A Population-Based Human *In Vitro* Approach to Characterize Inter-Individual Variability in Responses to Chemical Mixtures

Lucie Ford

Interdisciplinary Faculty of Toxicology, Texas A&M University

September 14th, 2021

TEXAS A&M UNIVERSITY
SUPERFUND
RESEARCH CENTER

Acknowledgments

- Dr. Ivan Rusyn - PI
- Dr. Weihsueh Chiu
- Dr. Fred Wright
- Dr. Stephen Safe
- Suji Jang
- Dr. Zunwei Chen

Rusyn Lab Members
Arlean Rohde

Collaborators:
Chiu Lab TAMU
Wright Lab NCSU

TEXAS A&M UNIVERSITY
Veterinary Medicine & Biomedical Sciences

TEXAS A&M UNIVERSITY
SUPERFUND
RESEARCH CENTER

Funding Sources

NIH
NIEHS

Grant: P42 ES027704

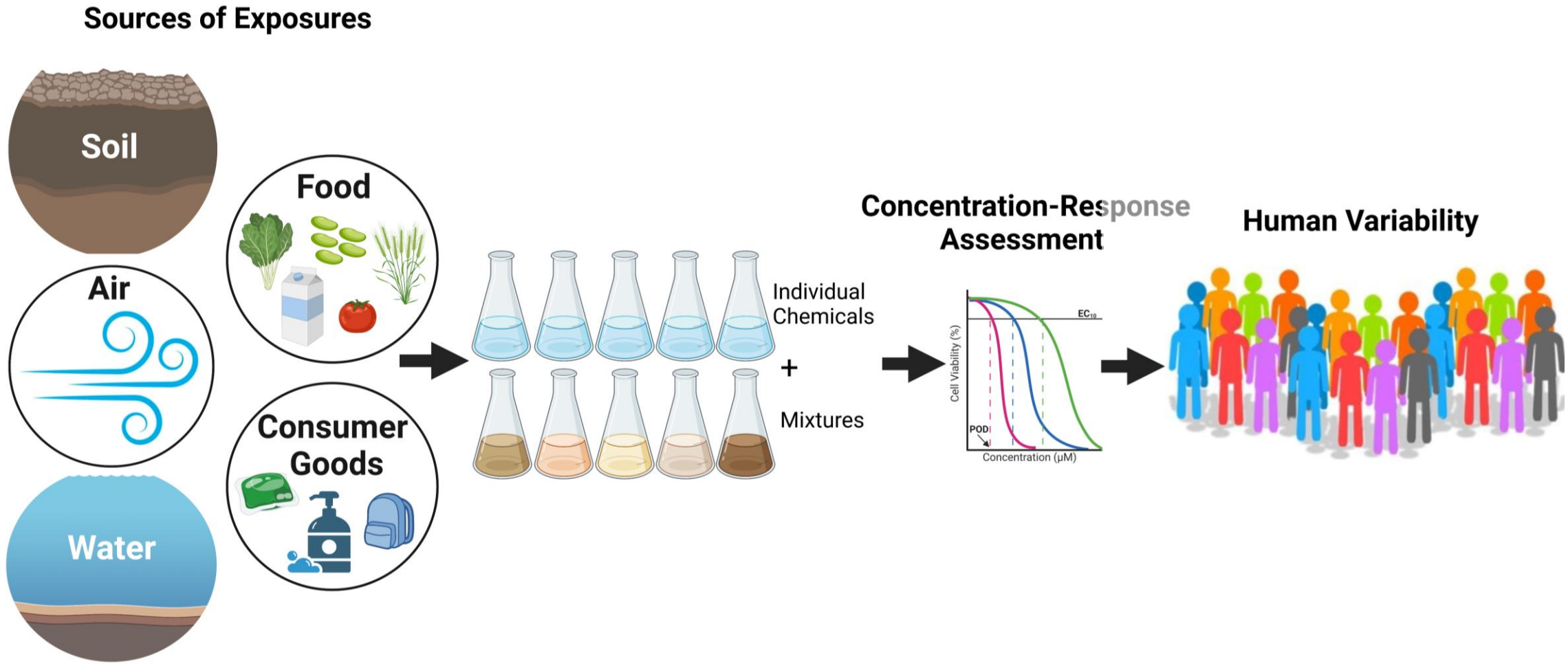
Grant: T32 ES026568

TEXAS A&M UNIVERSITY

Texas A&M University
Merit Fellowship

TEXAS A&M UNIVERSITY
SUPERFUND
RESEARCH CENTER

Various Exposures and Unknown Outcomes



How can mixtures be assessed?

Current challenges in mixtures risk assessment:

1) Health effects
2) Unknown composition of the mixtures
3) Exposure assessment

- Existing risk assessment methods rely on data from individual chemicals
- No standardized approach to assess risk of mixtures

Two proposed methods:

1) Whole-mixture approach
2) Component-based approach

GUIDANCE

EFSA Journal

ADOPTED: 17 November 2021

doi: 10.2903/j.efsa.2021.7033

Guidance Document on Scientific criteria for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals

EFSA Scientific Committee,
Simon John More, Vasileios Bampidis, Diane Benford, Claude Bragard, Antonio Hernandez-Jerez, Susanne Hougaard Bennekou, Thorhallur Ingi Halldorsson, Konstantinos Panagiotis Koutsoumanis, Claude Lambré, Kyriaki Machera, Hanspeter Naegeli, Søren Saxmose Nielsen, Josef Rudolf Schlatter, Dieter Schrenk, Vittorio Silano, Dominique Turck, Maged Younes, Emilio Benfenati, Amélie Crépet, Jan Dirk Te Biesebeek, Emanuela Testai, Bruno Dujardin, Jean Lou CM Dorne and Christer Hogstrand

Abstract

This guidance document provides harmonised and flexible methodologies to apply scientific criteria and prioritisation methods for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals. In the context of EFSA's risk assessments, the problem formulation step defines the chemicals to be assessed in the terms of reference usually through regulatory criteria often set by risk managers based on legislative requirements. Scientific criteria such as hazard-driven criteria can be used to group these chemicals into assessment groups. In this guidance document, a framework is proposed to apply hazard-driven criteria for grouping of chemicals into assessment groups using mechanistic information on toxicity as the gold standard where available (i.e. common mode of action or adverse outcome pathway) through a structured weight of evidence approach. However, when such mechanistic data are not available, grouping may be performed using a common adverse outcome. Toxicokinetic data can also be useful for grouping, particularly when metabolism information is available for a class of compounds and common toxicologically relevant metabolites are shared. In addition, prioritisation methods provide means to identify low-priority chemicals and reduce the number of chemicals in an assessment group. Prioritisation methods include combined risk-based approaches, risk-based approaches for single chemicals and exposure-driven approaches. Case studies have been provided to illustrate the practical application of hazard-driven criteria and the use of prioritisation methods for grouping of chemicals in assessment groups. Recommendations for future work are discussed.

