IN VITRO SCREENING FOR INTER-INDIVIDUAL AND POPULATION VARIABILITY IN TOXICITY OF PESTICIDE MIXTURES

Nour Abdo, DVM, MPH
PhD Candidate
Department of Environmental Sciences and Engineering
University of North Carolina at Chapel Hill

WHAT WILL WE DO WITH *IN VITRO* DATA ON THOUSANDS OF CHEMICALS?

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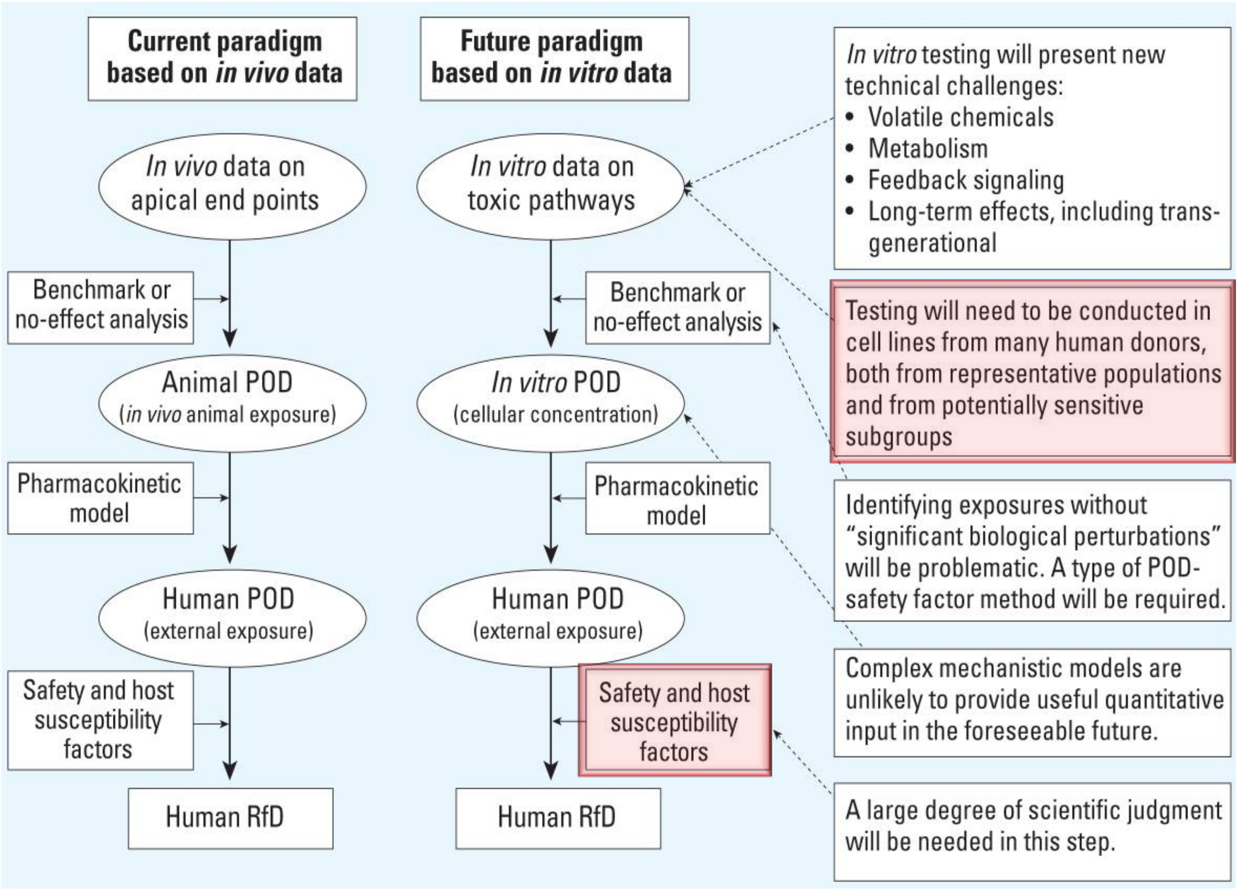
The Future Use of *in Vitro* Data in Risk Assessment to Set Human Exposure Standards: Challenging Problems and Familiar Solutions

Kenny S. Crump,[1] Chao Chen,[2] and Thomas A. Louis[3]

[1]Louisiana Tech University, Ruston, Louisiana, USA; [2]National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC, USA; [3]Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

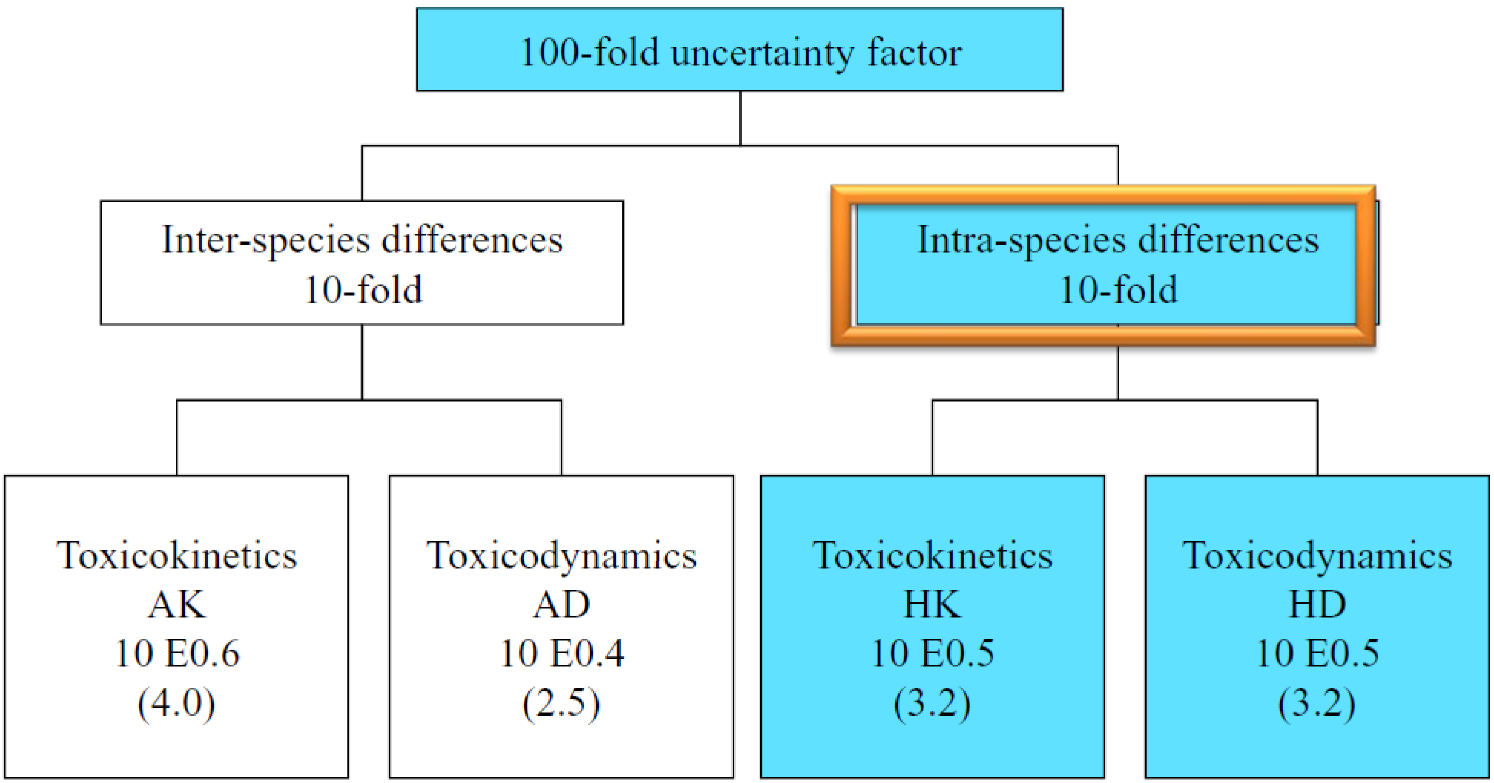


Harmonization Project Document No. 2

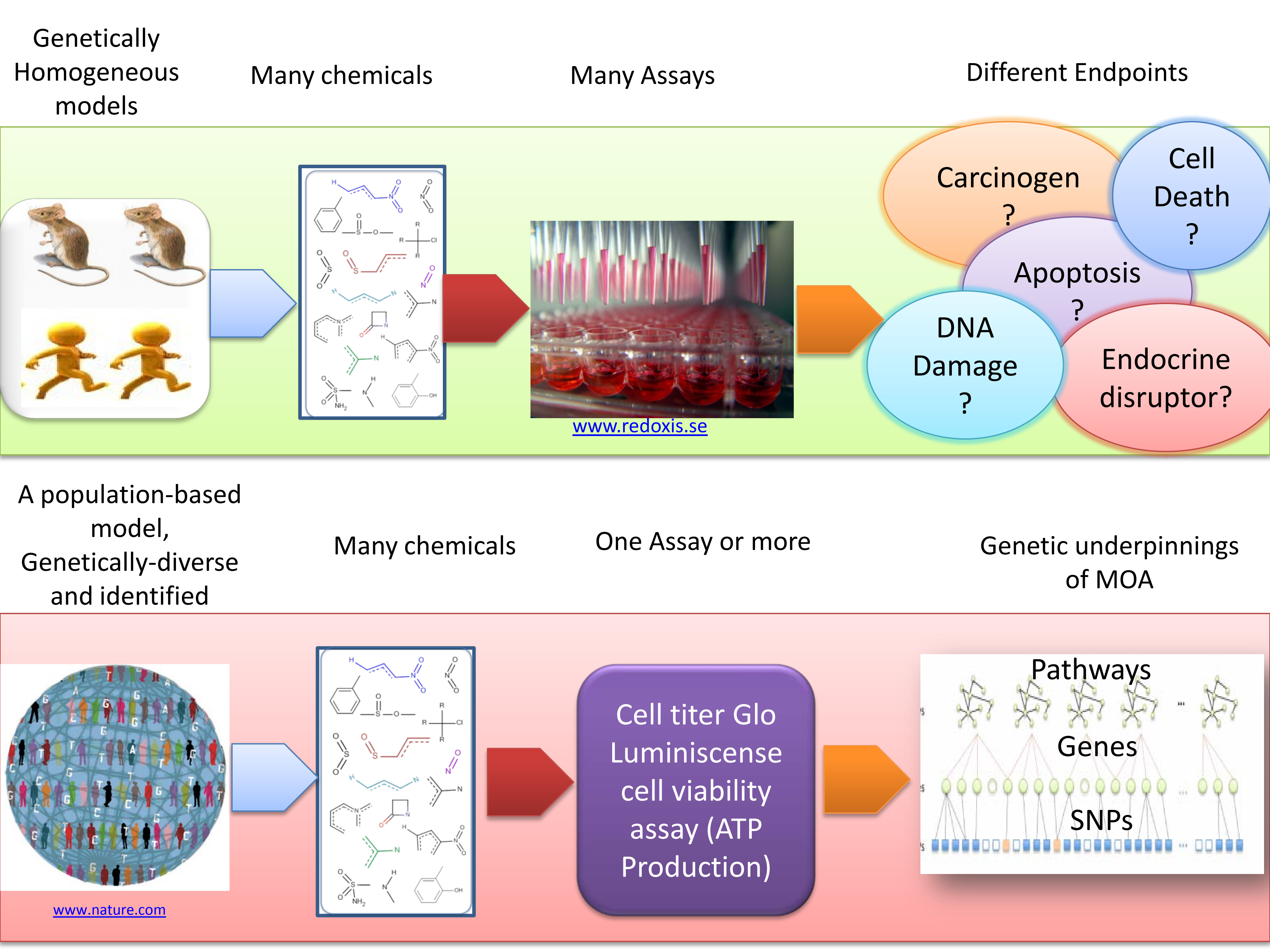
CHEMICAL-SPECIFIC ADJUSTMENT FACTORS FOR
INTERSPECIES DIFFERENCES AND HUMAN VARIABILITY:
GUIDANCE DOCUMENT FOR USE OF DATA IN
DOSE/CONCENTRATION–RESPONSE ASSESSMENT

