped for >10^6 SNPs

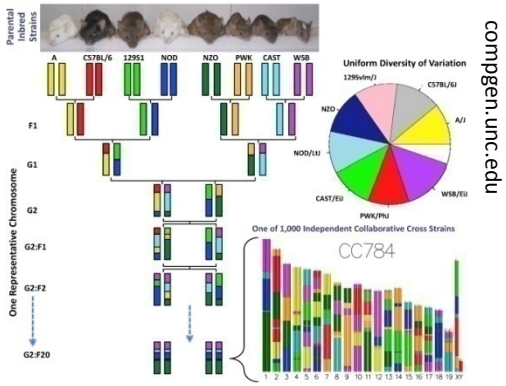


Genome-wide plots of available GWAS results for all associations P = 0.0001. (*BMC Medical Genetics* 2009)

***In vivo* animal studies:**
hundreds of inbred mouse strains have been genotyped for ~10^7 SNPs

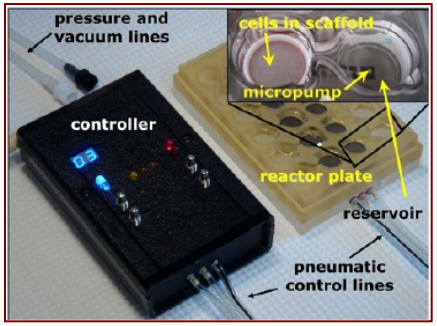
www.niehs.nih.gov/crg/

ornl.gov



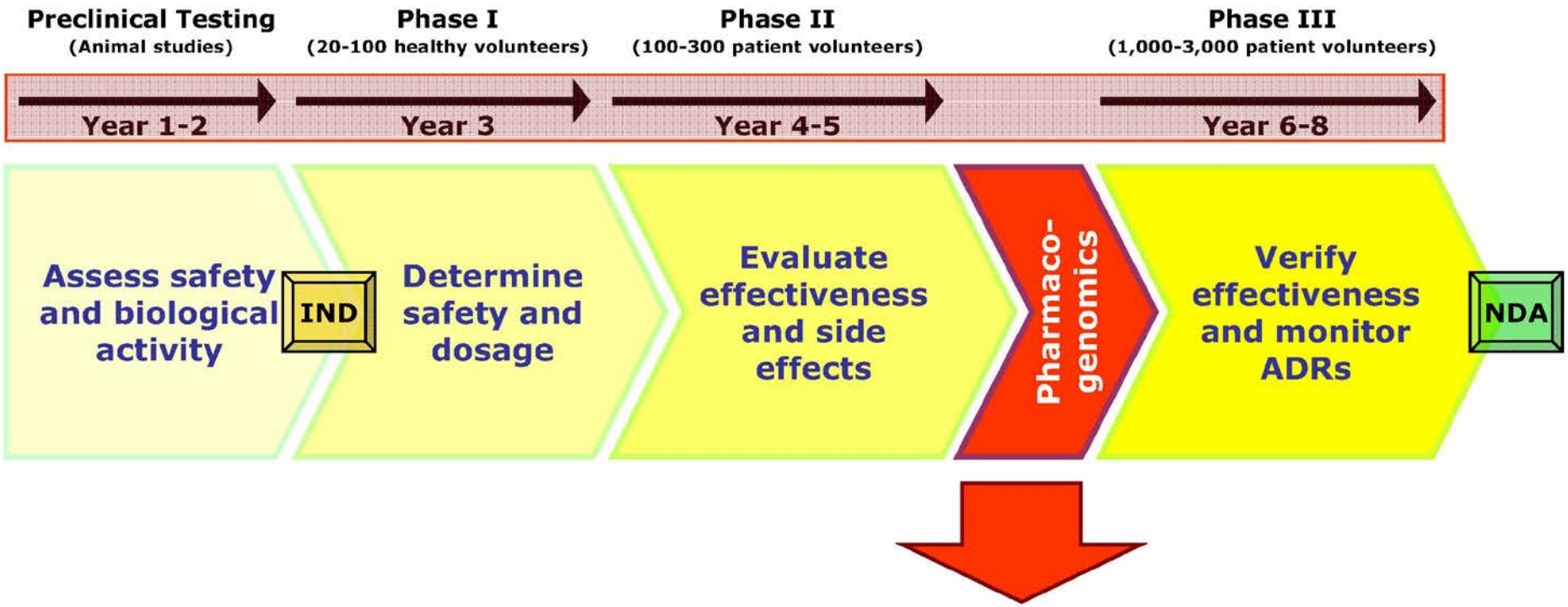
compgen.unc.edu

***In vitro* studies:**
Thousands of cell lines have been deep sequenced or genotyped for ~10^7 SNPs



INTER-INDIVIDUAL VARIABILITY AND "REAL-LIFE" TOXICOLOGY:

UNDERSTANDING DRUG-INDUCED ADVERSE HEALTH EFFECTS

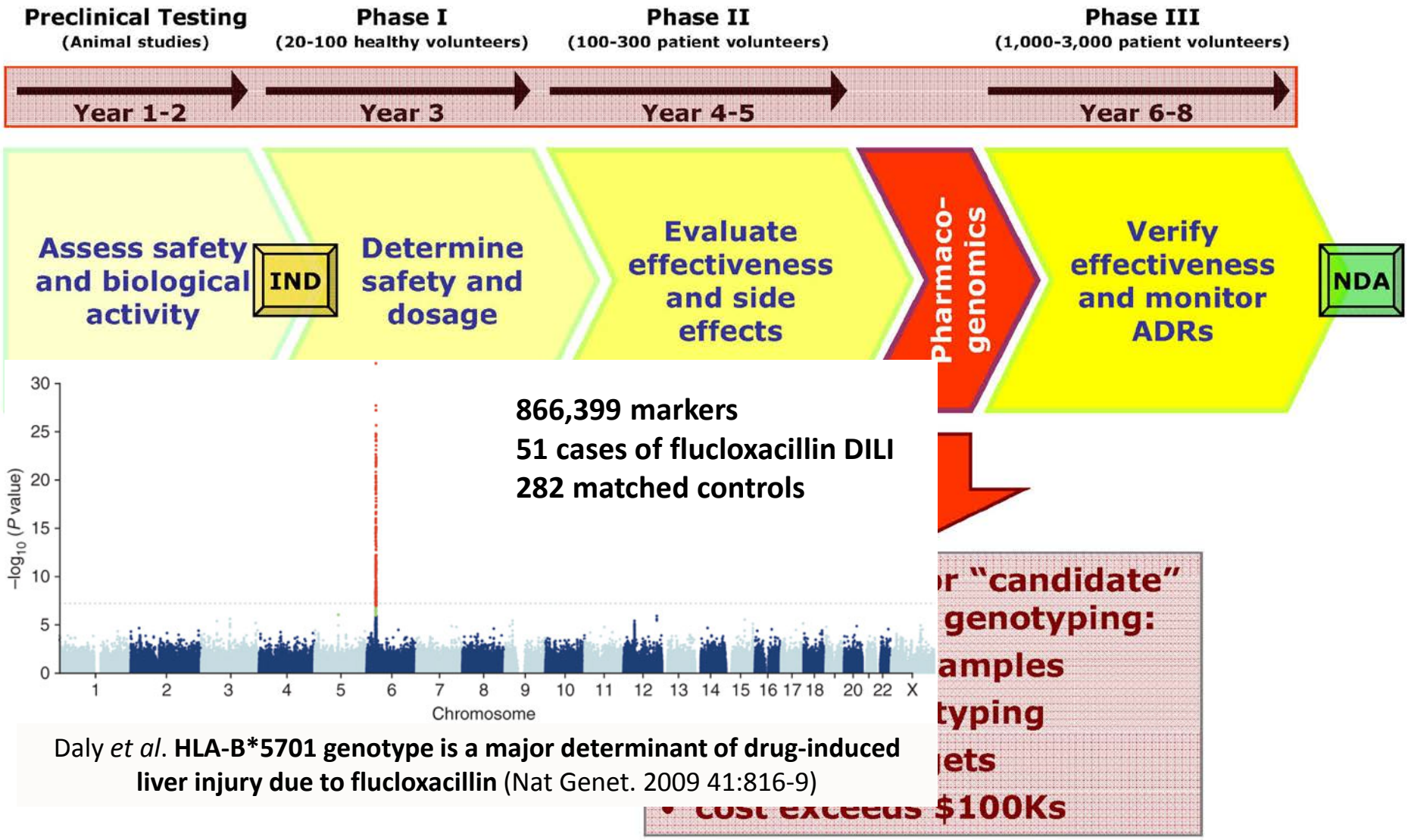


Genome-wide or "candidate" gene-based genotyping:

- hundreds of samples
- *de novo* genotyping
- unknown targets
- cost exceeds $100Ks

INTER-INDIVIDUAL VARIABILITY AND "REAL-LIFE" TOXICOLOGY:

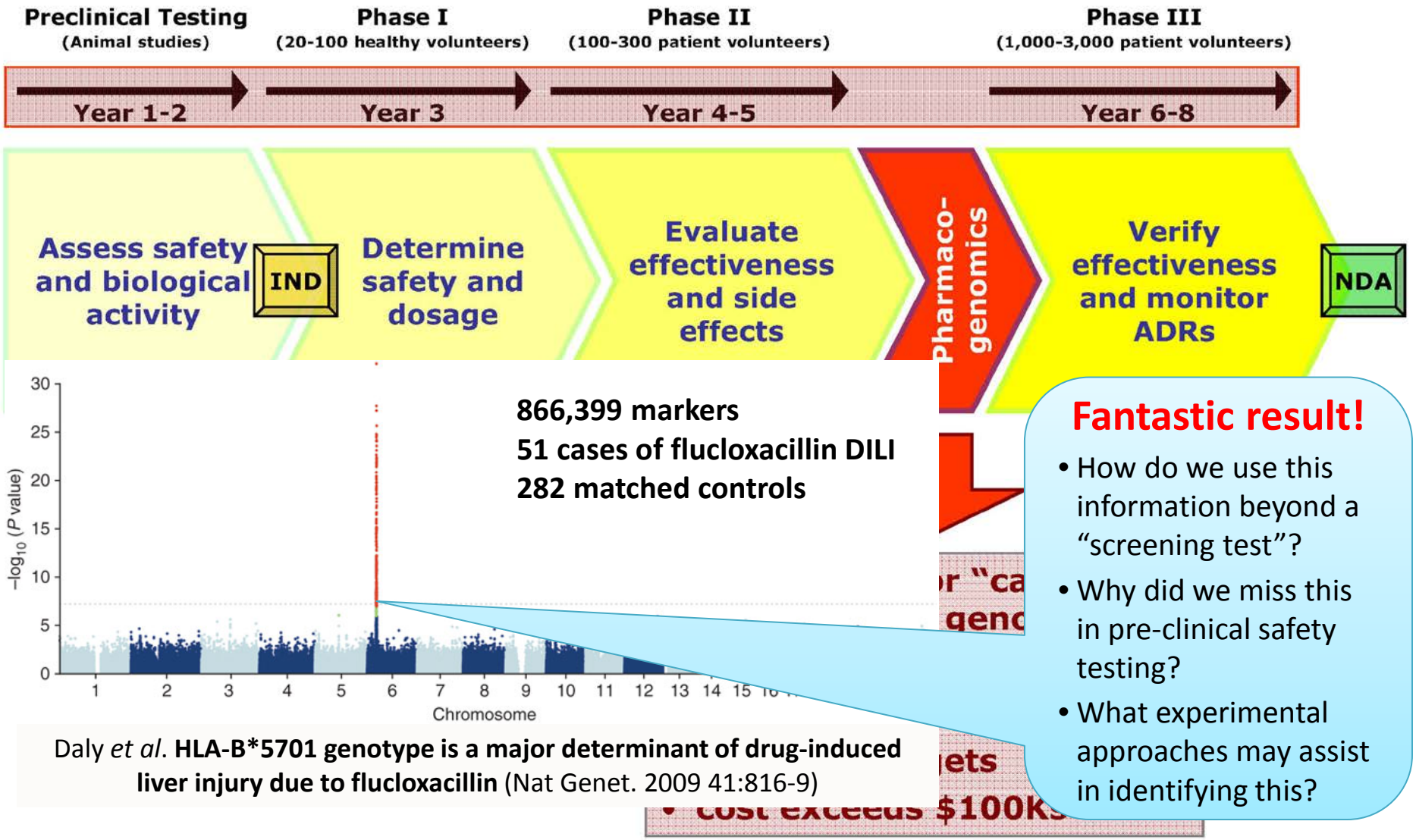
UNDERSTANDING DRUG-INDUCED ADVERSE HEALTH EFFECTS



Daly *et al*. **HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin** (Nat Genet. 2009 41:816-9)

INTER-INDIVIDUAL VARIABILITY AND "REAL-LIFE" TOXICOLOGY:

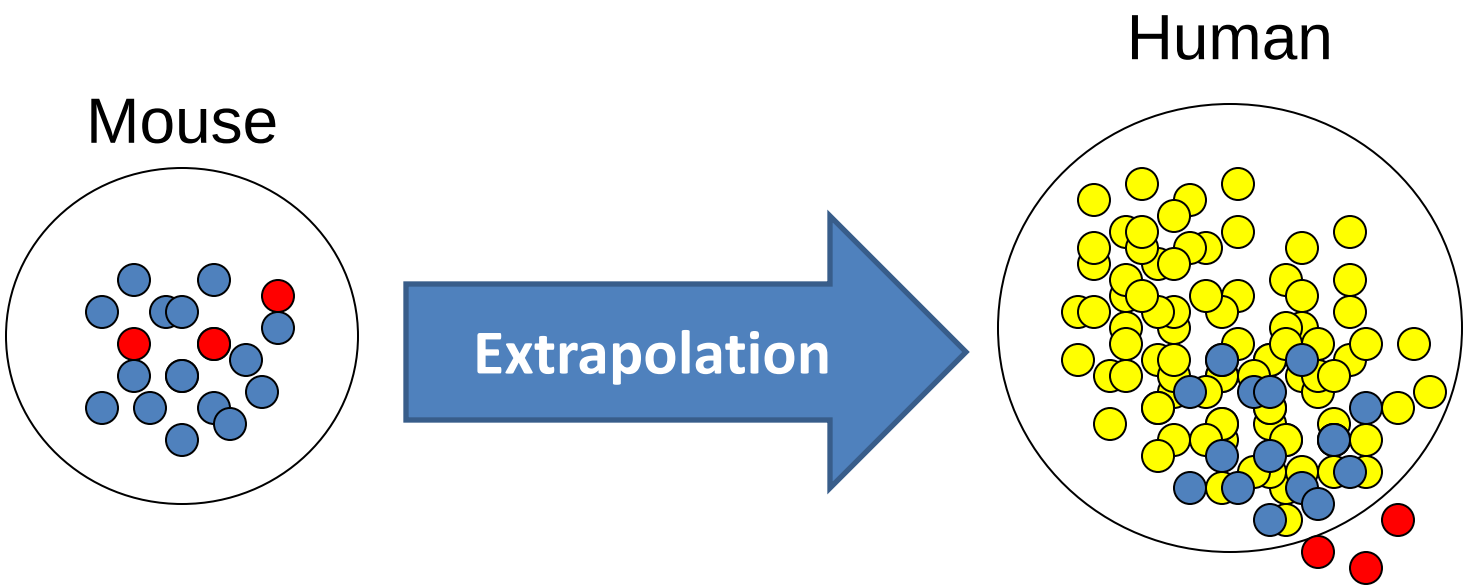
UNDERSTANDING DRUG-INDUCED ADVERSE HEALTH EFFECTS



Daly *et al*. **HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin** (Nat Genet. 2009 41:816-9)

How can we increase the odds of finding a "successful" animal model for human health assessment?

- **Single genetic variant** = risk of picking one that happens to be a poor model for the human population
- With a **genetically diverse test population**, one is more likely to "hit the target"



"*Poor*" models of humans

"*Good*" models of humans

Slide courtesy of Dr. Weihsueh Chiu (TAMU)

"*Human Health Assessment of Chemicals*" is...

The evaluation of *scientific information* on:

- the hazardous properties of chemicals → **Hazard assessment**
- the dose-response relationship → **Dose-response assessment**
- the extent of human exposure to those agents → **Exposure assessment**

+mode of action

"The product of the risk assessment is a statement regarding the probability that populations or individuals so exposed will be harmed and to what degree" → **Risk characterization (uncertainty factors and the "*margin of safety*")**

Modified from *National Research Council, 1983*

Observational Data

Models and Theories

Experimental Data

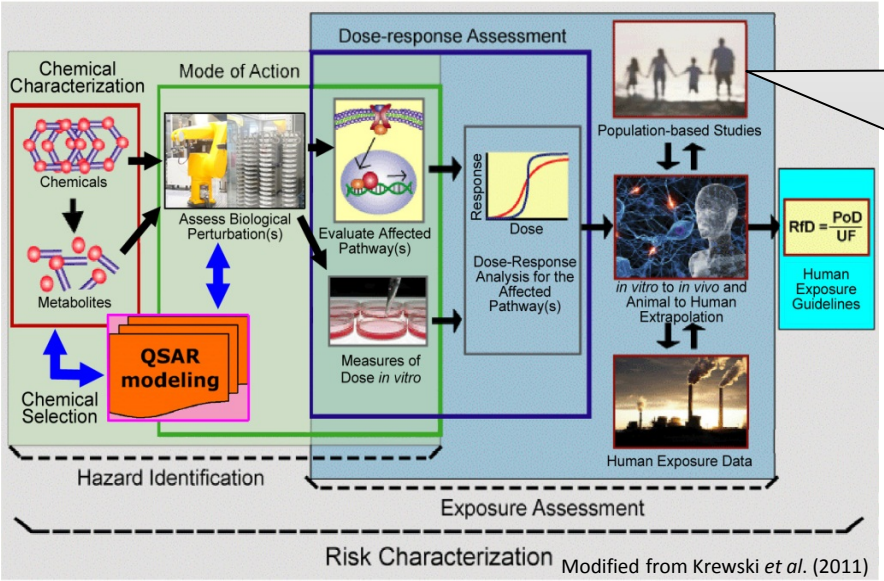
Evaluation, Analysis, Extrapolation, Synthesis

Human Health Risk Assessment

- **Hazard ID**
- **Dose-Response**
- **MOA analysis**

Toxicity has usually been [*and still is*] evaluated in test systems (experimental animals or *in vitro*) that are homogeneous in all aspects, including genetics