Keywords: harmonised methodologies, human risk assessment, combined exposure to multiple chemicals, scientific criteria, grouping, assessment groups, dose addition

Requestor: EFSA

Question number: EFSA-Q-2019-00517

Correspondence: sc.secretariat@efsa.europa.eu

implementation plan will be extended to other chemicals.

- In view of increasing the outreach towards national competent authorities, EFSA will promote the implementation of the methods and tools developed and provide adequate training, where necessary.

European Food Safety Authority
Via Carlo Magno 1A – 43126 Parma, Italy
Tel. +39 0521 036 111 | www.efsa.europa.eu

TEXAS A&M UNIVERSITY SUPERFUND RESEARCH CENTER

Why are we interested in population-based *in vitro* methods?

Traditional Toxicity Testing Methods: *In Vivo*

- Time and labor-intensive, expensive, and low throughput
- Challenges with extrapolation to humans
- Models often overlook inter-individual variability
- Ethical concerns

New Approach Methods for Toxicity Testing: *In Vitro*

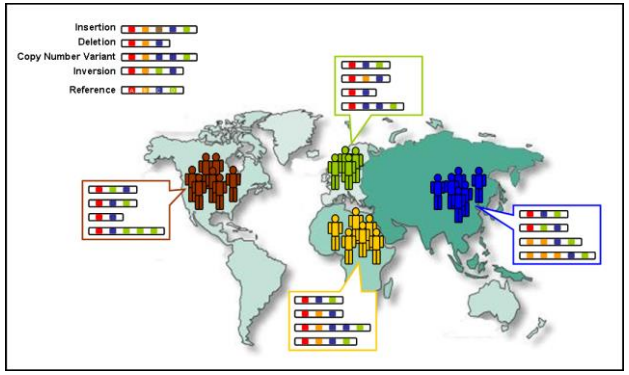
- Faster, cheaper, and higher-throughput
- Ability to look at biologically-relevant phenotypes
- Can evaluate inter-individual and inter-species variability
- Reduces use of animal testing

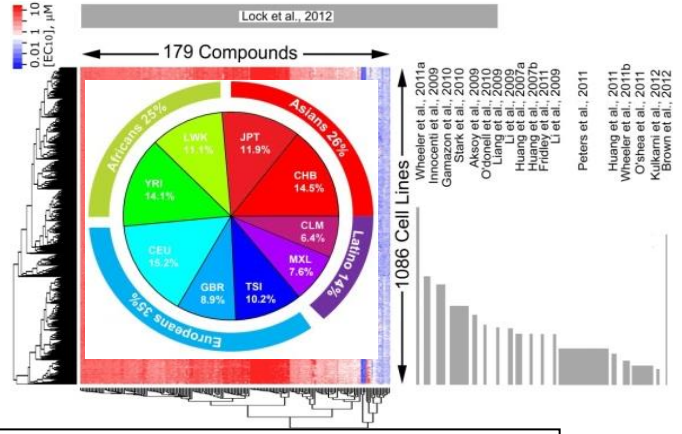
Population-Based Human *In Vitro* Models

- Human lymphoblast cell lines (1,000+ donors)
- Human induced pluripotent stem cell-derived cardiomyocytes (~43 donors)
- Assess inter-individual and chemical-specific variability
- Translation to humans

TEXAS A&M UNIVERSITY
SUPERFUND
RESEARCH CENTER

Why do toxicity testing in human lymphoblast cell lines?