World Health Organization
Geneva, 2005

Chemical Specific Adjustment Factor (CSAF)



Slide from Michael Dourson (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/


Genetically Homogeneous models
Many chemicals
Many Assays
Different Endpoints
www.redoxis.se
Carcinogen ?
Cell Death ?
Apoptosis ?
DNA Damage ?
Endocrine disruptor?
A population-based model, Genetically-diverse and identified
Many chemicals
One Assay or more
Genetic underpinnings of MOA
www.nature.com
Cell titer Glo Luminiscense cell viability assay (ATP Production)
Pathways
Genes
SNPs

Population genetics effort enables *in vitro* toxicity testing

http://blog-epi.grants.cancer.gov/2012/08/27/what-have-we-learned-from-epidemiology-cohorts-and-where-should-we-go-next/

http://www.sciencedaily.com/releases/2010/10/101027133238.htm

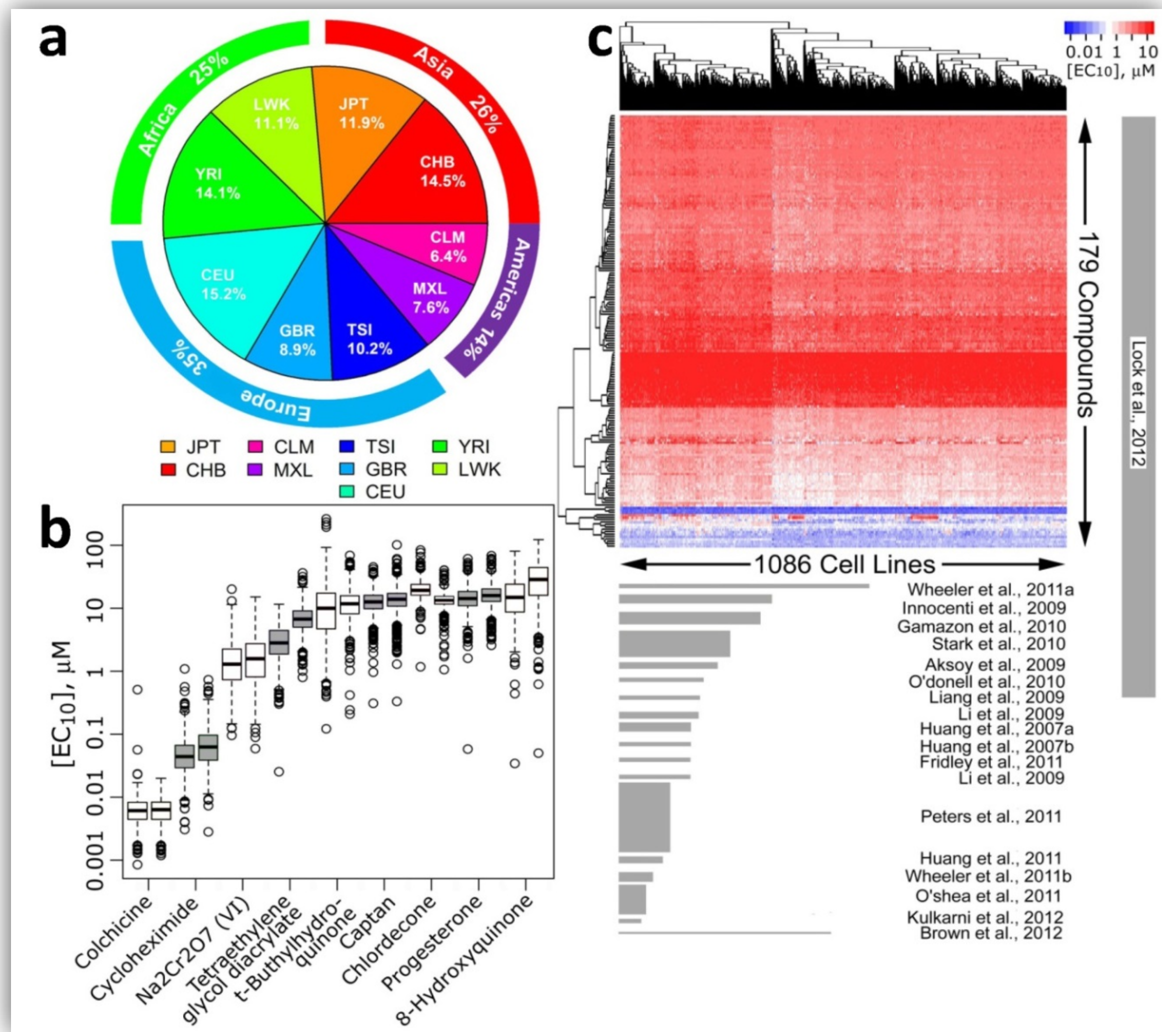
The 1000 Genomes and HapMap Projects have established thousands of immortalized cell lines LCLs (B-lymphocyte derived) from geographically and genetically diverse human populations

http://www.buzzle.com/articles/blood-donation-side-effects.html

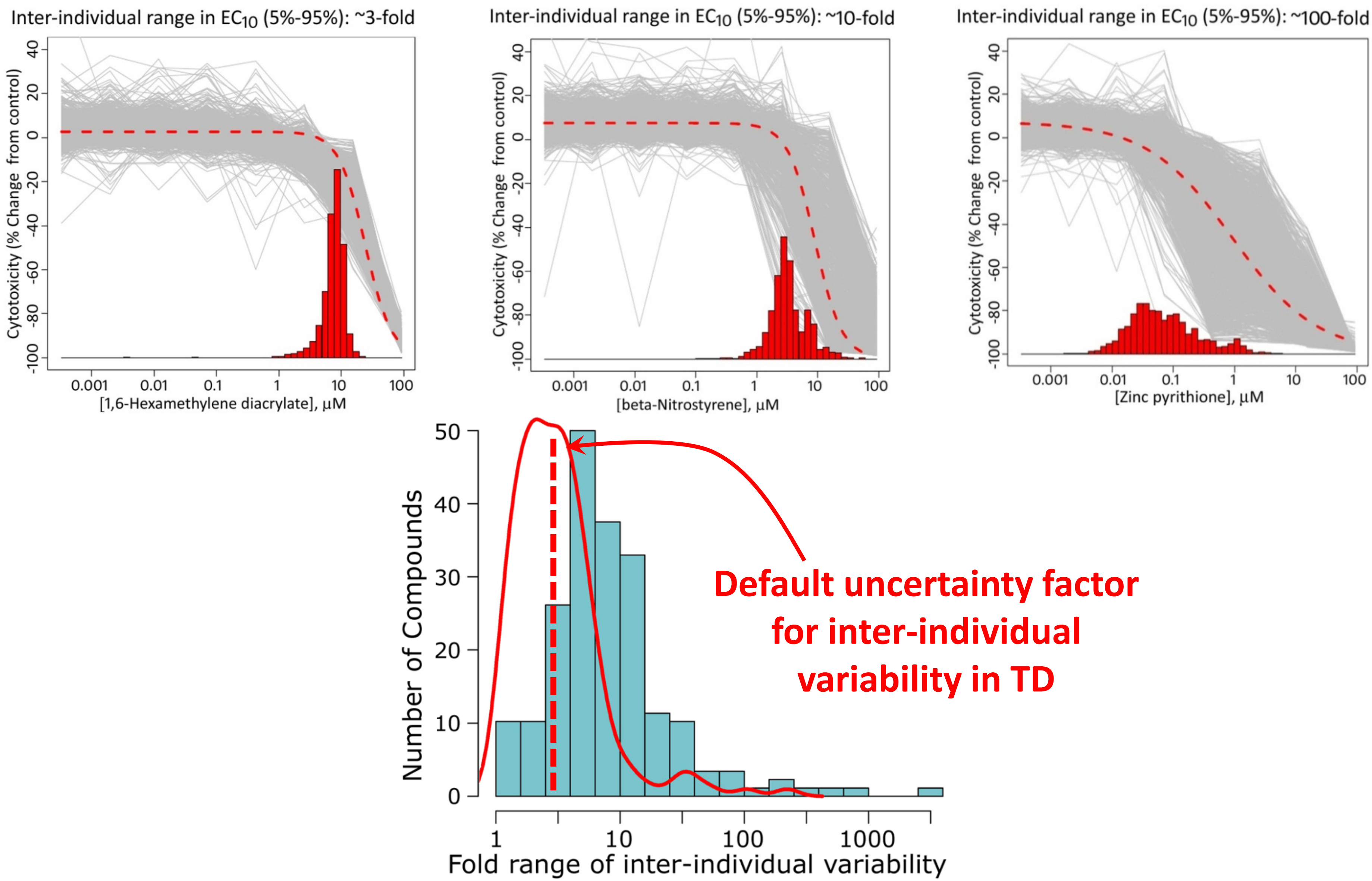
http://www.shutterstock.com/pic-94660003/stock-photo-handling-of-cell-culture-plates-for-cultivation-of-immortalized-cancer-cell-lines-in-life-science.html