"New directions in toxicity testing"



Modified from Krewski *et al.* (2011)

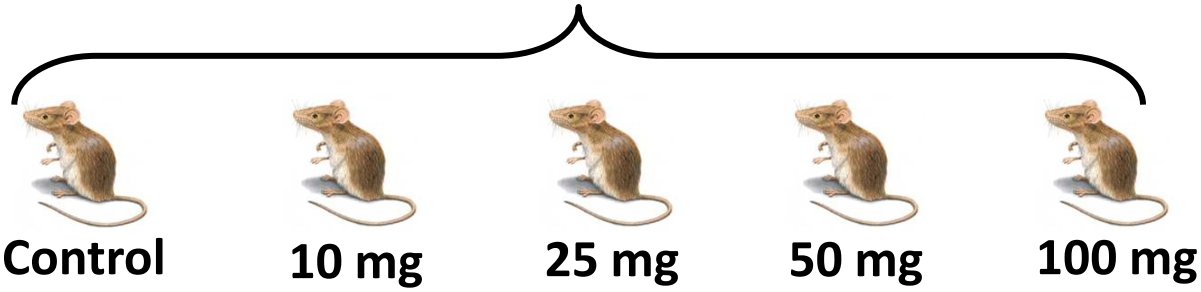
New experimental systems <u>can</u> incorporate genetic diversity:

while still controlling most variables in terms of age, treatment, etc., one can be using populations with

defined genetic heterogeneity

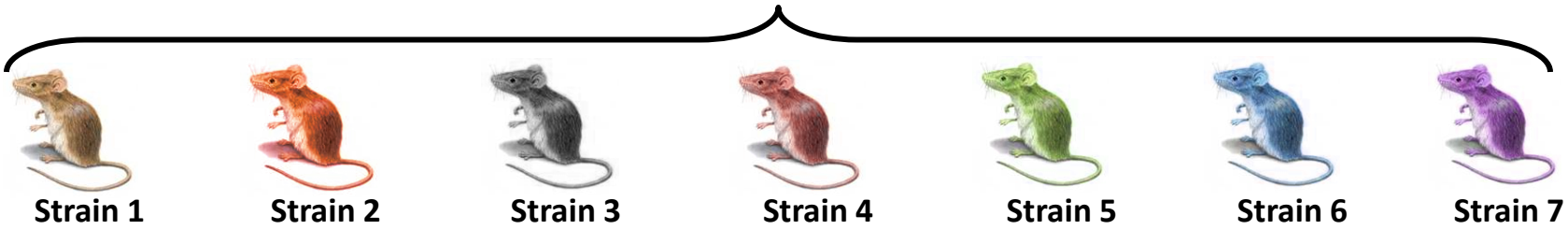
POPULATION-BASED STUDIES: A MOUSE MODEL FILLS CRITICAL GAPS

Single Strain: Constant Genotype



Vary the environment (e.g., treatment)

Many Strains: Varied Genotype



Fix the environment (same treatment), vary the genotype

POPULATION-BASED STUDIES: WHAT IS POSSIBLE ON A *reasonable* BUDGET?

Inbred Mouse Strain Studies			
Acetaminophen (oral)	One dose/1 time point	Liver, Serum	36 inbred, 1 hybrid
Ethanol (intragastric)	One dose/1 time point	Liver, Serum	15 inbred
TCE (oral)	One dose/3 time points (acute)	Liver, Kidney, Serum	15 inbred, 1 hybrid
TCE (oral)	One dose/1 time point (sub-acute)	Liver, Kidney, Serum	7 inbred
TCE (oral)	Two doses/3 time points (subchronic)	Liver, Kidney, Serum	2 inbred
Choline- and folate-deficiency (diet)	One protocol/time point	Liver, Serum, Plasma	7 strains
Butadiene (inhalation)	Two concentrations/1 time point	Liver, Kidney, Lung, BM	7 strains

Collaborative Cross Mouse Strain Studies			
TCE (oral)	Dose-response/1 time point	Liver, Kidney, Serum	50 strains, Males (1 mouse/group)
TCE (oral)	One dose/ 1 time point	Liver, Kidney, Serum	20 strains, M (1 mouse/group)
TCE (oral)	One dose/time-course	Liver, Kidney, Serum	6 strains, M/F (1 mouse/group)
PERC (oral)	One dose/time-course	Liver, Kidney, Serum	45 strains, M (1 mouse/group)
Butadiene (inhal.)	Two concentrations/1 time	Liver, Kidney, Lung, BM	50 strains, M (1 moue/group)

***In Vitro* Human Lymphoblast Studies**			
14 chemicals	Concentr.-response/1 time point	Cytotoxicity, Apoptosis	87 cell lines (one population)
240 chemicals	Concentr.-response/1 time point	Cytotoxicity	87 cell lines (one population)
179 chemicals	Concentr.-response/1 time point	Cytotoxicity	1086 cell lines (9 populations)

POPULATION-BASED STUDIES: WHO NEEDS THEM? *REALLY*…

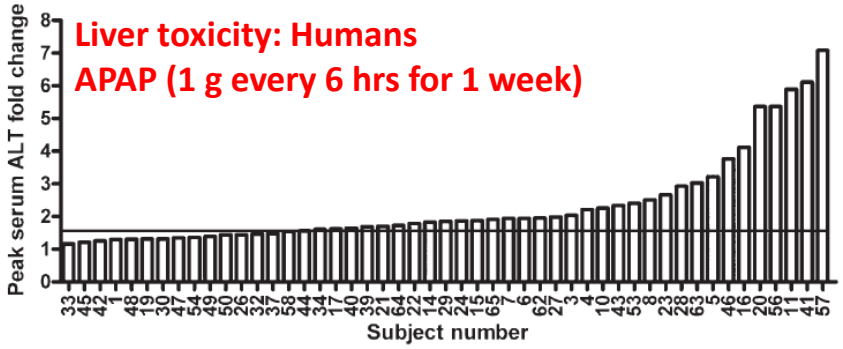
Frustrated human health assessor (*a.k.a.* regulator):
"Wait, isn't what you are proposing going to create another mountain of data? I already have enough to think about when I have the data on one mouse strain… Now I will have to interpret the data from many strains???"

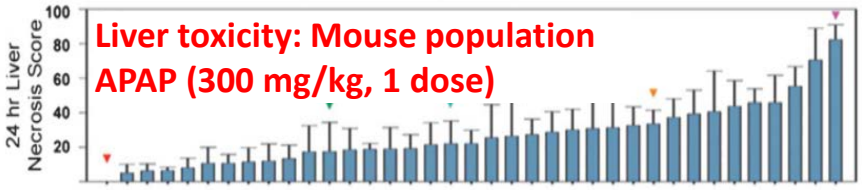
Equally frustrated chemical industry toxicologist:
"I'm not sure how the population variability has ended up in the proposal after so much discussion […]. Talking about "various tissues and disease states to emulate the anatomical and pathophysiological diversity of human organisms" and "…[cells] from [many] individuals to enable population based assessment of toxicity phenotypes" makes me really wonder ***"why??"*** *– i.e., is this really needed to meet our objectives,* ***is there really <u>a need</u> to build population variability into*** *[human health decisions]?"*

How a population-based study design can help, not hurt?

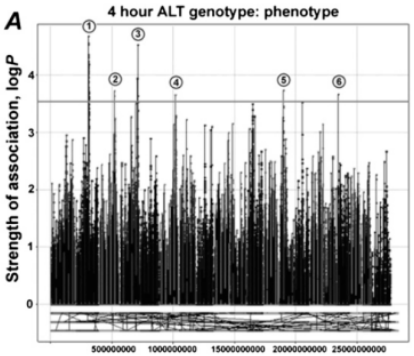
REPLACING DEFAULTS IN THE MODE OF ACTION ASSUMPTIONS THROUGH POPULATION-BASED MODEL STUDIES: IDENTIFYING "SUSCEPTIBILITY" GENES AND PATHWAYS