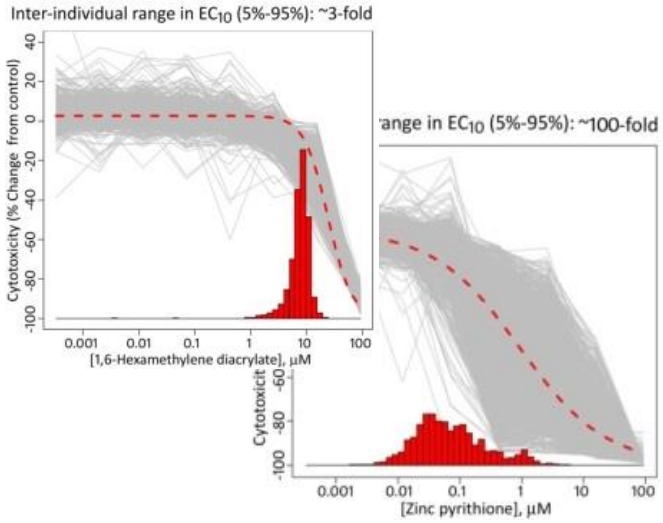


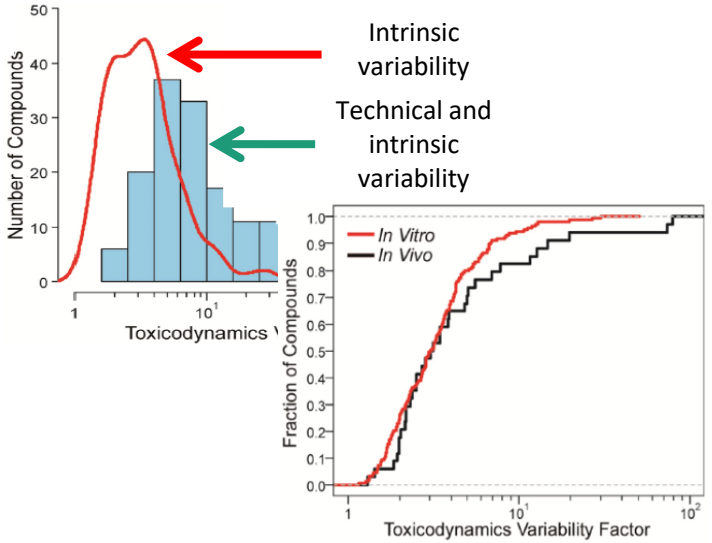


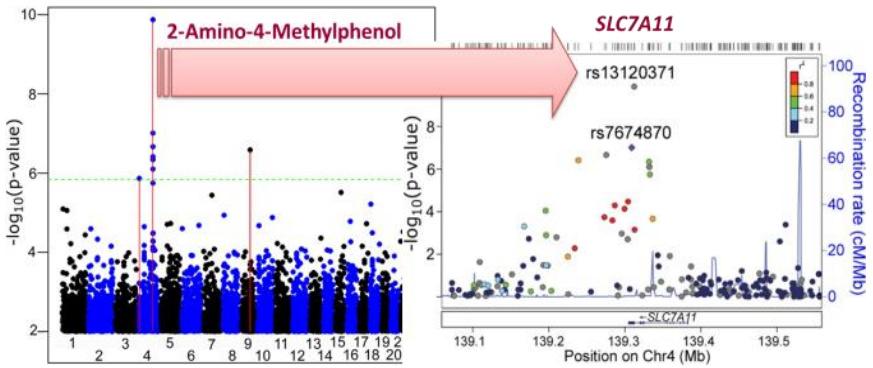
Hazard and Dose-Response Assessments

Ability to Assess Variability and Relevant to Humans

Mechanistic Hypotheses and Identification of Susceptible Genes







- Abdo et al Environ Int 85:147-55, 2015
- Abdo et al Environ Health Perspect 123(5):458-66,
- Eduati et al Nat Biotechnol 33(9):933-40, 2015
- Chiu et al ALTEX 34(3):377-388, 20172015

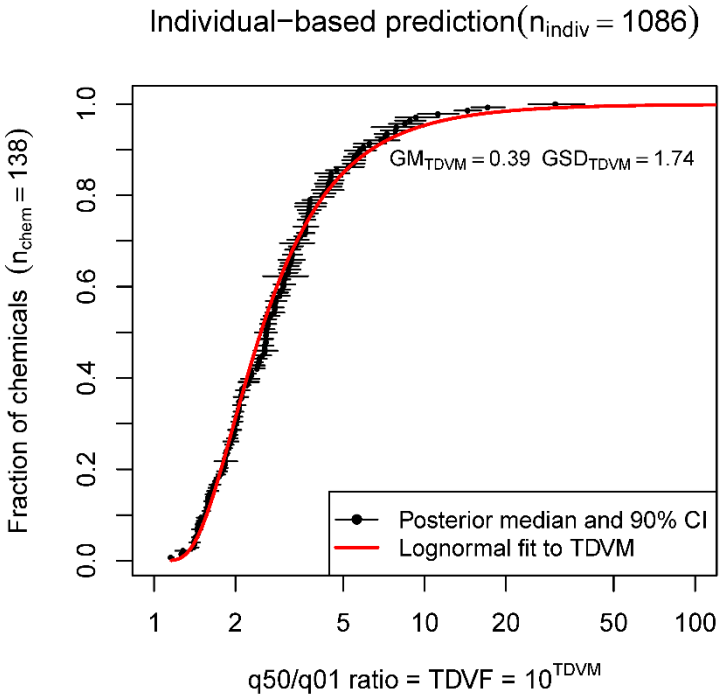
TEXAS A&M UNIVERSITY SUPERFUND RESEARCH CENTER

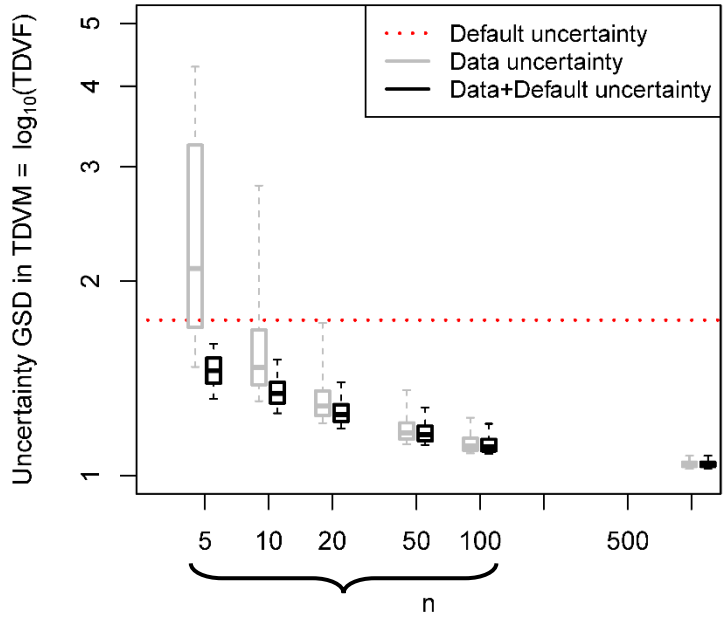
Slide adapted from Ivan Rusyn

Do we need 1000+ cell lines to study human variability?

How many individuals do we really need for *in vitro* screening?