1000 Genomes Toxicogenetics Project (UNC-NTP-NCATS):

Addressing chemical toxicity and population variability in a human *in vitro* model system

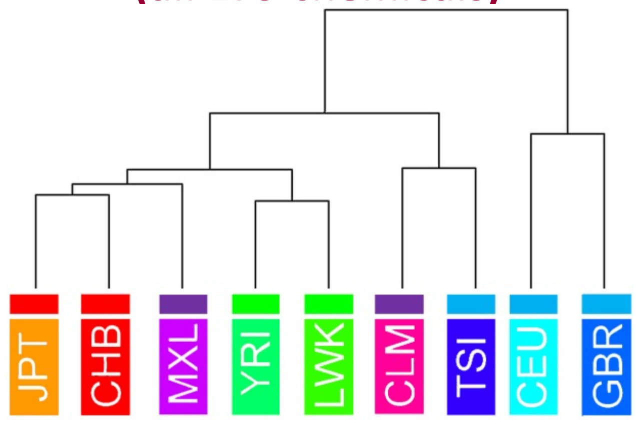


"WHY SHOULD I CARE?" REASON #1: ASSESSING HAZARD AND INTER-INDIVIDUAL VARIABILITY IN TOXICODYNAMICS FOR INDIVIDUAL CHEMICALS

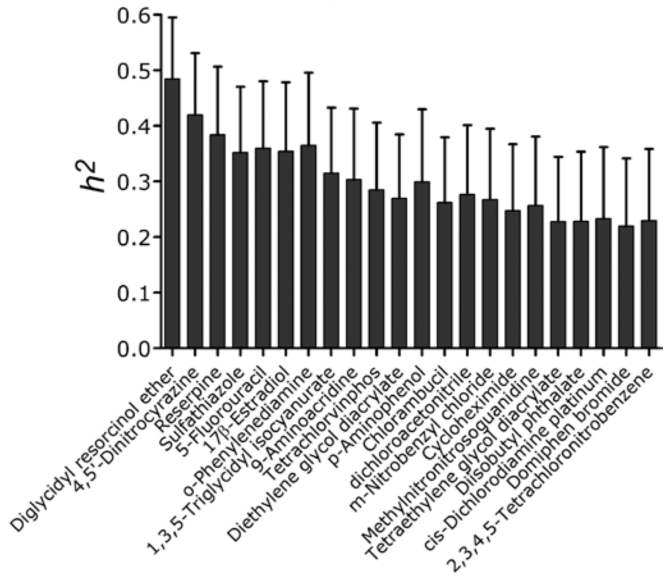


“WHY SHOULD I CARE?” REASON #2:
IDENTIFYING SUSCEPTIBLE SUB-POPULATIONS

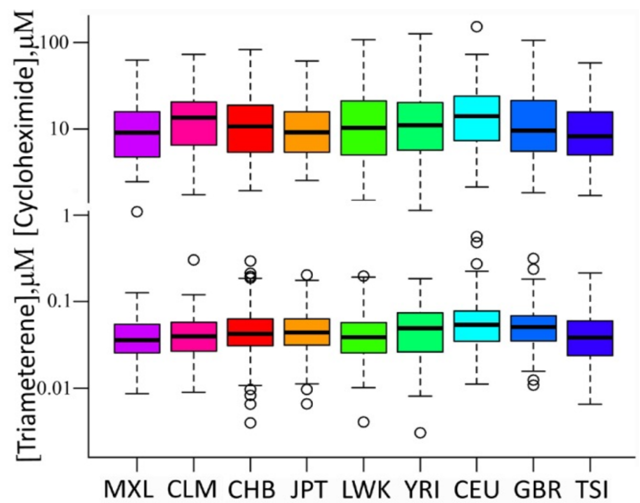
Subpopulation-specific profiles (all 179 chemicals)



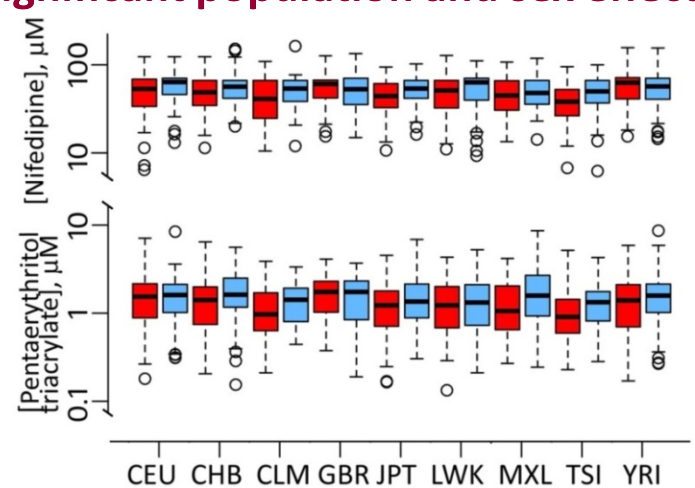
High heritability → genetic determinants



Significant population effect (2 of 79 chemicals shown)



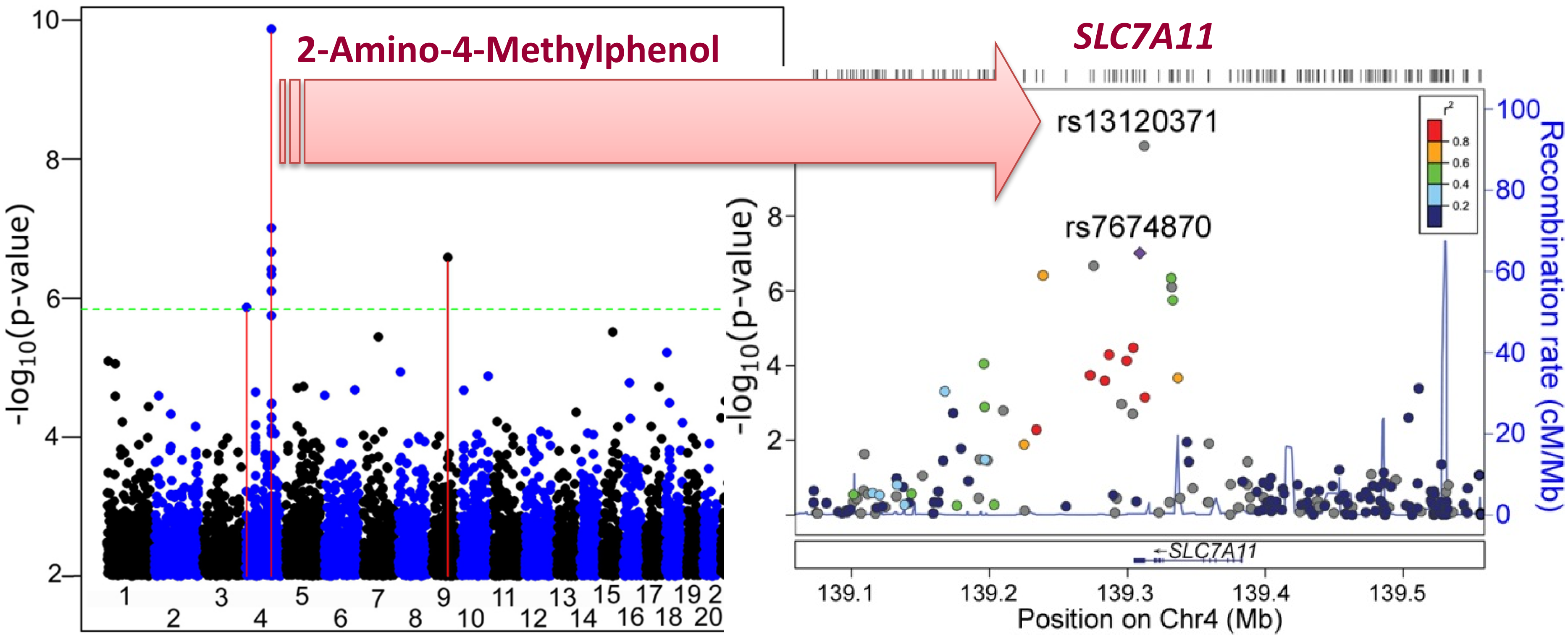
Significant population and sex effect



“WHY SHOULD I CARE?” REASON #3:

UNDERSTANDING GENETIC DETERMINANTS OF INTER-INDIVIDUAL VARIABILITY

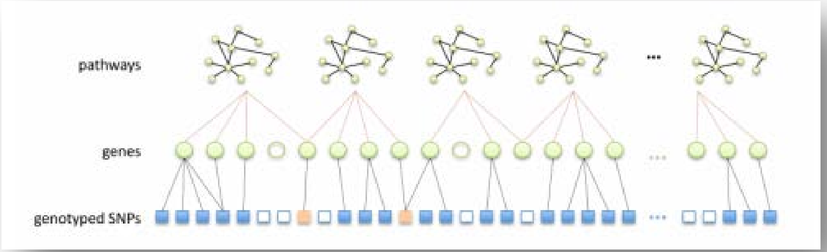
	ChemicalName	ChrNum	RSID	$-\log_{10}(p)$	Genes
1	2-Amino-4-methylphenol	4	rs13120371	9.9	SLC7A11
2	o-Aminophenol	16	rs1800566	8.9	NQO1
3	13-Dicyclohexylcarbodiimide	8	rs28437300	8.9	^{u}SLC39A14
4	N-Isopropyl-N-phenyl-p-phenylenediamine	7	rs1159874	8.8	None
5	Methylmercuric(II)chloride	4	rs13120371	7.9	SLC7A11
6	Aldrin	3	rs340251	7.8	^{d}MFSD1
7	Titanocenedichloride	15	rs62009303	7.7	SNP not known
8	Reserpine	4	rs13143102	7.6	None
9	Cycloheximide	16	rs8053118	7.5	WWOX
10	Dieldrin	1	rs504504	7.5	MCOLN2



"WHY SHOULD I CARE?" REASON #4:

GENERATE TESTABLE HYPOTHESES ABOUT TOXICITY PATHWAYS

GWAS-based Pathway Analysis:



Chemical	Gene set	Gene Set Name	Num	P (fwer)	P (raw)	P (fdr)
2-Pivalyl-1,3-indandione	GO.BP	Cellular response to dexamethasone stimulus	7	0.323	1.69E-05	0.0705
8-Hydroxyquinoline	KEGG	Steroid hormone biosynthesis	52	0.02	0.0006	0.0652
Cadmium chloride	GO.BP	Gonadotropin secretion	8	0.132	2.80E-06	0.0057
Pentaerythritol triacrylate	GO.BP	Cellular response to dexamethasone stimulus	7	0.215	6.10E-06	0.0254
Triamterene	GO.BP	Negative regulation of sterol transport	8	0.19	5.46E-06	0.0228