Acetaminophen-induced liver injury

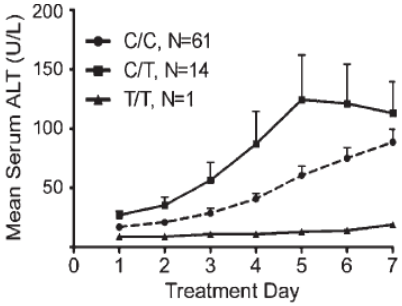




GWAS in mouse strains

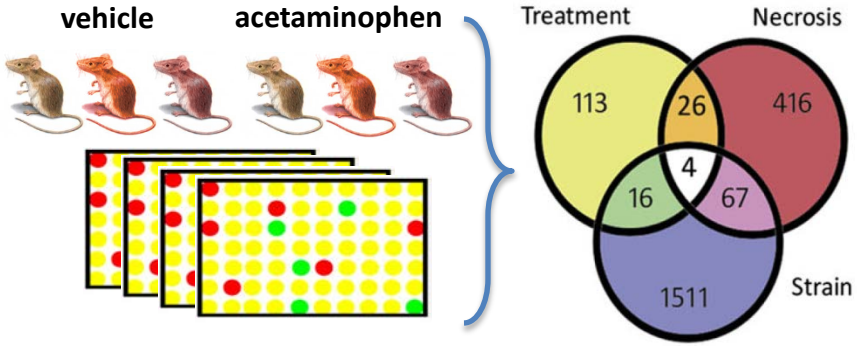


Confirm genetic susceptibility in humans

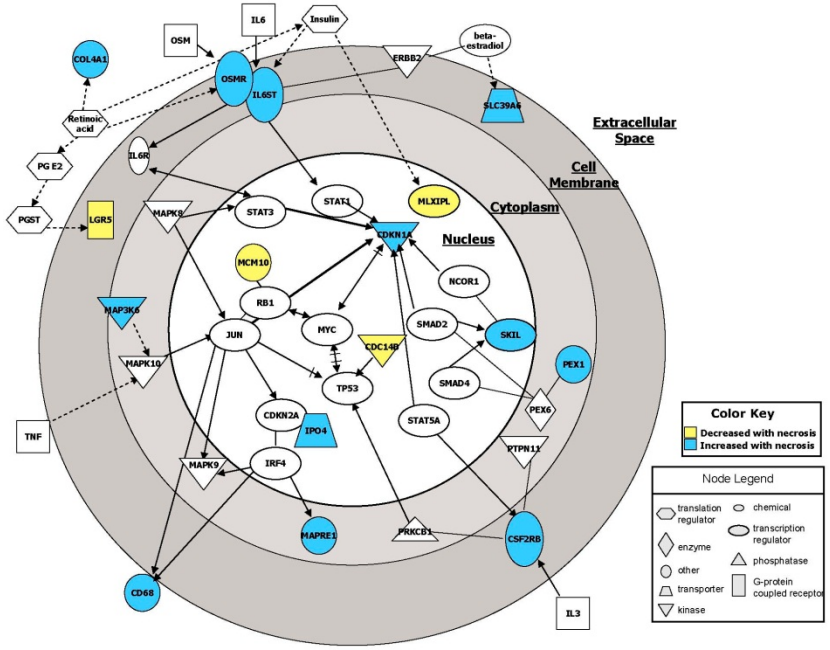


Harrill *et al.* (Genome Research, 2009)

Population-based toxicogenomics

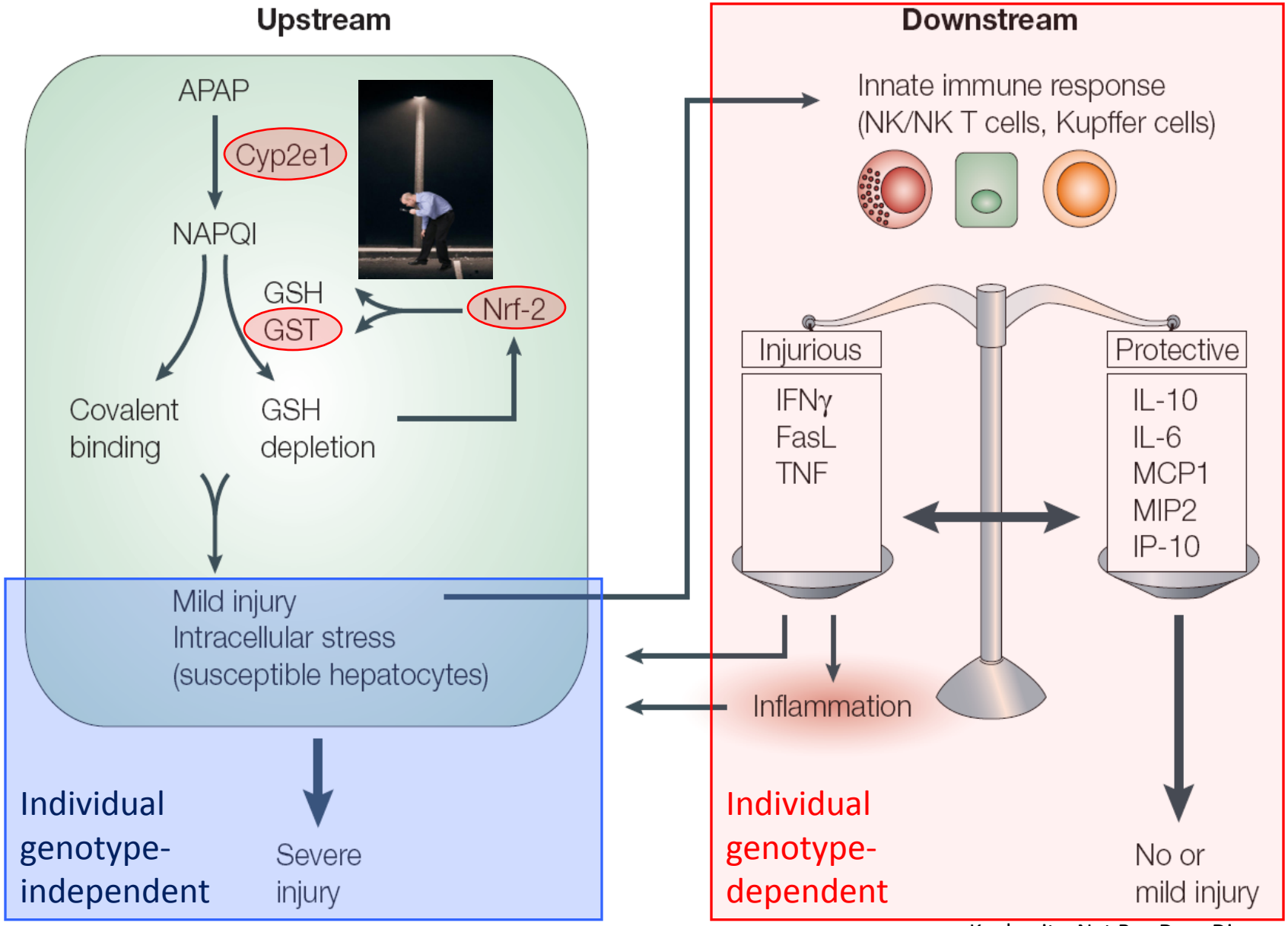


Genetic Background-Independent Pathway: *Cell Death and Proliferation*



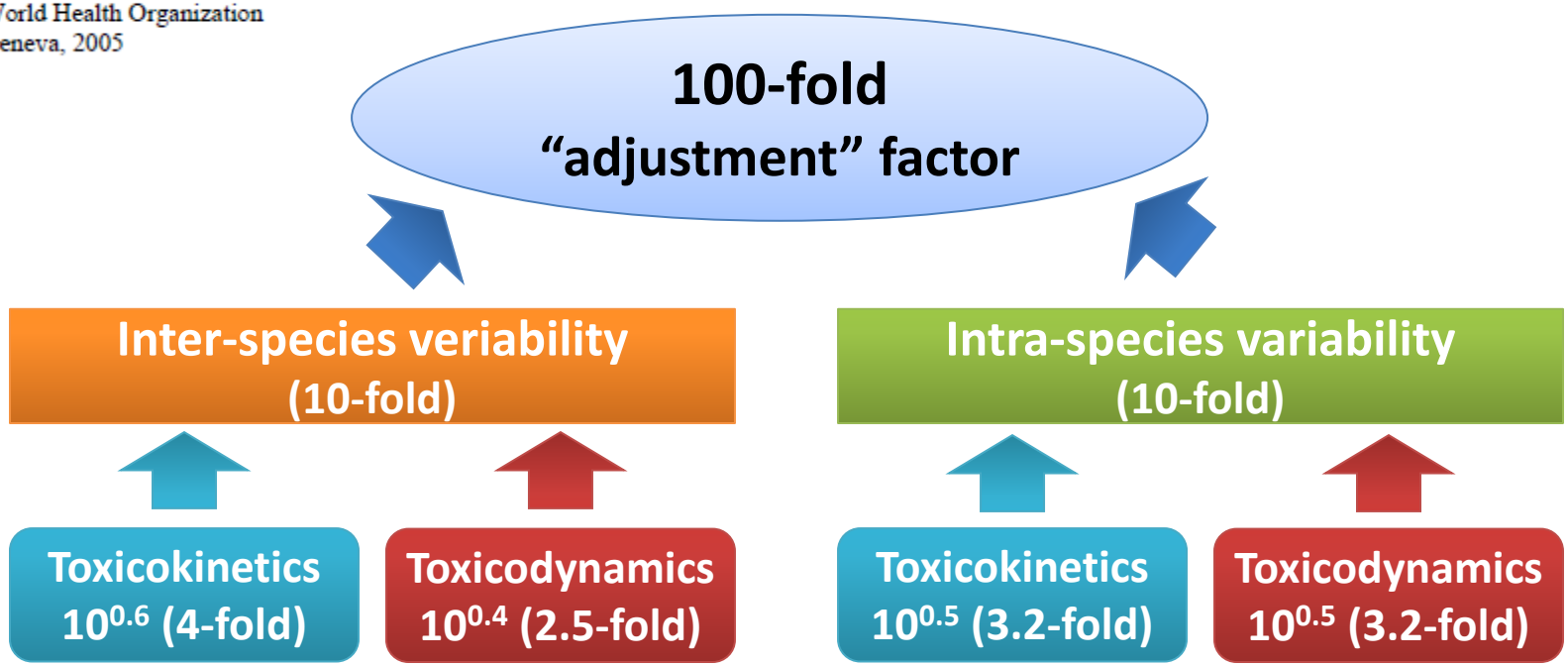
Harrill *et al.* (Toxicological Sciences, 2009)