- Power calculations based on data from Abdo et al (2015)
  - Resampled 1000+ individuals
  - Resampling across all tested chemicals





Sample sizes as small as 5 donors can be informative

Reliable estimates with 20-100 individuals

TEXAS A&M UNIVERSITY SUPERFUND RESEARCH CENTER

Chiu et al ALTEX 34(3):377-388, 20172015

Slide adapted from Ivan Rusyn

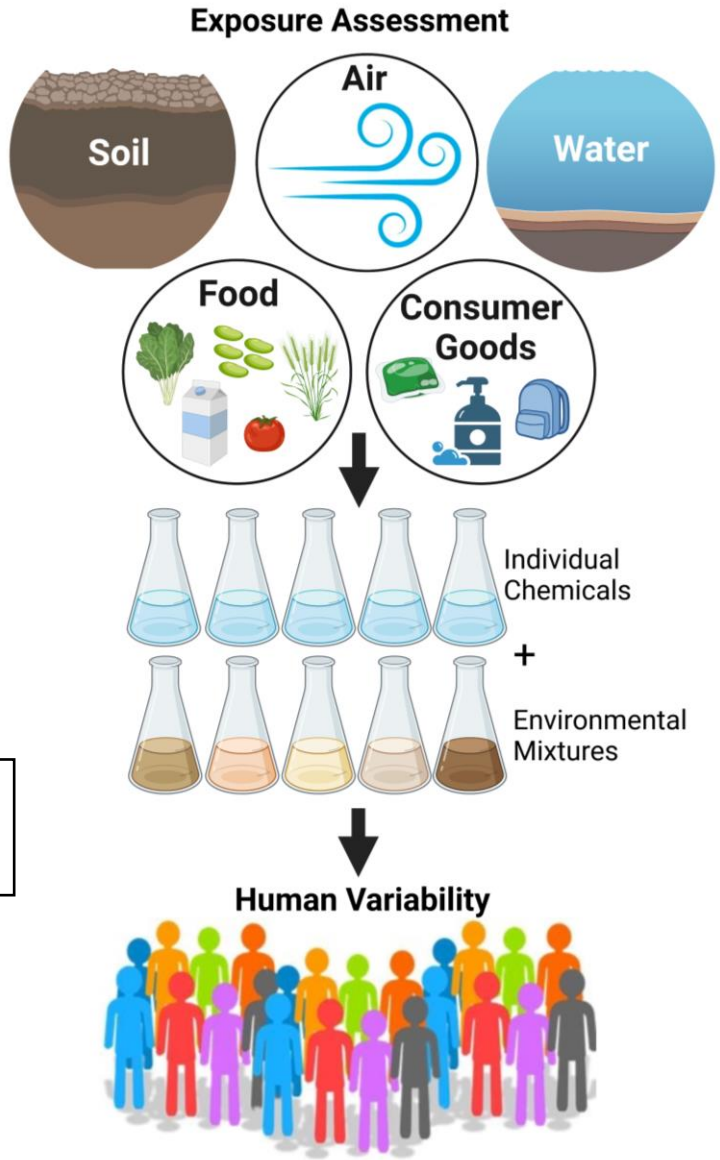
Why are we interested?

- Because there is no standard approach to test human variability in mixtures risk assessment
- Can we estimate the extent of population variability for mixtures?
  - Do we need to test both individual constituents and mixtures?
  - Is the extent of variability greater for mixtures than for chemicals?
  - Can we apply the same uncertainty factor to mixtures and chemicals?

What is our approach?

Apply population-based *in vitro* methods to assess potential toxicity of component-based mixtures

- Test defined mixtures and the individual constituents
- Use a population-based human *in vitro* model of LCLs
- Quantify toxicodynamic variability for chemicals and mixtures
- Identify potential drivers of variability through a GWAS



TEXAS A&M UNIVERSITY
SUPERFUND
RESEARCH CENTER

Experimental Design

Lymphoblast cell lines from 4 populations (N = 146)

⬇

Testing of cytotoxicity in concentration response

⬇

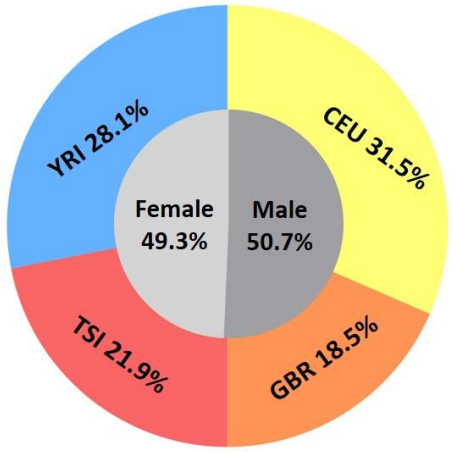
Cell line-specific concentration response modeling

⬇

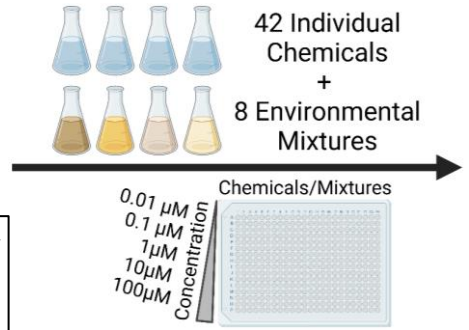
Cumulative Distribution of $TDVF_{05}$

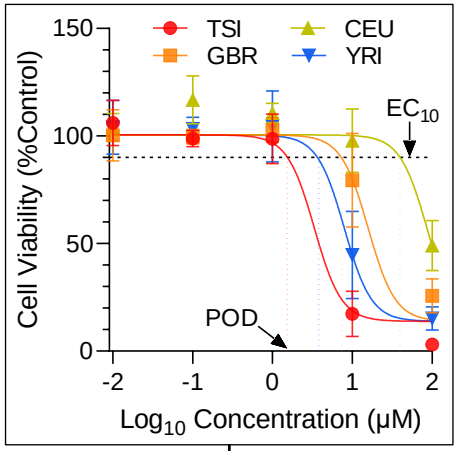
⬇

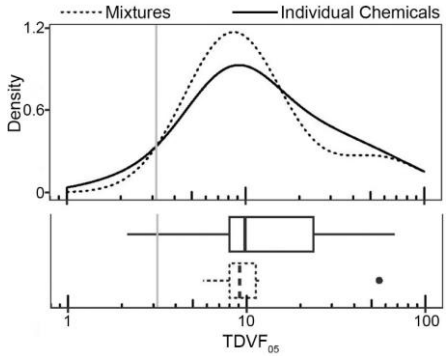
GWAS using cell line-specific PODs

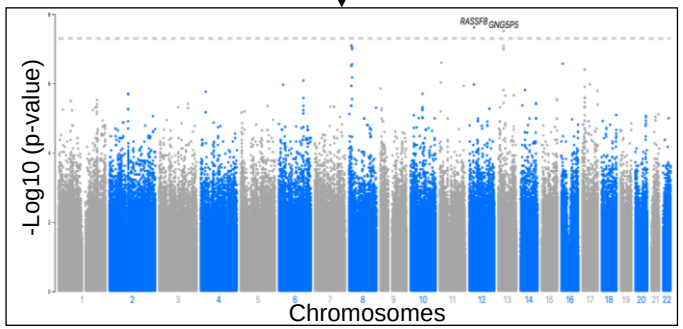


CEU- Utah residents with European ancestry
GBR- British from England and Scotland
TSI- Tuscans in Italy
YRI- Yoruban from Ibadan, Nigeria