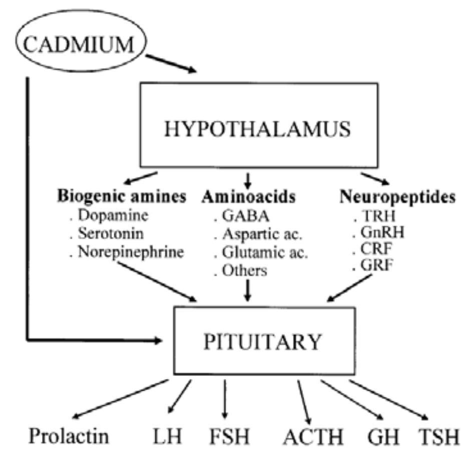
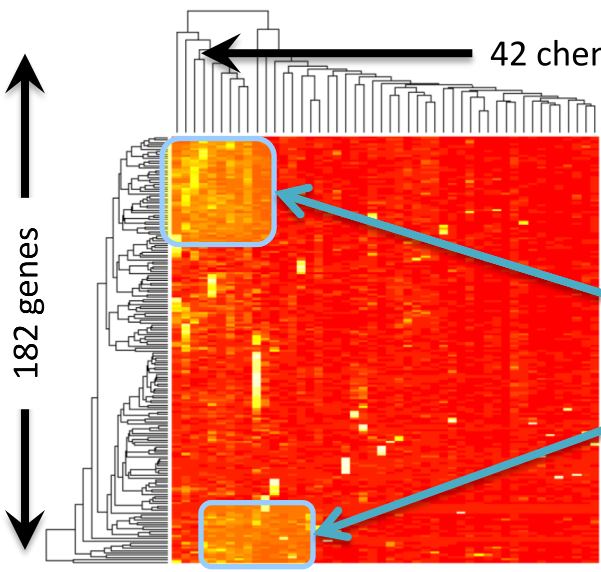


Fig. 5. Cadmium can affect the hypothalamic–pituitary axis at the hypothalamic and/or pituitary level.

Image from: Lafuente and Esquifino (1999)



Correlation analysis of basal gene expression across 350 cell lines (RNA-sequencing) and chemical-specific cytotoxicity phenotypes:

- Common toxicity pathways
- Similar susceptibility drivers

"WHY SHOULD I CARE?" REASON #5: CAN WE BE PREDICTIVE *IN SILICO*?

NIEHS-NCATS-UNC DREAM8: *Toxicogenetics Challenge*

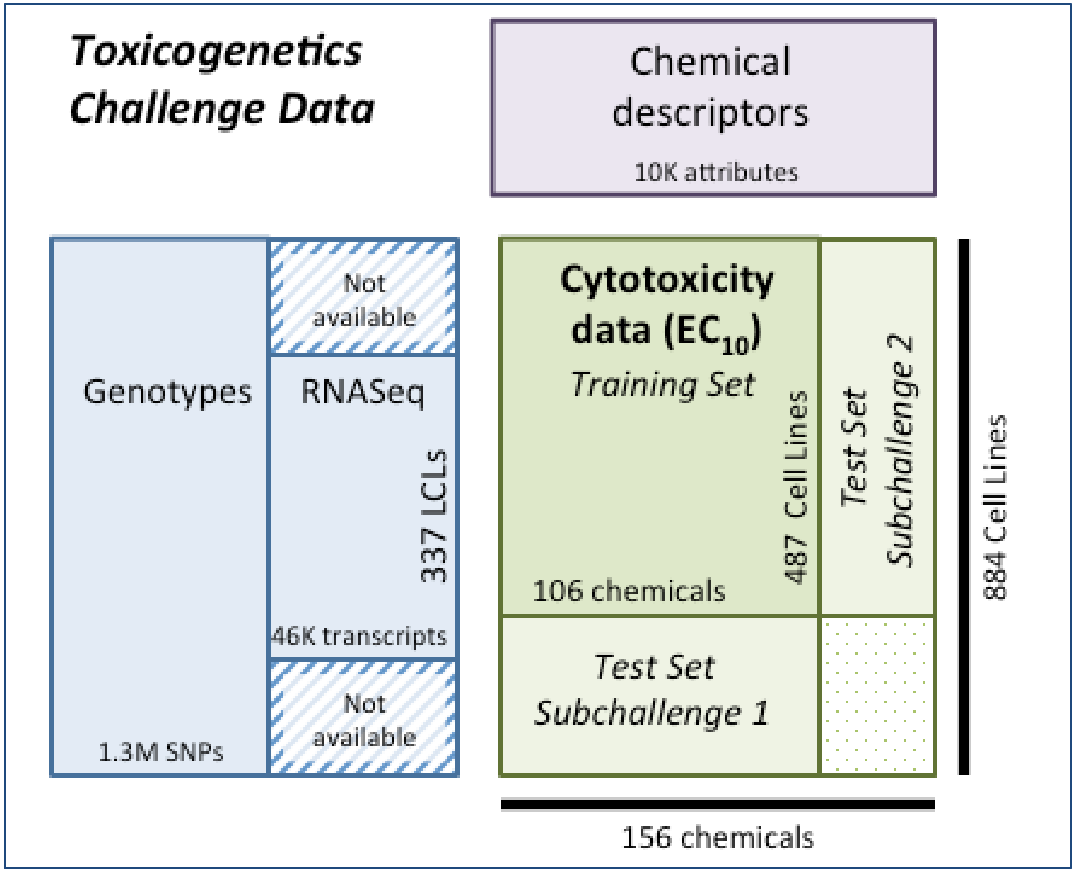
THE UNIVERSITY *of* NORTH CAROLINA *at* CHAPEL HILL

NIH National Center for Advancing Translational Sciences

NIH National Institute of Environmental Health Sciences

Sage BIONETWORKS

DREAM



- 232 registered participants
- 99 submissions from 34 teams for SC1
- 91 submissions from 24 teams for SC2
- Nature Biotechnology will consider an overview paper describing the results and insights

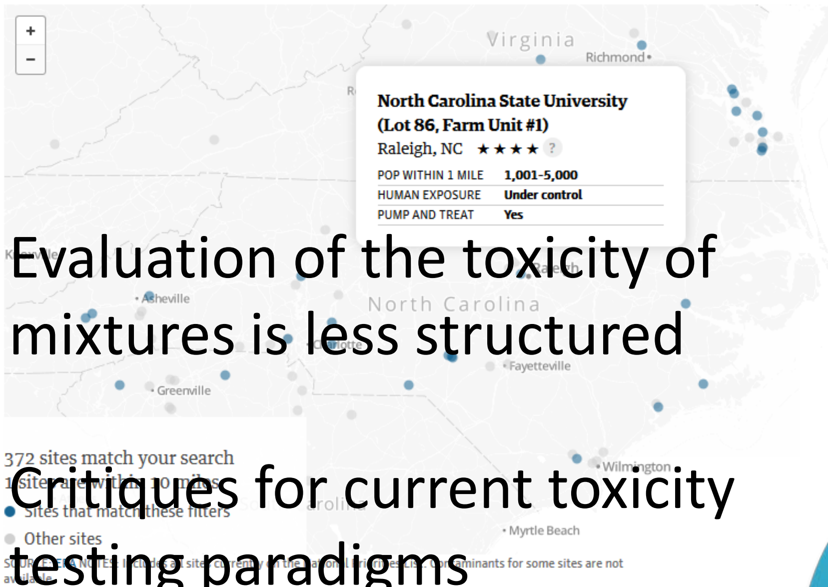
Can we expand our *in vitro* population-based model to address environmental chemical mixtures?

Real Chemical Mixtures

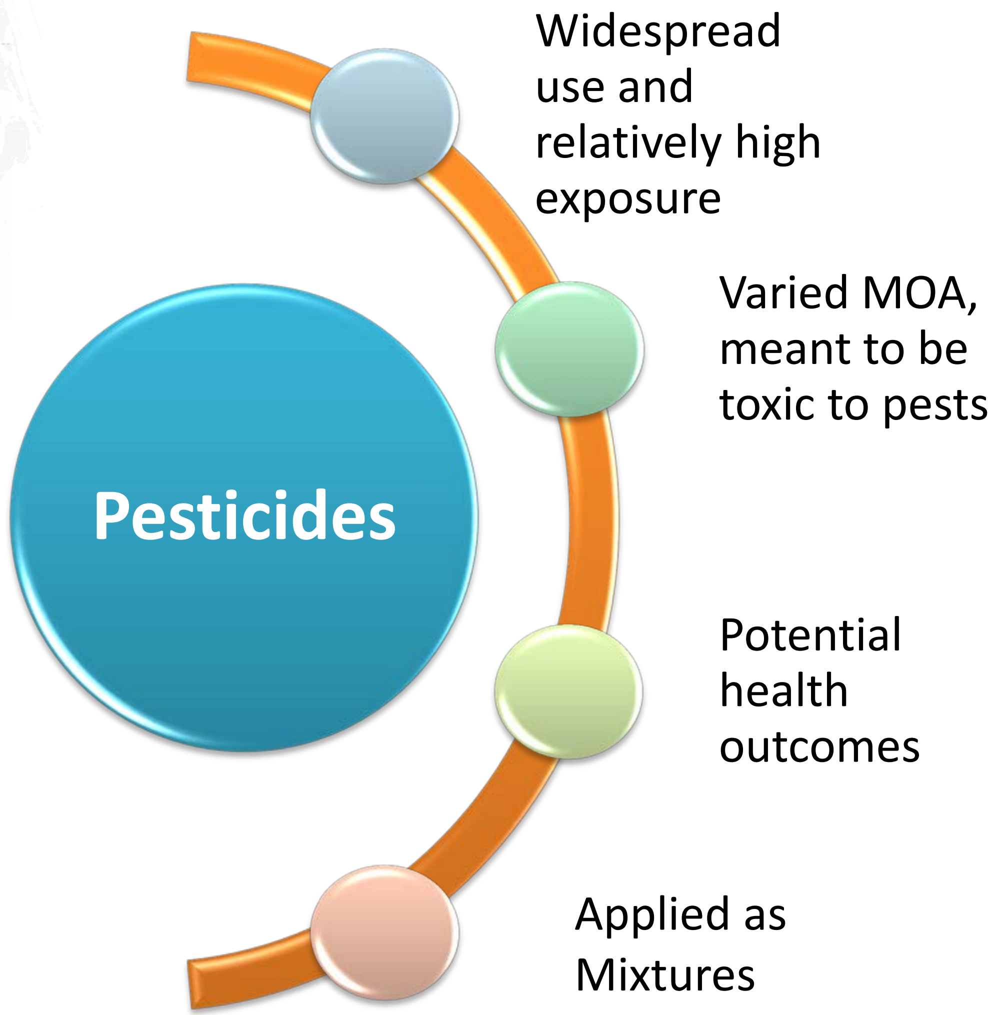
www.eweb.org

Lab Chemical Mixtures

Background

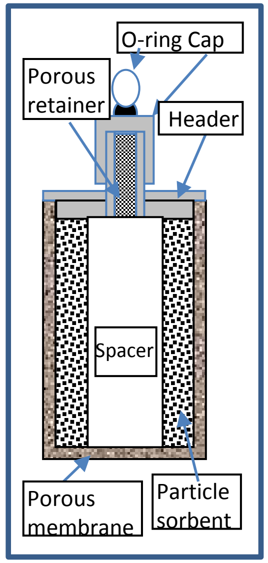


- Evaluation of the toxicity of mixtures is less structured
- Critiques for current toxicity testing paradigms
  - co-exposures
  - population variability
- Whole animal testing is difficult to employ for testing chemical mixtures.