REPLACING DEFAULTS IN THE MODE OF ACTION ASSUMPTIONS THROUGH POPULATION-BASED MODEL STUDIES: IDENTIFYING "SUSCEPTIBILITY" GENES AND PATHWAYS



Kaplowitz. Nat Rev Drug Discov. (2005)

World Health Organization
Geneva, 2005

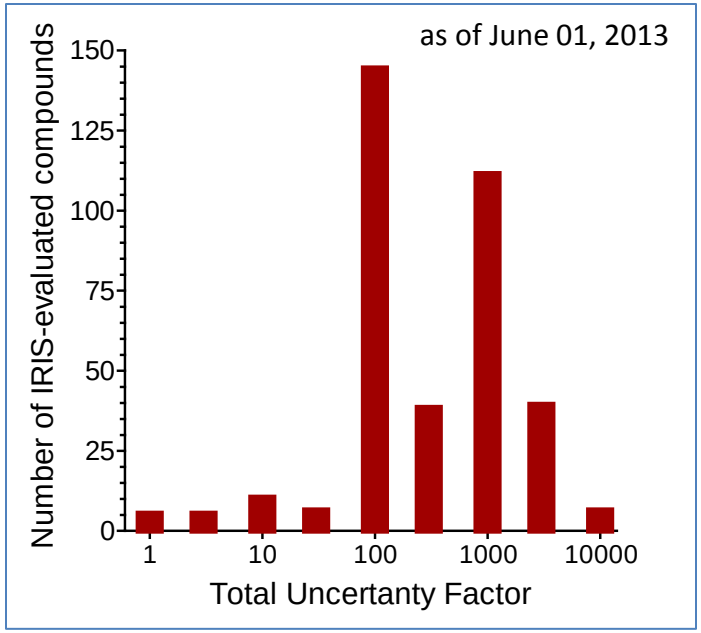


Uncertainty in extrapolating from animals to humans:

Dichloromethane IRIS: "The use of ***PBPK models*** to extrapolate internal doses from rats to humans ***reduces toxicokinetic uncertainty*** in extrapolating from the rat liver lesion data ***but does not account for the possibility that humans may be more sensitive than rats to dichloromethane due to toxicodynamic differences***."

Uncertainty in human variation (from average to sensitive):

Dichloromethane IRIS: "The probabilistic ***human PBPK model*** used in this assessment incorporates the best available information about variability in ***toxicokinetic disposition*** of dichloromethane in humans ***but does not account for humans who may be sensitive due to toxicodynamic factors***."



Inter-individual Variability in Toxicokinetics

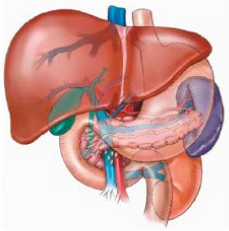
System specific factors

Demographic and genetic factors

Age
Weight
Height
Sex
Genetics
Race
Disease

+

Anatomical and physiological factors



Organ size
Blood flow
Enzymes
Transporters
Plasma protein
Haematocrit
Transit time
pH

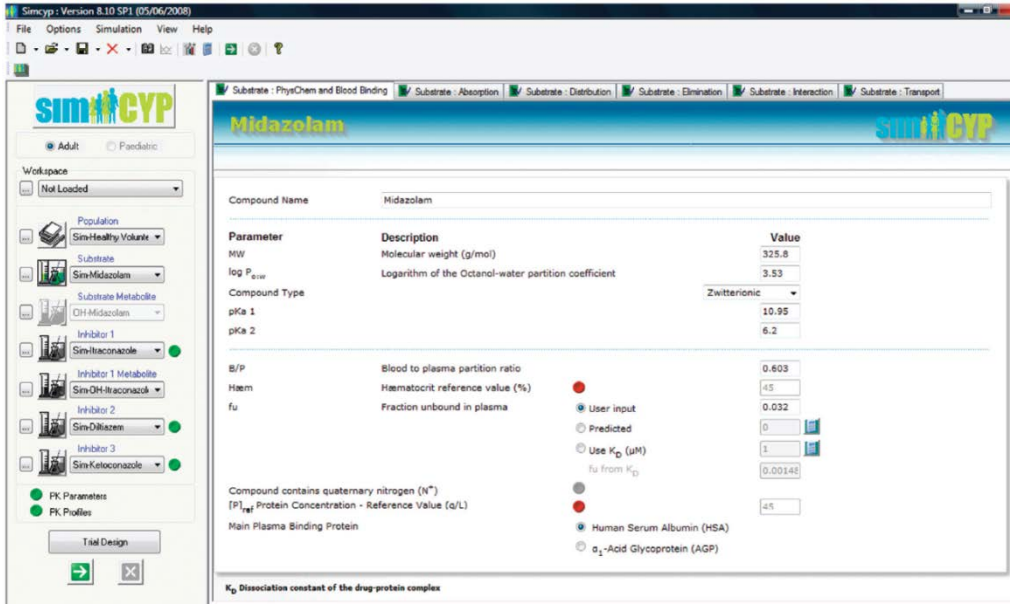
+

Drug specific factors

MW
pK_a
Log P
PSA
Solubility
Permeability
K_m, V_{max}
fu, B:P, fu_{inc}

Simulation of Human Variability: Simcyp

- Developed at U. Sheffield
  - Geoff Tucker, Amin Rostami-Hodjegan
- Drug industry funded
  - Pfizer, Roche, others
- Supports *in vitro* to *in vivo* extrapolation (IVIVE)
  - Prediction of *in vivo* absorption and clearance
- Incorporates data on population variability / pediatrics
- Designed to predict metabolic drug-drug interactions (DDI)
  - Uses PBPK structure
- Some limitations for environmental chemicals
  - Data on Cyps and UGTs, but not GSTs or esterases
  - IVIVE for absorption calibrated with data on "druglike" compounds
  - Expensive (or free)
- Other similar pharma-oriented simulation platforms available
  - GastroPlus
  - PKSim



Slide from Harvey Clewell (presented at NRC workshop on **Individual Variability: Biological Factors that Underlie Individual Susceptibility to Environmental Stressors and Their Implications for Decision-Making** April 18-19, 2012)
http://nas-sites.org/emergingscience/meetings/individual-variability/

Expert Opin Drug Metab Toxicol 2009 5:211-23

Inter-individual Variability in Toxicodynamics: A Persistent Challenge

- Rarely sufficient data to replace this component; more likely to be human data that addresses both kinetics and dynamics
  - cannot distinguish between them
- Data could be obtained from *in vitro* studies in tissue of critical effect from average versus sensitive humans

Slide from Harvey Clewell (presented at NRC workshop on Individual Variability: Biological Factors that Underlie Individual Susceptibility to Environmental Stressors and Their Implications for Decision-Making, April 18-19, 2012)
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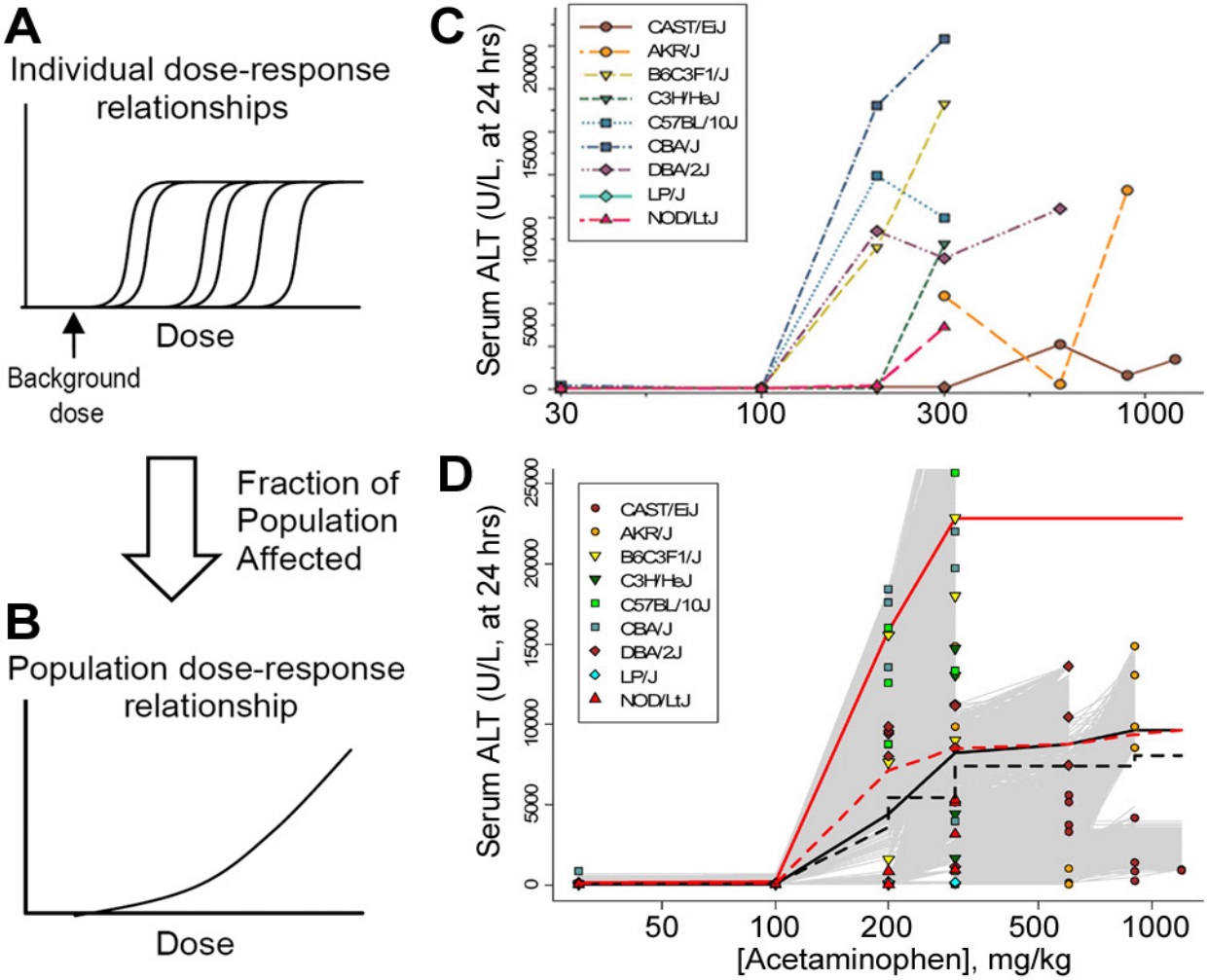
A MOUSE MODEL FILLS CRITICAL GAPS:

USING ACTUAL DATA FOR DOSE-RESPONSE IN INDIVIDUALS AND POPULATIONS

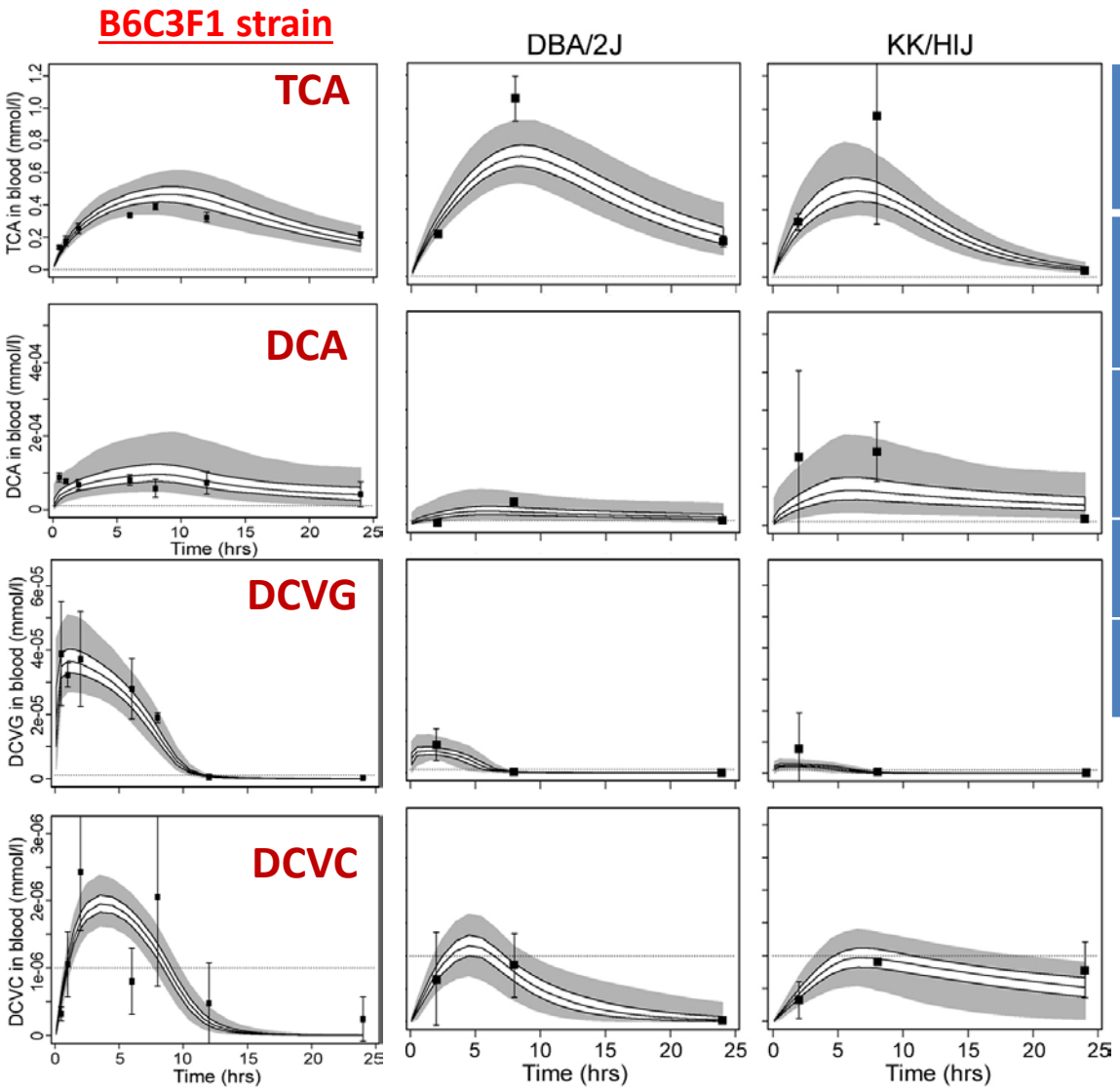
Acetaminophen-induced liver injury
(mouse population, 24 hr time-point)



National Research Council (2009)
Science and Decisions: Advancing Risk Assessment

Harrill *et al*. (Genome Res., 2009)

REPLACING DEFAULTS IN THE DOSE-RESPONSE ANALYSIS THROUGH POPULATION-BASED MODEL STUDIES: "SIMULATED" VARIABILITY OR REAL DATA?



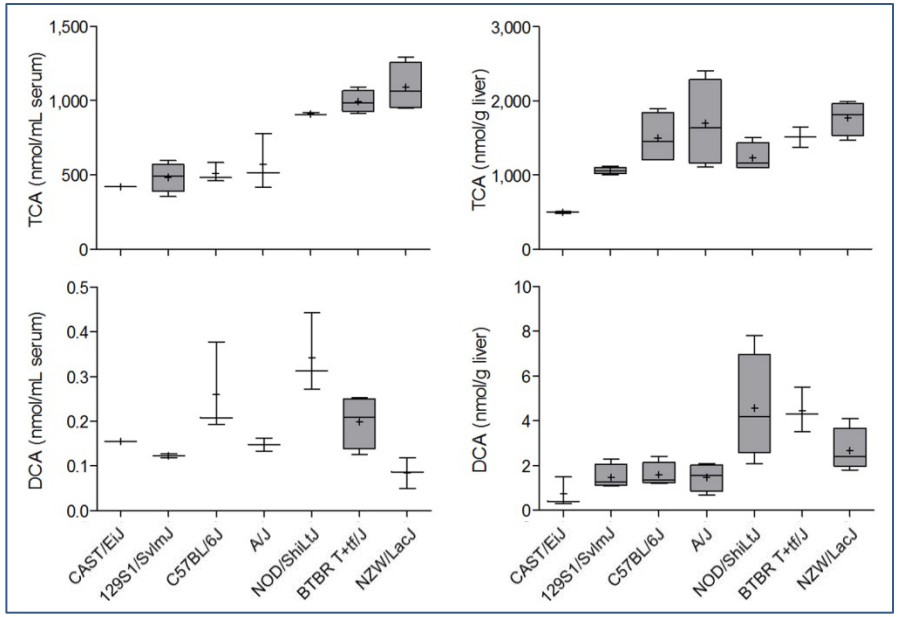
	Ratio of 95th percentile/50th percentile individual or strain*	
	Human inter-individual variability	**Mouse** inter-strain variability
TCE oxidized by P450	**1.11** (1.05, 1.22)	**1.05** (1.01, 1.27)
Total TCA produced	**2.09** (1.81, 2.51)	**1.77** (1.36, 2.99)
TCE conj. with GSH	**6.61** (3.95, 11.17)	**7.12** (3.43, 20.7)

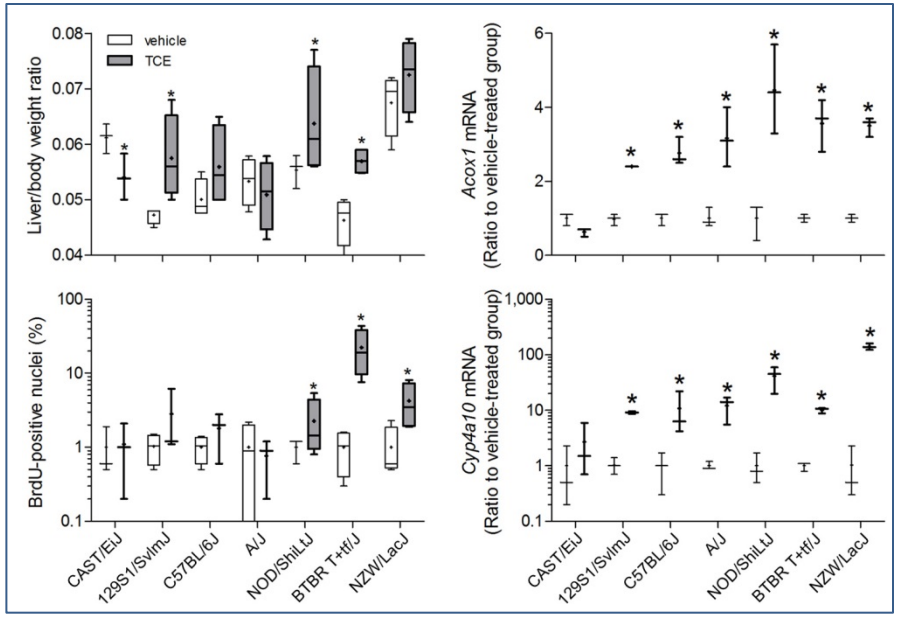
Mouse population variability in toxicokinetics is nearly identical to that in humans