TEXAS A&M UNIVERSITY SUPERFUND RESEARCH CENTER

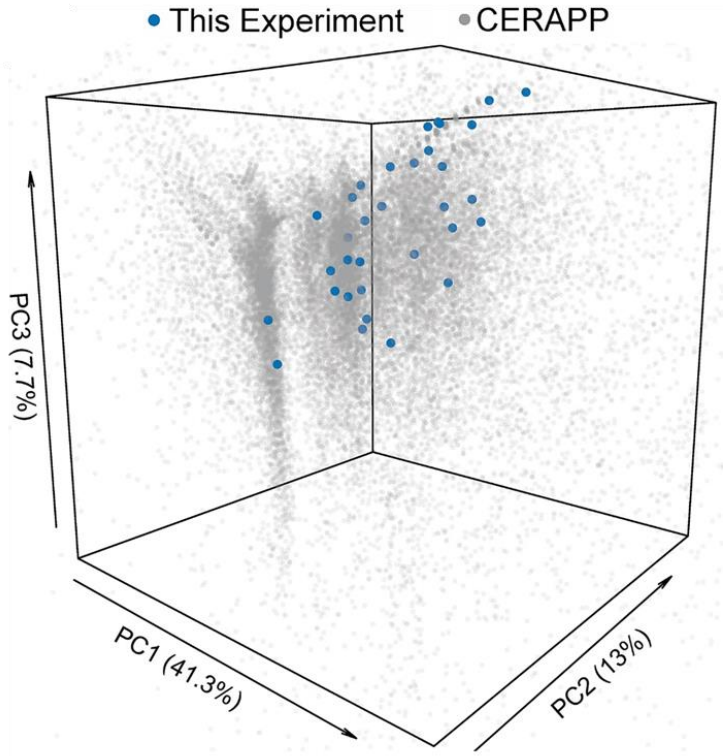
Figures adapted from Ford *et al.*, 2022 (PMID: 6006120)

Experimental Design

Chemical Selection and Design Mixtures

ID	SUBSTANCE NAME	CAS	Class
1	BENZO(A)ANTHRACENE	56-55-3	PAH
2	NAPHTHALENE	91-20-3	PAH
3	FLUORANTHENE	206-44-0	PAH
4	DDT, P,P'-	50-29-3	Pest
5	DIELDRIN	60-57-1	Pest
6	ALDRIN	309-00-2	Pest
7	HEPTACHLOR	76-44-8	Pest
8	LINDANE	58-89-9	Pest
9	DISULFOTON	298-04-4	Pest
10	ENDRIN	72-20-8	Pest
11	DIAZINON	333-41-5	Pest
12	HEPTACHLOR EPOXIDE	1024-57-3	Pest
13	PENTACHLOROPHENOL	87-86-5	HPV
14	DI-N-BUTYL PHTHALATE	84-74-2	Plastiz
15	CHLORPYRIFOS	2921-88-2	Pest
16	DI(2-ETHYLHEXYL)PHTHALATE	117-81-7	Plastiz
17	2,4,6-TRICHLOROPHENOL	88-06-2	HPV
18	ETHION	563-12-2	Pest
19	AZINPHOS-METHYL	86-50-0	Pest
20	2,4,5-TRICHLOROPHENOL	95-95-4	HPV
21	PARATHION	56-38-2	Pest

ID	SUBSTANCE NAME	CAS	Class
22	BENZO(B)FLUORANTHENE	205-99-2	PAH
23	TRIFLURALIN	1582-09-8	Pest
24	ACENAPHTHENE	83-32-9	PAH
25	DDD, P,P'-	72-54-8	Pest
26	BENZIDINE	92-87-5	HPV
27	ENDOSULFAN	115-29-7	Pest
28	METHOXYCHLOR	72-43-5	Pest
29	2,4-DINITROPHENOL	51-28-5	Pest
30	2,4-DINITROTOLUENE	121-14-2	HPV
31	DICOFOL	115-32-2	Pest
32	CRESOL, PARA-	106-44-5	HPV
33	DDT, O,P'-	789-02-6	Pesticide
34	4,6-DINITRO-O-CRESOL	534-52-1	HPV
35	1,2,3-TRICHLOROBENZENE	87-61-6	HPV
36	LEAD NITRATE	10099-74-8	Metal
37	CADMIUM CHLORIDE	10108-64-2	Metal
38	ZINC CHLORIDE	7646-85-7	Metal
39	MERCURIC CHLORIDE	7487-94-7	Metal
40	POTASSIUM CHROMATE	7789-00-6	Metal
41	COBALT CHLORIDE	7646-79-9	Metal
42	NICKEL CHLORIDE	7718-54-9	Metal



Pesticides (n=20)
HPV (n=8)
Heavy Metals (n=7)
PAHs (n=5)
Phthalates (n=2)

TEXAS A&M UNIVERSITY SUPERFUND RESEARCH CENTER

Figures adapted from Ford *et al.*, 2022 (PMID: 6006120)