Experimental design

Surface water universal passive sampling device (Project 4):
Organochlorine pesticide environmental mixture

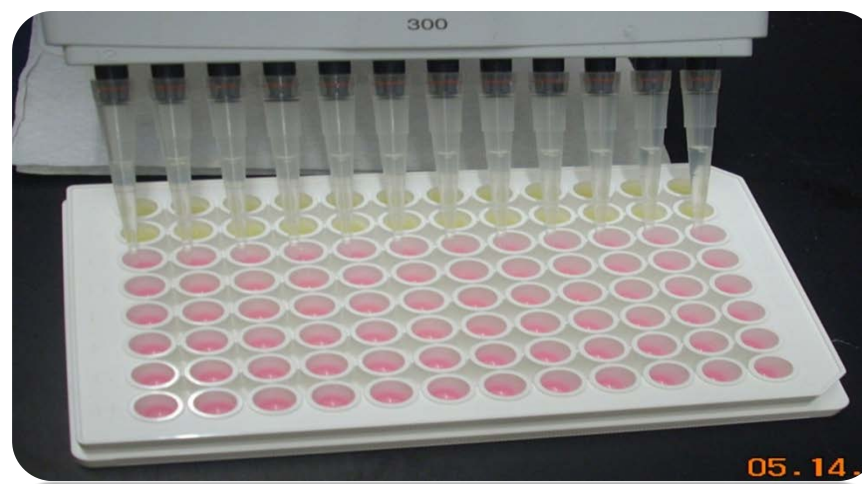


A mixture of 36 currently used pesticides

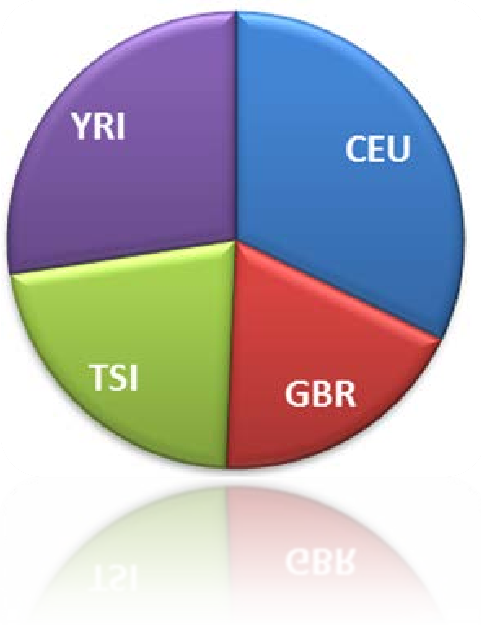
Human population-based *in vitro* toxicity screening (Project 2)

146 human lymphoblast cell lines
2 mixtures of pesticides (UNC Project 4)
8 concentrations (0.0003 to 330 μM)
All lines screened in 2+ plate replicates
1 assay (CellTiter-Glo®, ATP content)

~5,000 data points

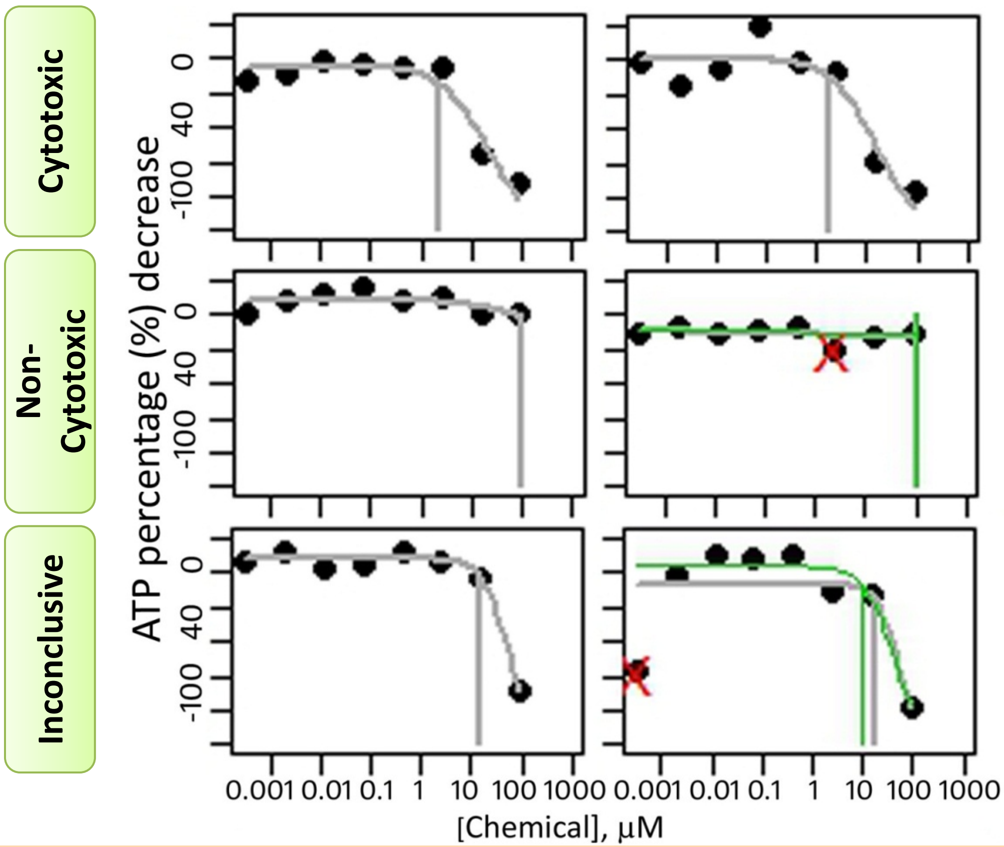


Populations Screened



Population	# (%)	Screened
CEU: Utah residents with Northern & Western European ancestry	76(22.9%)	47 (32.2%)
YRI: Yoruban in Ibadan, Nigeria	77(23.3%)	40(27.4%)
TSI: Tuscan in Italy	87(26.3%)	32(21.2%)
GBR: British from England & Scotland	91(27.5%)	27(18.5%)
Total	**331**	**146**

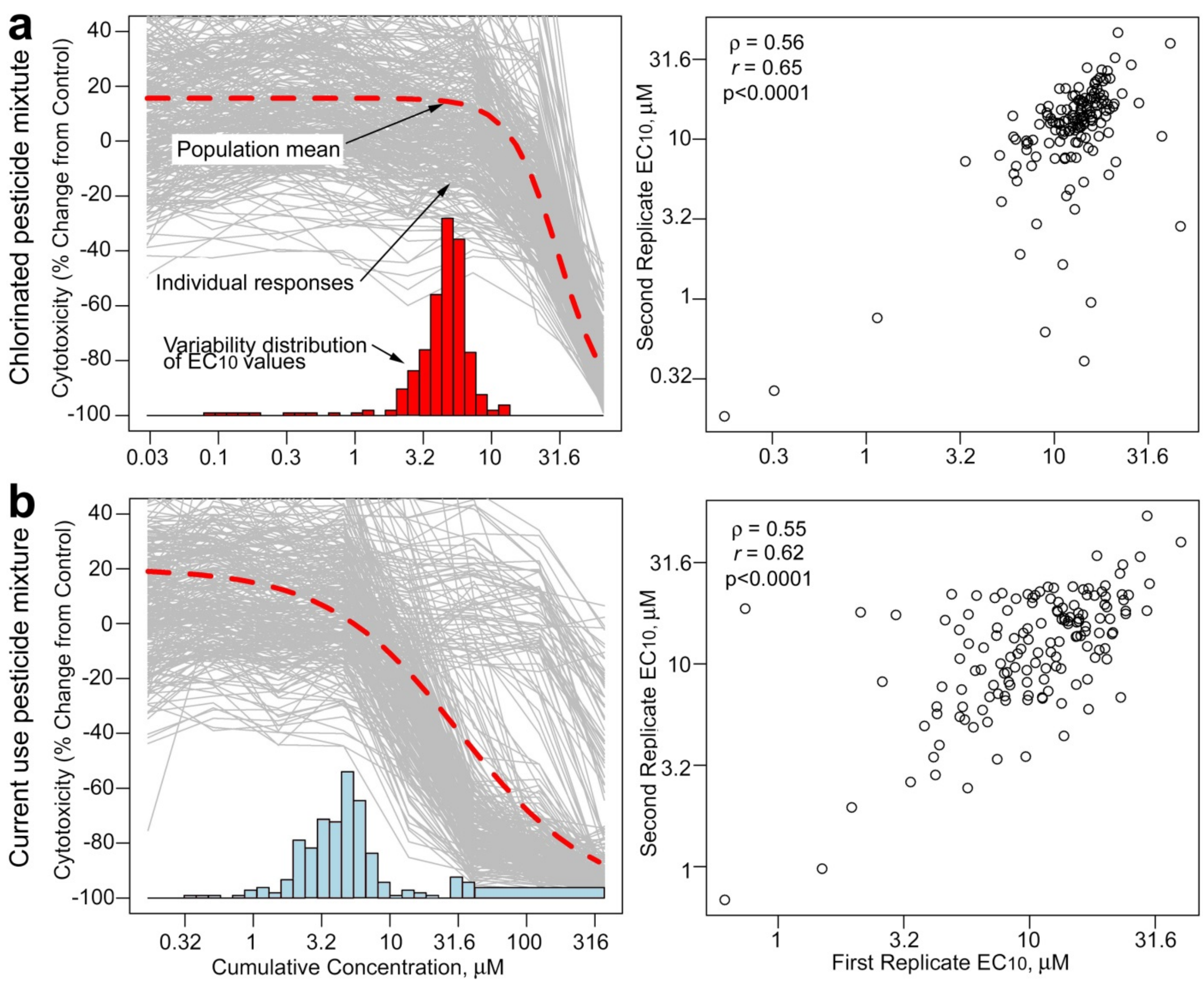
Deriving a Quantitative Toxicity Phenotype (EC10)