Chiu *et al.* (Environmental Health Perspectives, 2014)

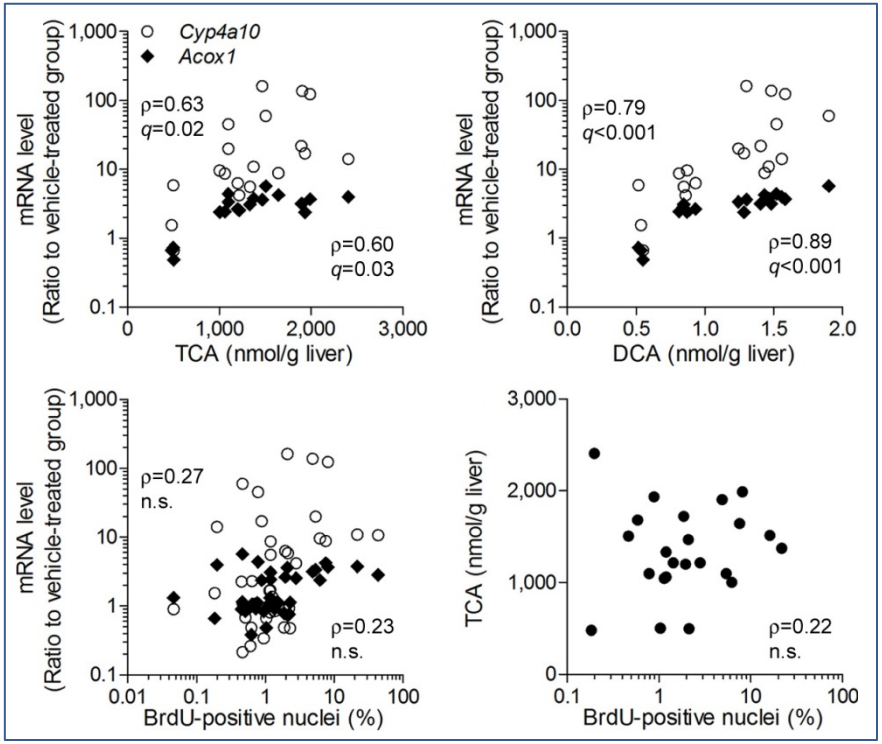
REPLACING DEFAULTS IN THE EXTRAPOLATION THROUGH POPULATION-BASED MODEL STUDIES:

Individual variability in metabolism ⟷ individual variability in liver toxicity



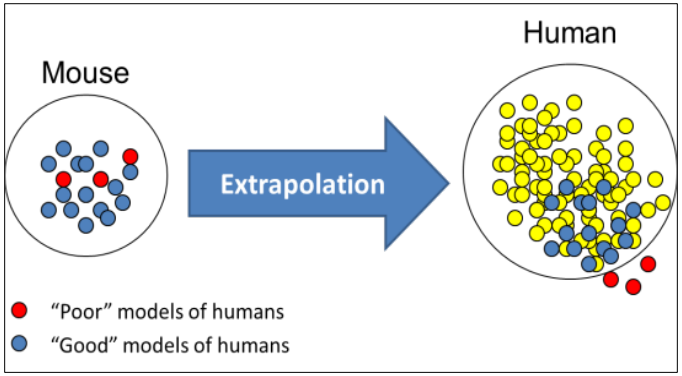


TCE (gavage, 5 days, 600 mg/kg/day)
Tissues collected 5 hr after last dose



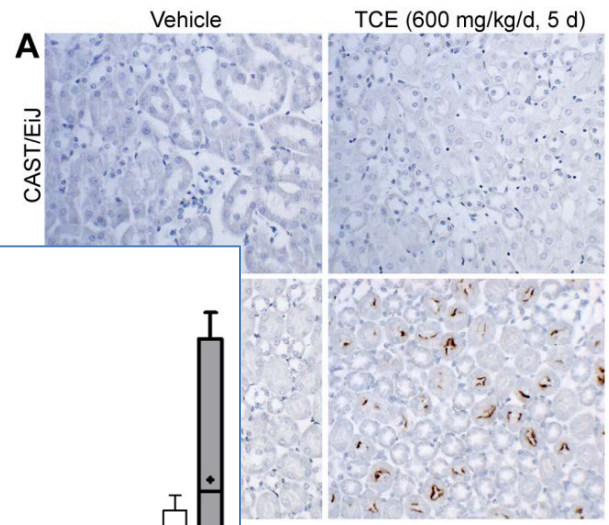
Yoo *et al.* (JTEHA, 2015)

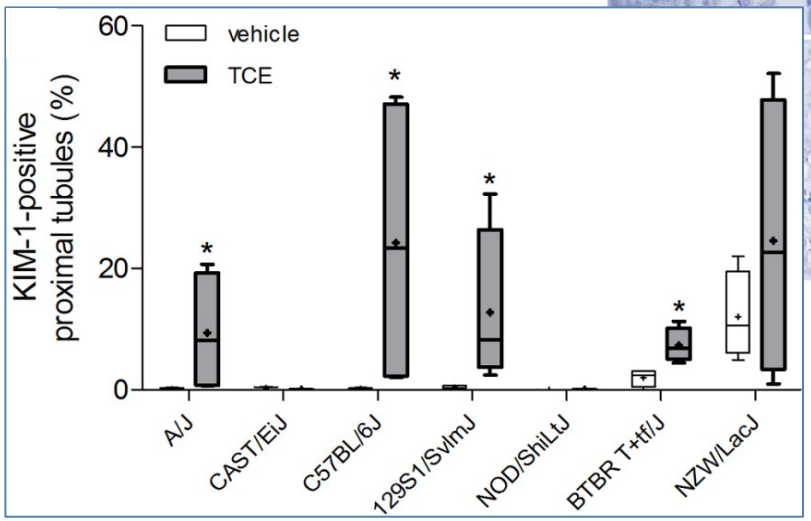
IMPROVED HAZARD IDENTIFICATION THROUGH POPULATION-BASED MODEL STUDIES: CREATING "GOOD" MODELS FOR HUMANS



Association between exposure to trichloroethylene (TCE) and tumor incidence in chronic animal and epidemiology studies

	Mouse		Rat		Human
	M	F	M	F	
Liver	Strong[1,2]	Strong[1,2]	-	-	Inconclusive[3]
Kidney	-	-	Strong[2]	-	Strong[3]





Yoo *et al.* (JTEHA, 2015)

Liver

- Liver cancer in mice (M, F)
- Weak association in epidemiological studies

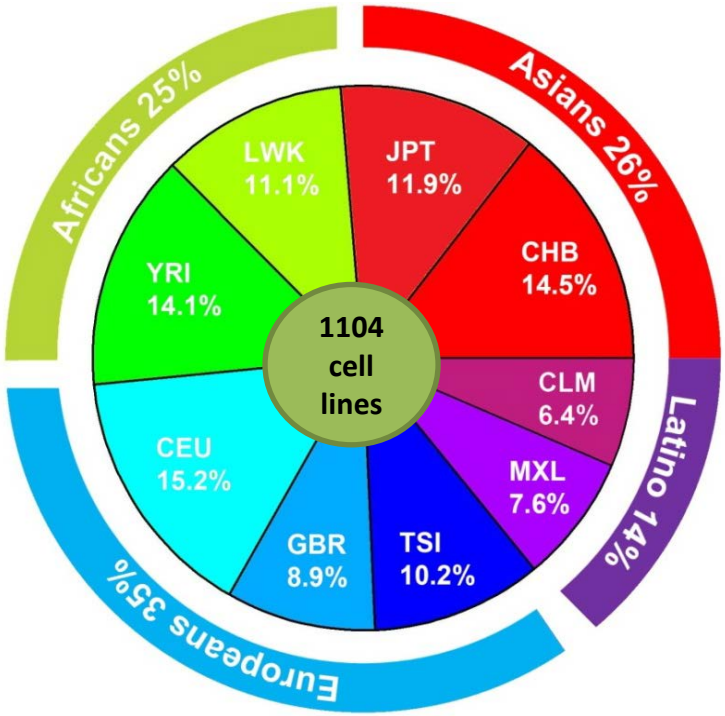
Kidney

- Renal cancer in male rats
- Toxic nephropathy including cytomegaly or hyperplasia was observed in both rats and mice
- TCE is one of the few environmental chemicals for which causal association was observed for kidney cancer in human epidemiological studies