Experimental Design

Chemical Selection and Design Mixtures

ID	SUBSTANCE NAME	CAS	Class
1	BENZO(A)ANTHRACENE	56-55-3	PAH
2	NAPHTHALENE	91-20-3	PAH
3	FLUORANTHENE	206-44-0	PAH
4	DDT, P,P'-	50-29-3	Pest
5	DIELDRIN	60-57-1	Pest
6	ALDRIN	309-00-2	Pest
7	HEPTACHLOR	76-44-8	Pest
8	LINDANE	58-89-9	Pest
9	DISULFOTON	298-04-4	Pest
10	ENDRIN	72-20-8	Pest
11	DIAZINON	333-41-5	Pest
12	HEPTACHLOR EPOXIDE	1024-57-3	Pest
13	PENTACHLOROPHENOL	87-86-5	HPV
14	DI-N-BUTYL PHTHALATE	84-74-2	Plastiz
15	CHLORPYRIFOS	2921-88-2	Pest
16	DI(2-ETHYLHEXYL)PHTHALATE	117-81-7	Plastiz
17	2,4,6-TRICHLOROPHENOL	88-06-2	HPV
18	ETHION	563-12-2	Pest
19	AZINPHOS-METHYL	86-50-0	Pest
20	2,4,5-TRICHLOROPHENOL	95-95-4	HPV
21	PARATHION	56-38-2	Pest

ID	SUBSTANCE NAME	CAS	Class
22	BENZO(B)FLUORANTHENE	205-99-2	PAH
23	TRIFLURALIN	1582-09-8	Pest
24	ACENAPHTHENE	83-32-9	PAH
25	DDD, P,P'-	72-54-8	Pest
26	BENZIDINE	92-87-5	HPV
27	ENDOSULFAN	115-29-7	Pest
28	METHOXYCHLOR	72-43-5	Pest
29	2,4-DINITROPHENOL	51-28-5	Pest
30	2,4-DINITROTOLUENE	121-14-2	HPV
31	DICOFOL	115-32-2	Pest
32	CRESOL, PARA-	106-44-5	HPV
33	DDT, O,P'-	789-02-6	Pesticide
34	4,6-DINITRO-O-CRESOL	534-52-1	HPV
35	1,2,3-TRICHLOROBENZENE	87-61-6	HPV
36	LEAD NITRATE	10099-74-8	Metal
37	CADMIUM CHLORIDE	10108-64-2	Metal
38	ZINC CHLORIDE	7646-85-7	Metal
39	MERCURIC CHLORIDE	7487-94-7	Metal
40	POTASSIUM CHROMATE	7789-00-6	Metal
41	COBALT CHLORIDE	7646-79-9	Metal
42	NICKEL CHLORIDE	7718-54-9	Metal

Preparation of 8 Defined Mixtures

Active Concentration 50% (AC_{50}) → *In vitro* toxicity data from ToxCast/ Tox21

Point of Departure (POD) → POD derived from experimental animal studies

Exposure (Expo) → Exposure estimates from ExpoCast

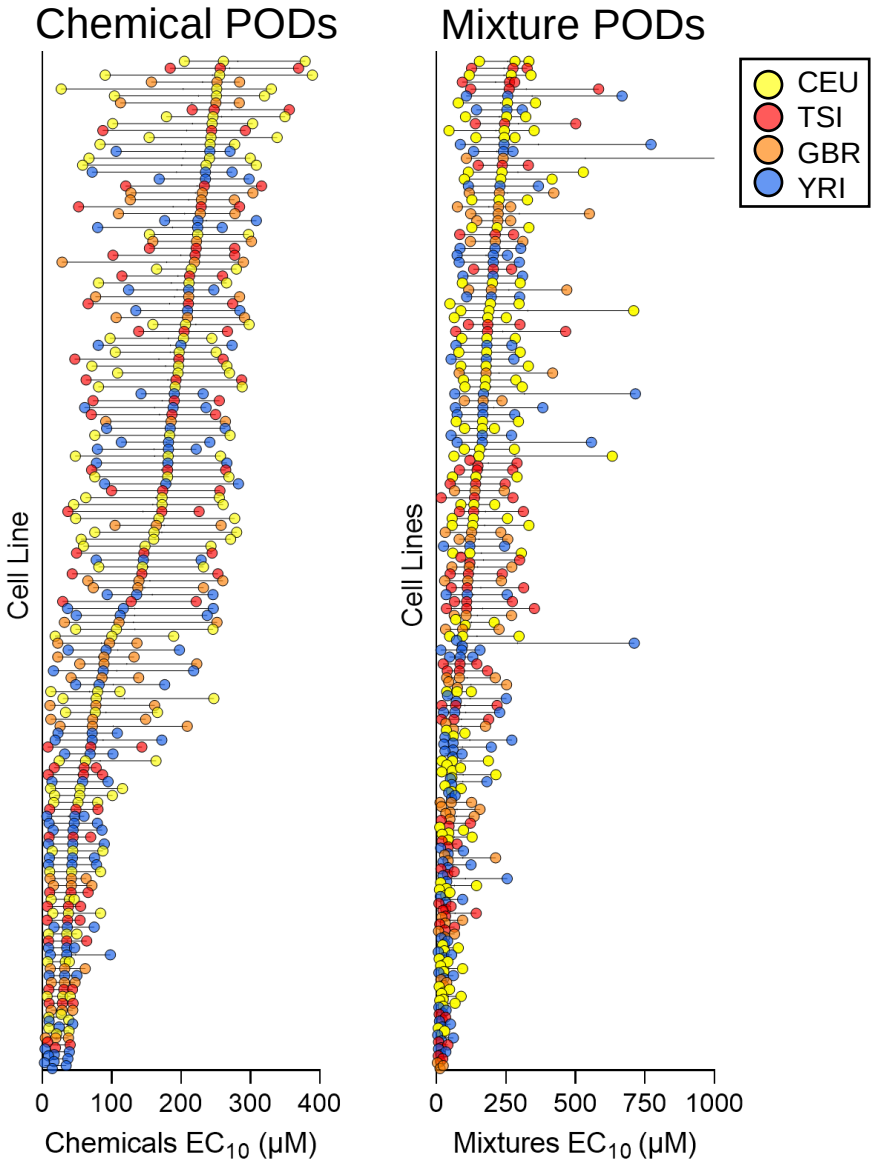
Reference Dose (RfD) → Experimental animal data converted to oral equivalent dose in humans