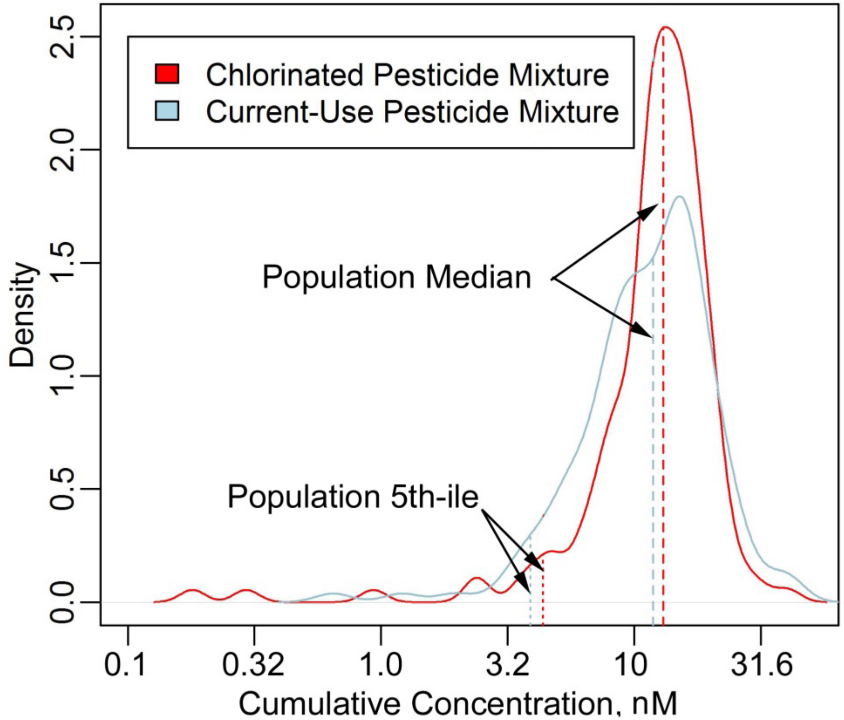
Deriving quantitative cytotoxicity phenotypes (EC_{10}):

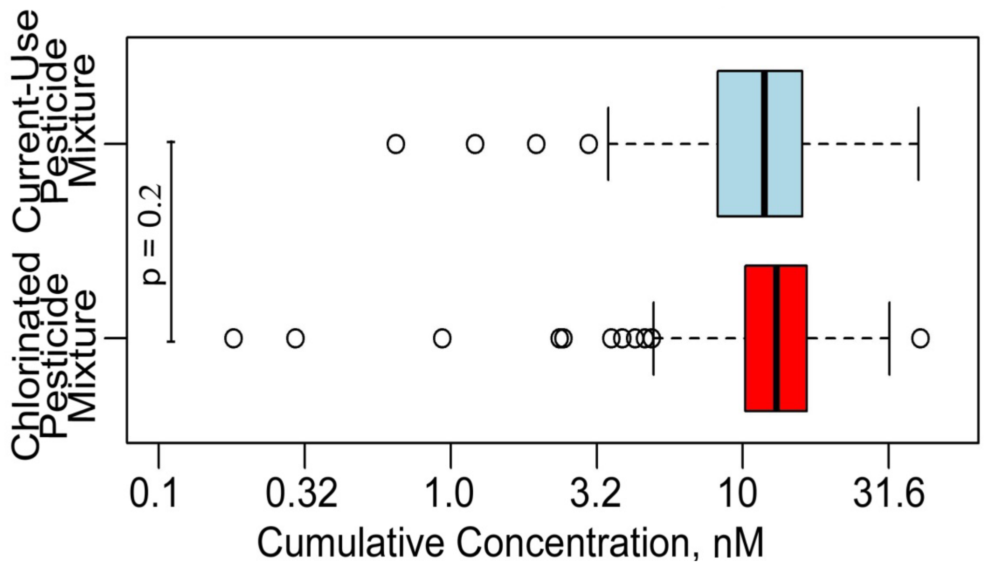
Curves were fit using a logistic model with baseline (lowest conc.) responses estimated from the data, and the maximum response value fixed at -100% (positive control). EC_{10} estimate is the concentration for which the estimated response dropped to 90% of the fitted value at the lowest concentration

Population Variability in Response to Pesticide Mixtures



Inter-individual variability in cytotoxic response across cell lines

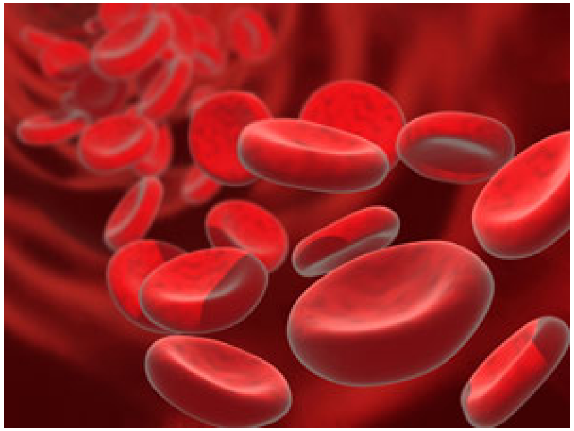




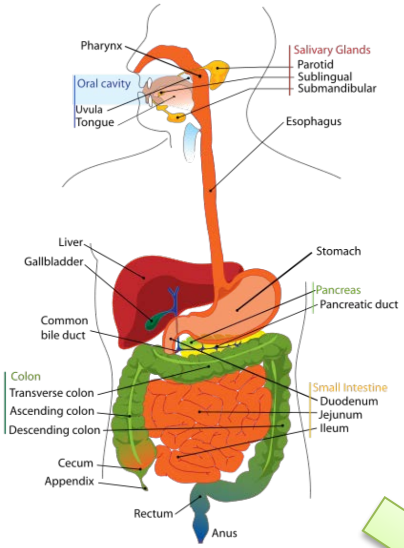
Pesticide Mixture	Mean	STD	Range	Median	Q05	Q95	UFk
Chlorinated Pesticide Mixture	11.6	1.96	(0.180-40.6)	13.1	4.36	21.7	3.00
Current Pesticide Mixture	11.1	1.85	(0.649-39.9)	11.9	3.89	24.7	3.05

What does "LCLs cytotoxicity" mean?
How to go from blood toxicity to exposure?

Blood concentration



Equivalent dose



Actual estimated exposure

What does "LCLs cytotoxicity" mean?

In vitro to *in vivo* extrapolation (IVIVE)

Hepatic Clearance

Non metabolic renal clearance

Blood binding

+

Population Simulation: 10,000 (20-50 yrs) males & females

=

Chemical specific steady-state blood concentration (Css)

Current-use pesticide mixture: 31 out of 36 chemicals

Chlorinated pesticide mixture: 4 out of 10 chemicals

How missing values were handled:

Scenario 1: "**Worst Case Scenario**"

- No hepatic clearance
- Only renal clearance
- High blood binding

→ Weighted by % of chemical in 1ml

→ Assuming Equal weight for each chemical

Scenario 2: "**Median**"
Assuming the median or highest Css value within each mixture for chemicals with missing values

→ Weighted by % of chemical in 1ml

→ Assuming Equal weight for each chemical

- Oral equivalent (OE) doses were calculated for each scenario using reverse dosimetry from the upper 95th % Css value:
  - OE was calculated for each cell line-chemical pair.
  - A cumulative OE was computed for each mixture for each cell line.

Wetmore et al., (2012). *Toxicol Sci, 125*(1), 157-174.

Predicted Exposure Limits

1936 chemicals evaluated by far-field mass balance &human exposure models

An indicator for indoor and/or consumer use

+

Bayesian analysis from urine concentrations for 82 chemicals reported in (NHANES)

=

Chemical specific predicted exposure

Current-use pesticide mixture:
35 out of 36 chemicals

Chlorinated pesticide mixture:
6 out of 10 chemicals

- Missing values were replaced by the highest predicted exposure within each mixture
- A cumulative predictive exposure was computed for each mixture from the upper 95th %.

Wambaugh et al., Environ Sci Technol. 2013 6;47(15):8479-88.