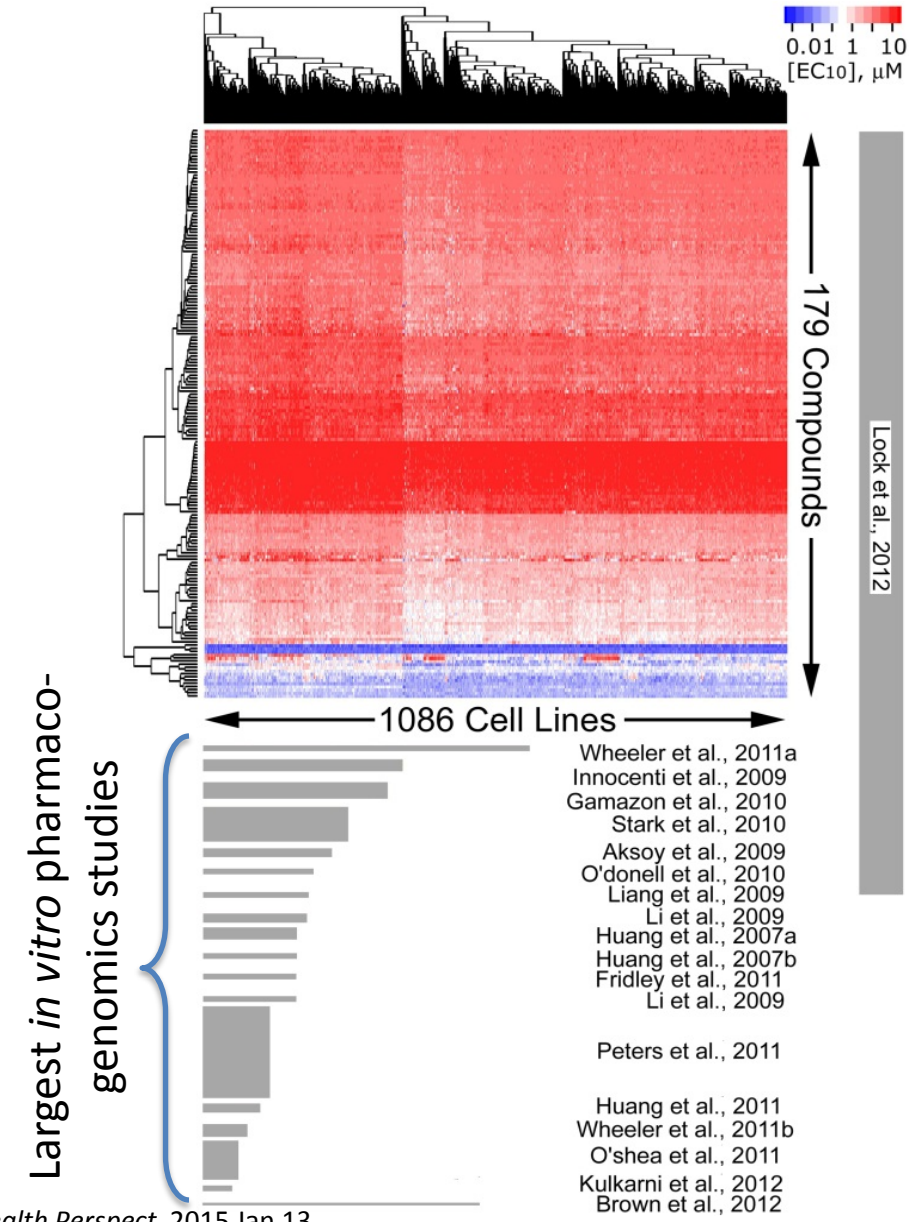
IN VITRO POPULATION-BASED MODEL FILLS CRITICAL GAPS:

PROBING RESPONSES USING WELL-POWERED POPULATION-WIDE STUDY DESIGN

NCGC-NTP-UNC Screening



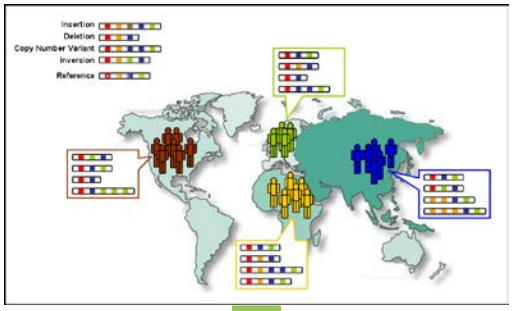
- CHB: Han Chinese in Beijing, China
- JPT: Japanese in Tokyo, Japan
- LWK: Luhya in Webuye, Kenya
- YRI: Yoruban in Ibadan, Nigeria
- CEU: Utah residents with European ancestry
- GBR: British From England and Scotland
- TSI: Tuscan in Italy
- MXL: Mexican ancestry in Los Angeles, California
- CLM: Colombian in Medellin, Colombia

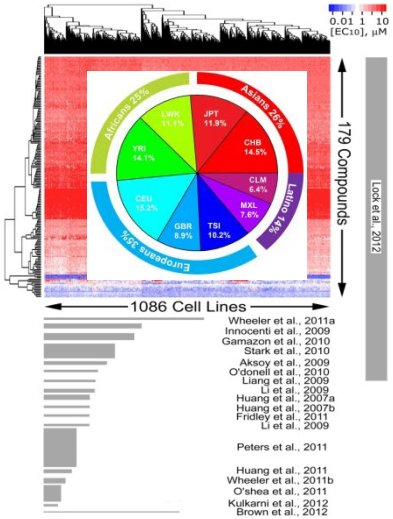


Abdo et al. *Environ Health Perspect*. 2015 Jan 13.

Why do toxicity testing in *in vitro* population-based models?

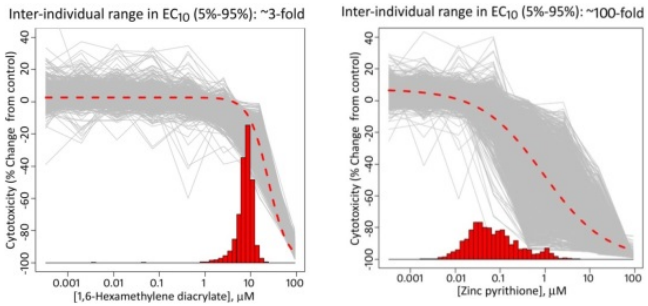
Because "Yes, we can!"



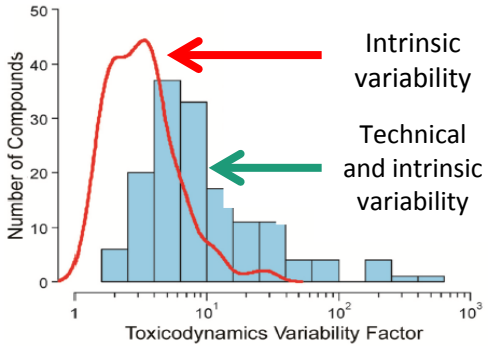


Because it generates more informative data!

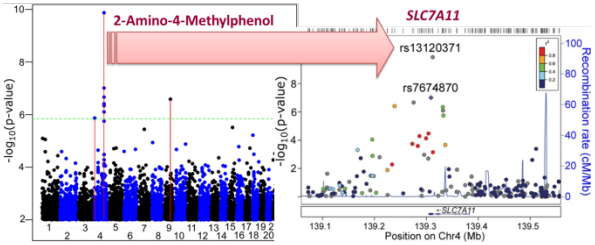
- Hazard
- Dose-response



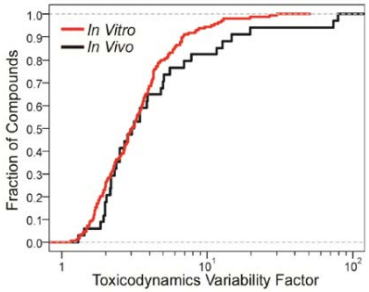
- Chemical-specific variability



- Mechanistic hypotheses
- Susceptibility genes



- Human relevance



Abdo et al. *Environ Health Perspect*. 2015 Jan 13.

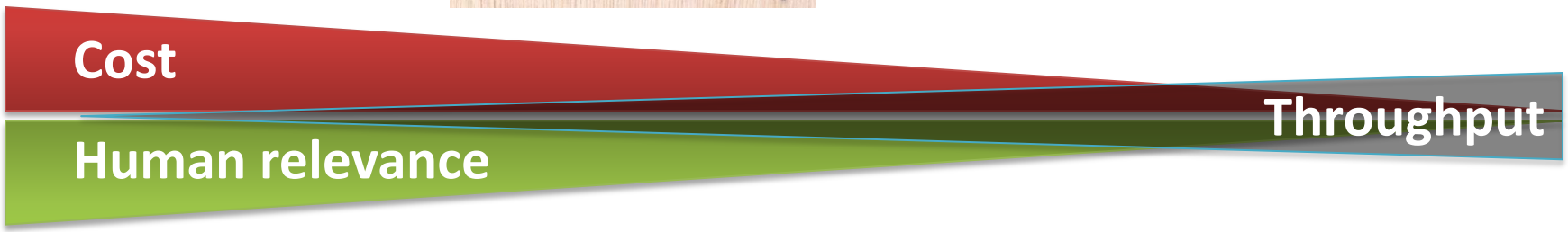
Population-based resources for toxicology:

If you build it, will they come?

	Pharmaceuticals	Environmental chemicals
How many?	One or several	Hundreds to thousands
Who pays?	$$$$$s already spent	"*The government*"?
What is the "?"	What is the SNP/gene/locus?	Extent of variability

Epidemiology studies

Large mouse population studies

Smaller mouse population studies

In vitro population studies