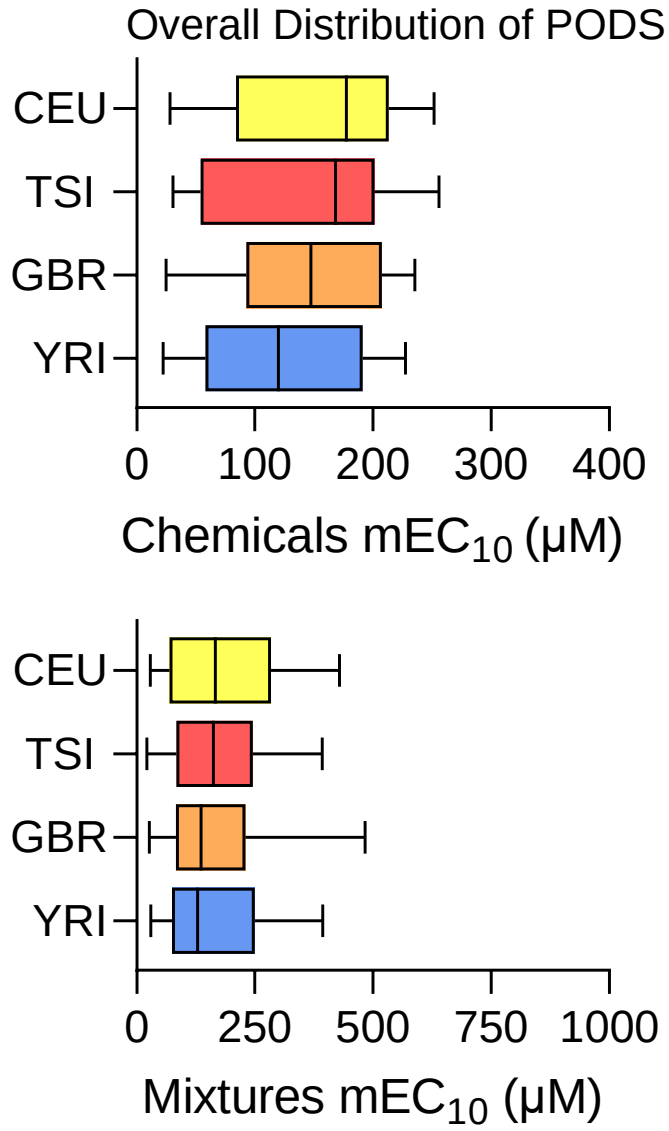
LOW: median values for each assumption

HIGH: 95th percentile for each assumption

TEXAS A&M UNIVERSITY SUPERFUND RESEARCH CENTER

Donor-Specific Concentration-Response Profiling



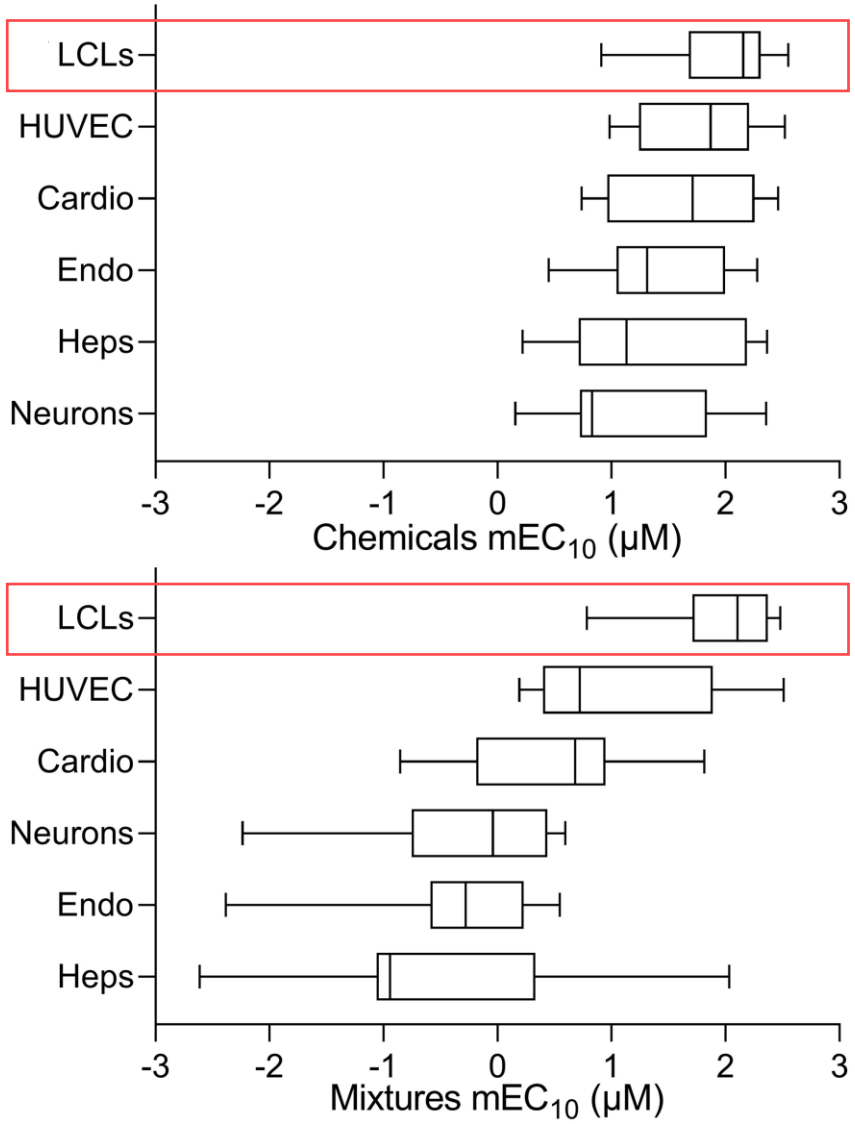


- Comparison of PODs across populations
- No significant differences across the 4 subpopulations
- YRI (subpopulation from African descent) lowest median PODs
- None of the subpopulations significantly more or less susceptible