In vitro to *in vivo* extrapolation

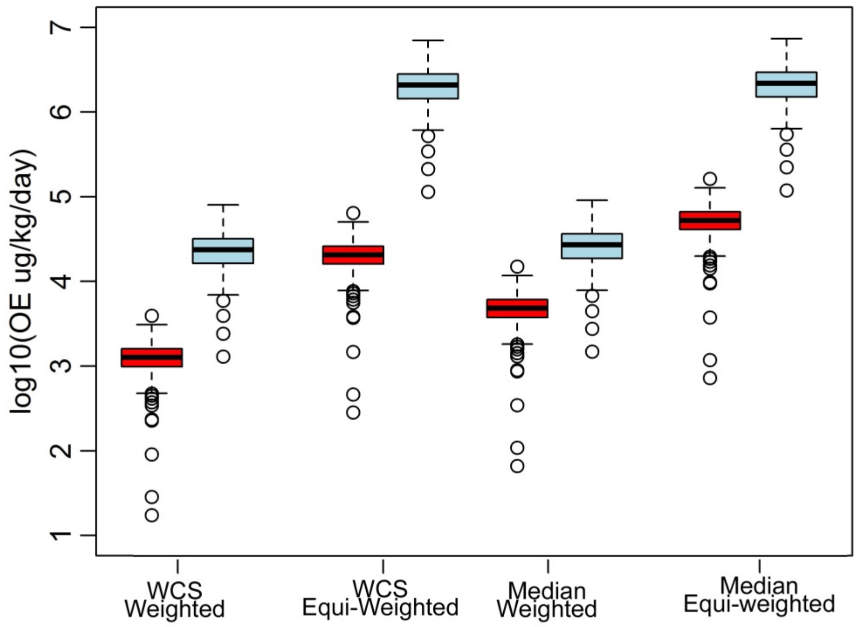


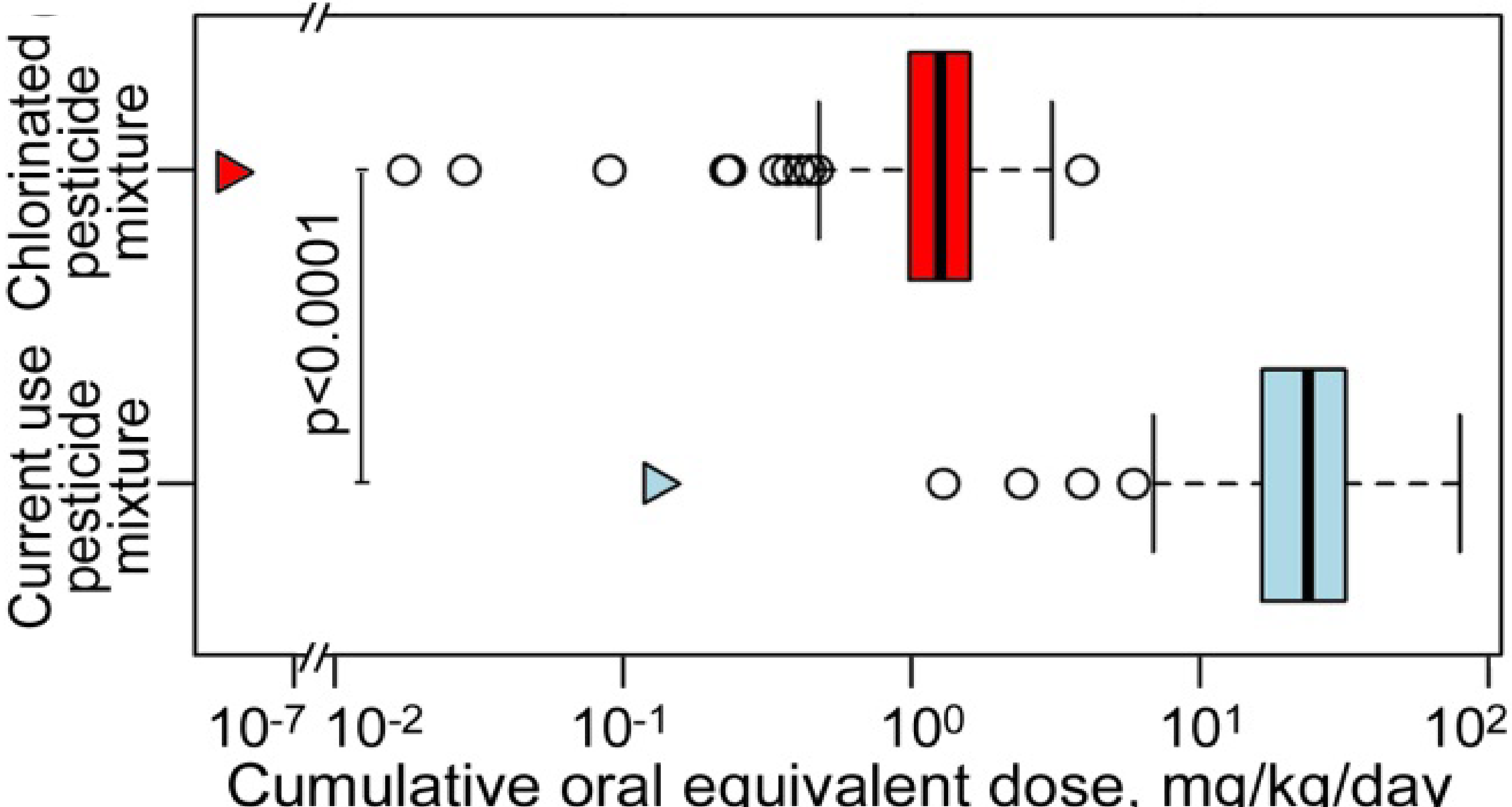
Table 5 **Current-Use Pesticide Mixture**

Scenario		Cumulative WCS	Cumulative Median	95th percentile WCS	95th percentile Median	Median WCS	Median Median
		Margin of Exposure					
Weighted by chemical %	Worst Case Scenario	1.0	1.1	1.7	2.1	4.0	4.1
	Median	1.1	1.2	1.8	2.2	4.1	4.1
Equally Weighted	Worst Case Scenario	2.9	3.1	3.6	4.0	6.0	6.0
	Median	3.0	3.1	3.7	4.0	6.0	6.0

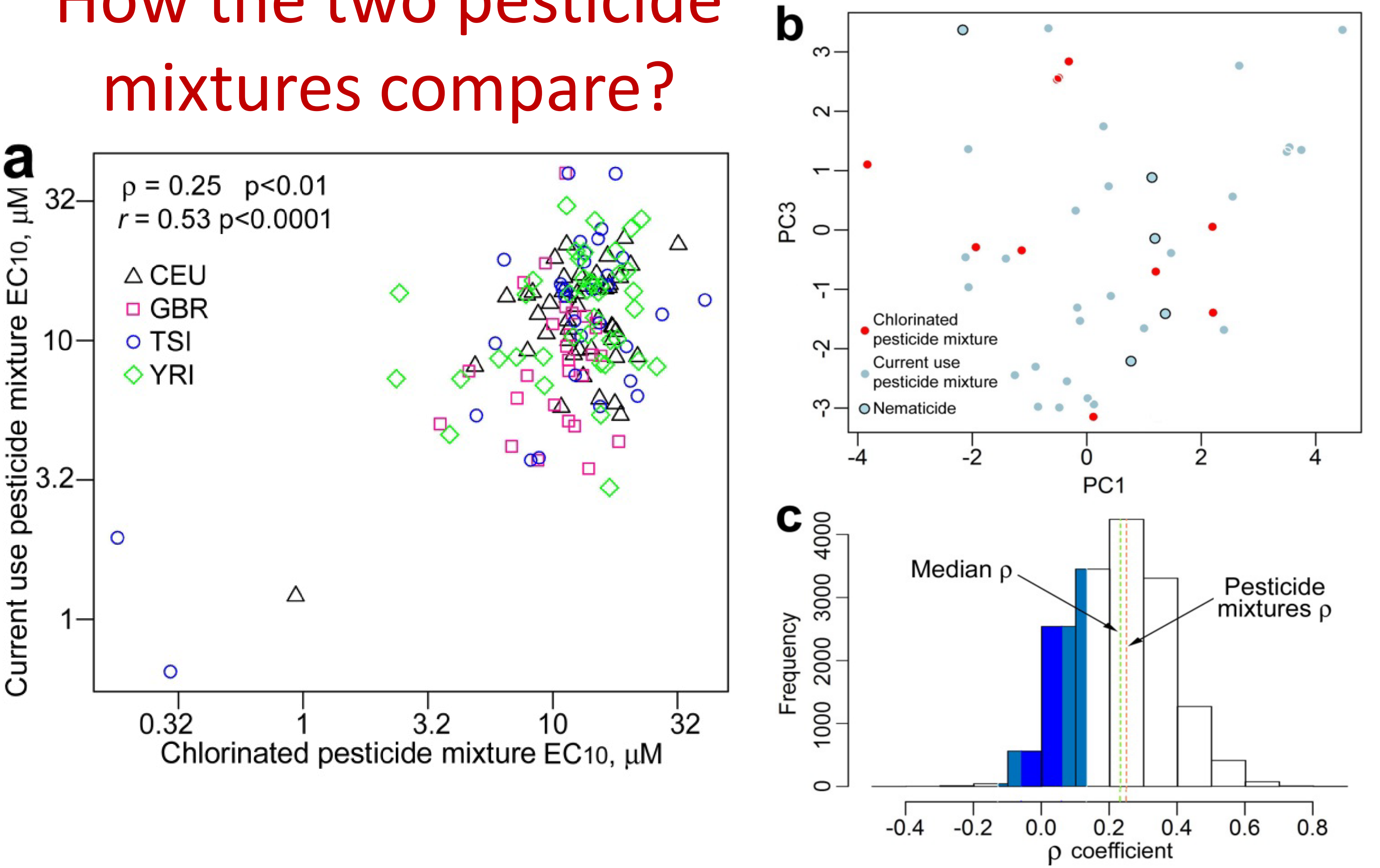
Table 6 **Chlorinated Pesticide Mixture**

Scenario		Cumulative WCS	Cumulative Median	95th percentile WCS	95th percentile Median	Median WCS	Median Median
		Margin of Exposure					
Weighted by chemical %	Worst Case Scenario	5.9	6.0	6.7	6.4	6.8	7.0
	Median	6.4	6.6	7.3	6.9	7.4	7.6
Equally Weighted	Worst Case Scenario	7.1	7.2	7.9	7.6	8.0	8.2
	Median	7.5	7.6	8.4	8.0	8.4	8.6

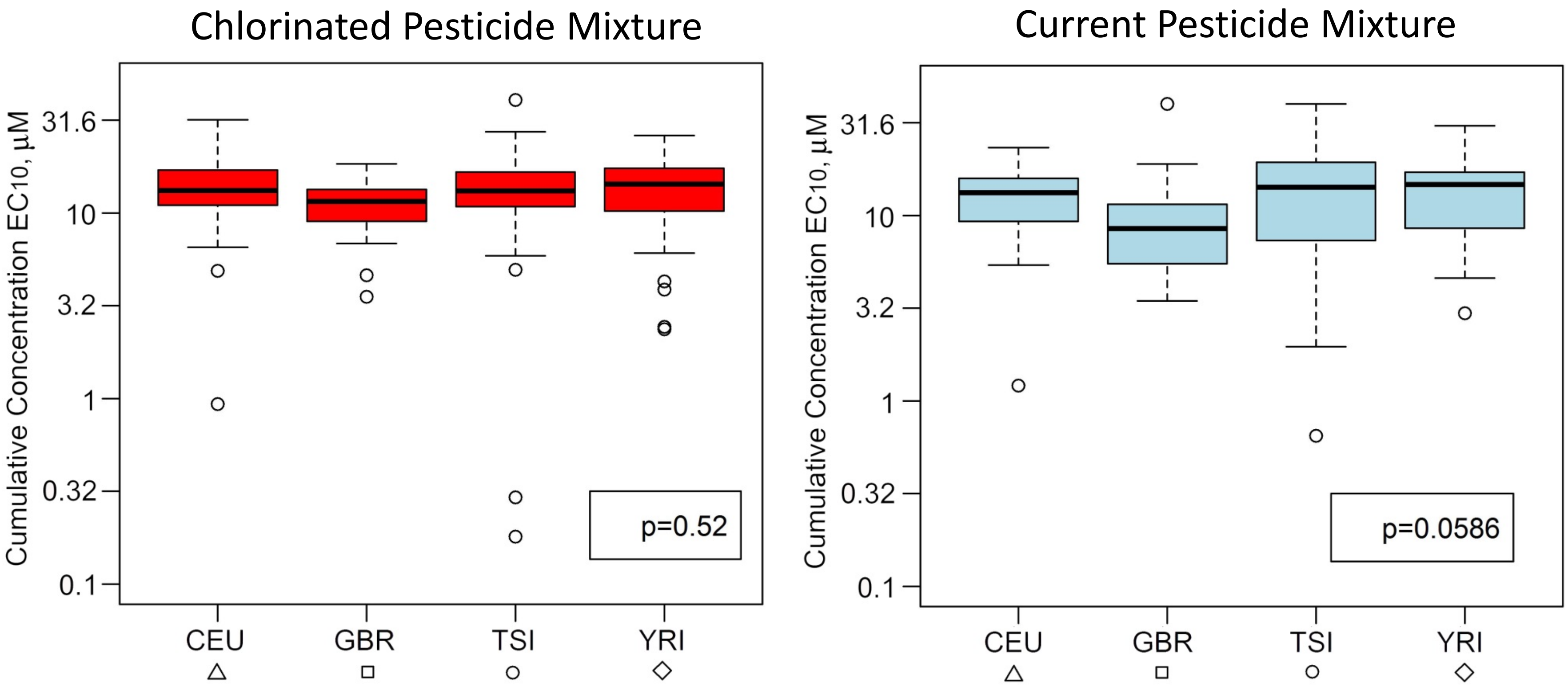
What does “LCLs cytotoxicity” mean?
In vitro to *in vivo* extrapolation



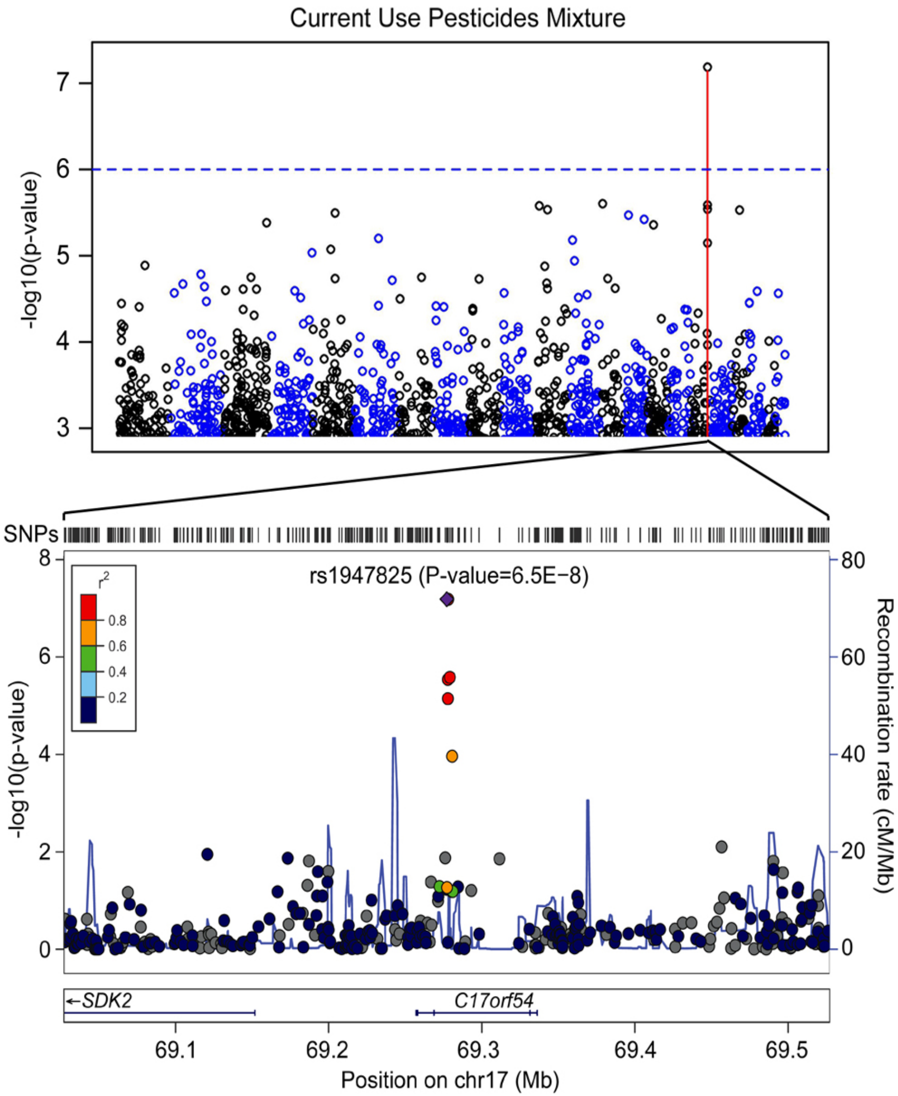
How the two pesticide mixtures compare?

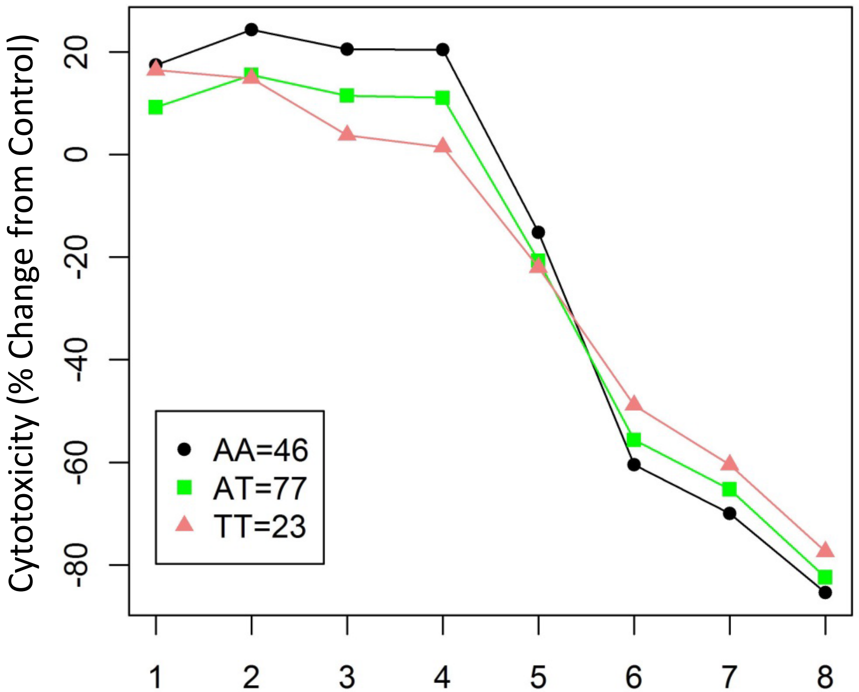


Identifying susceptible sub-populations

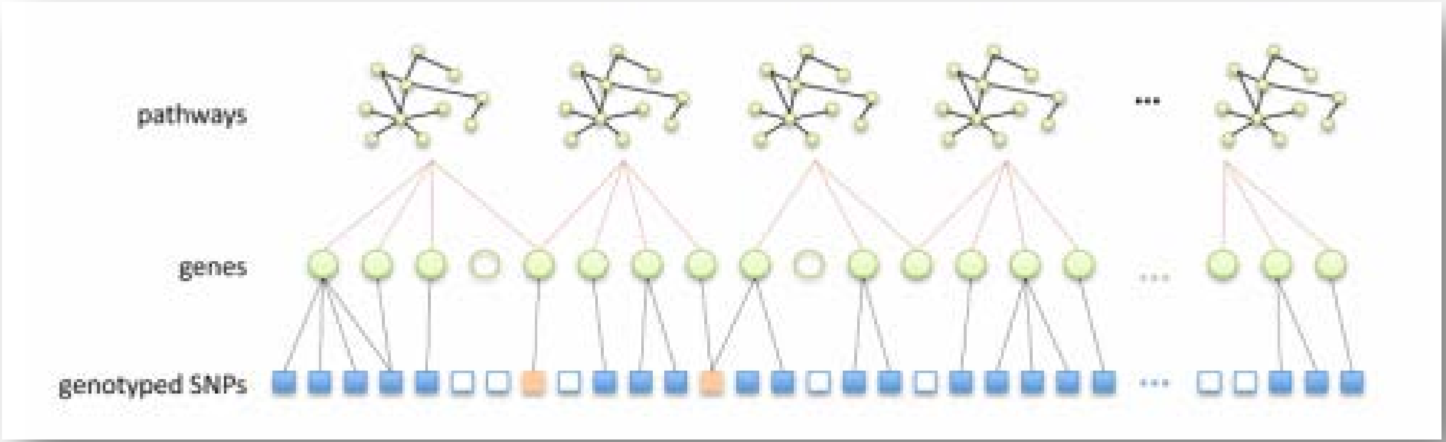


Genome-wide association with cytotoxicity to current use pesticide mixture (36 pesticides)





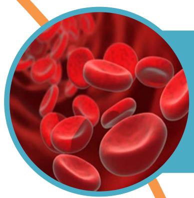
Pathway Analysis



Term	N Genes	Top 7 genes
Ascorbate and aldarate metabolism	22	UGT2B11, UGT2B7,UGT1A3, UGT1A7, UGT1A4, UGT1A5, UGT1A6
Starch and sucrose metabolism	48	UGT2B11, UGT2B7,UGT1A3,UGT1A7, UGT1A4, UGT1A5, UGT1A6
Porphyrin and chlorophyll metabolism	39	EARS2, UGT2B11, UGT2B7, BLVRA UGT1A3, UGT1A7, UGT1A4
Pentose and glucuronate interconversions	28	UGT2B11, UGT2B7,UGT1A3,UGT1A7, UGT1A4, UGT1A5, UGT1A6
Nitrogen metabolism	23	CA6, GLUL, CA2,CA4, HAL, CTH, CA5A

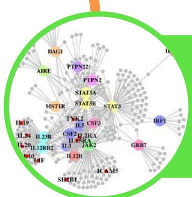
P FWER<0.1

Why is population-based toxicity screening more powerful than traditional approaches?



Quantitatively assess hazard and population variability in **chemical mixtures** *in vitro*

Identify susceptible sub-populations



Understand genetic underpinning and probe toxicity pathways

Extrapolating the in vitro POD to oral equivalent dose

Assessing risk by comparing to estimated human exposure

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http://comptox.unc.edu

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NCGC NIH Chemical Genomics Center
NTP National Toxicology Program
Srp Superfund Research Program