TEXAS A&M UNIVERSITY SUPERFUND RESEARCH CENTER

Figures adapted from Ford *et al.*, 2022 (PMID: 6006120)

Comparison Across Various *In Vitro* Models

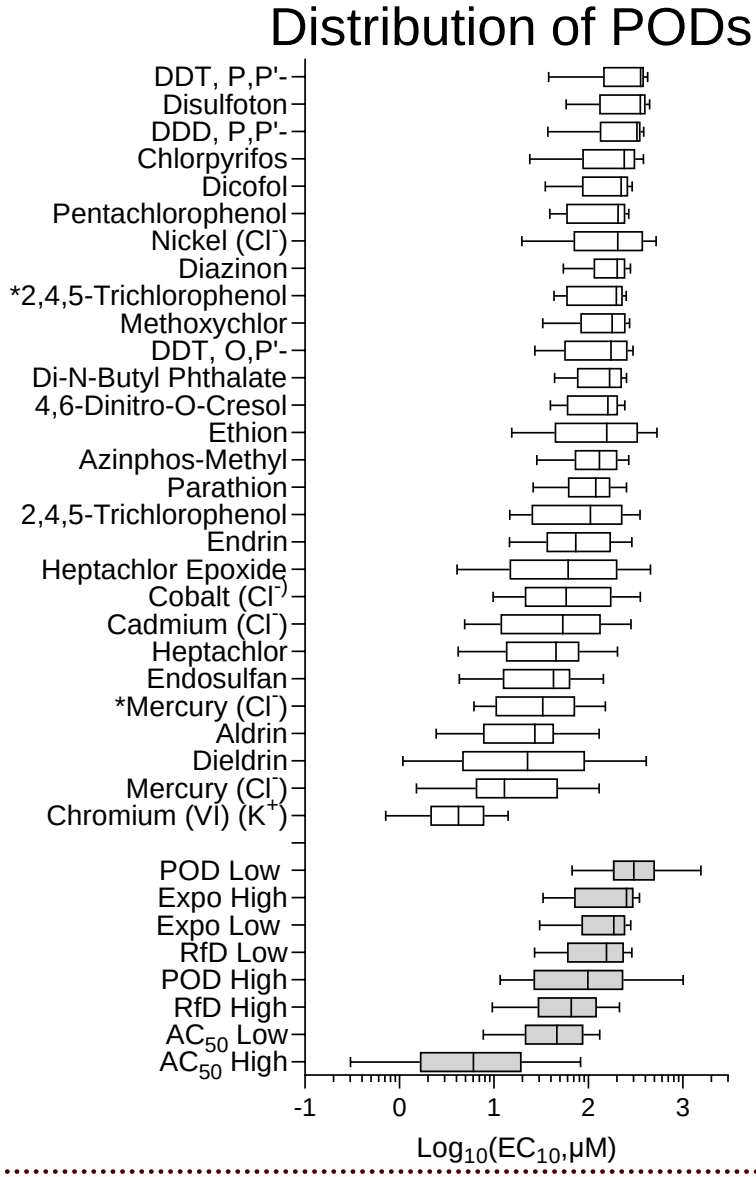


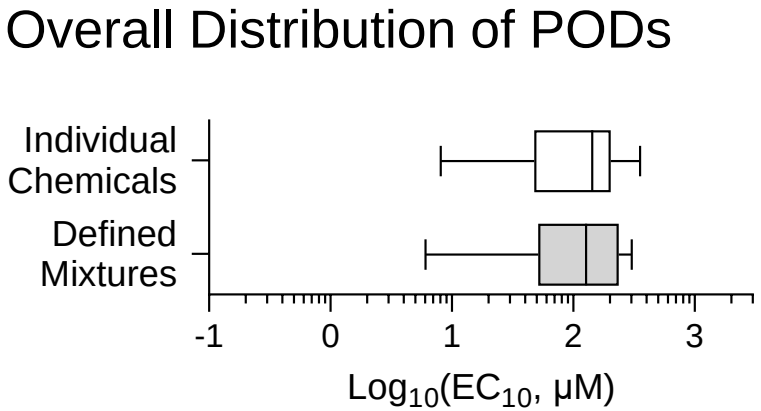
- Comparison of cytotoxic phenotypes for all models
- Chemicals and mixtures previously screened using 5 human *in vitro* models (PMID: 33395322)
- LCL within range of other *in vitro* models

TEXAS A&M UNIVERSITY SUPERFUND RESEARCH CENTER

Figures adapted from Ford *et al.*, 2022 (PMID: 6006120)

Chemical-Specific Concentration-Response Profiling





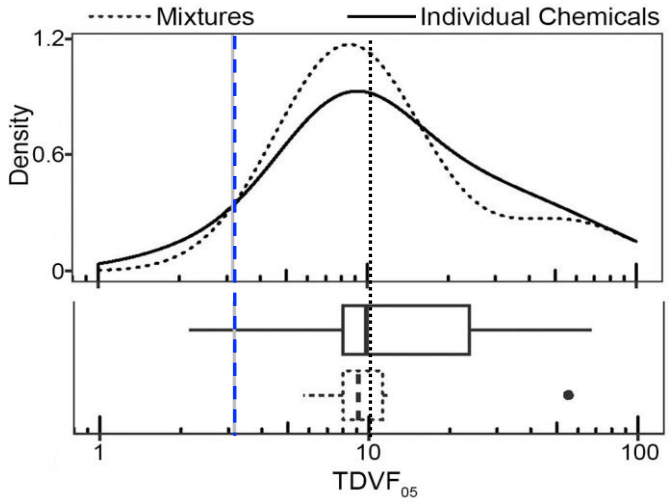
- 28 chemicals exhibited cytotoxic effects
- 17/28 chemicals were pesticides
- Heavy metals had the lowest EC_{10}
- AC_{50} high had the lowest EC_{10} for mixtures and the largest variability across all cells
- Median PODs for chemicals and mixtures were similar

TEXAS A&M UNIVERSITY SUPERFUND RESEARCH CENTER

Figures adapted from Ford *et al.,* 2022 (PMID: 6006120)

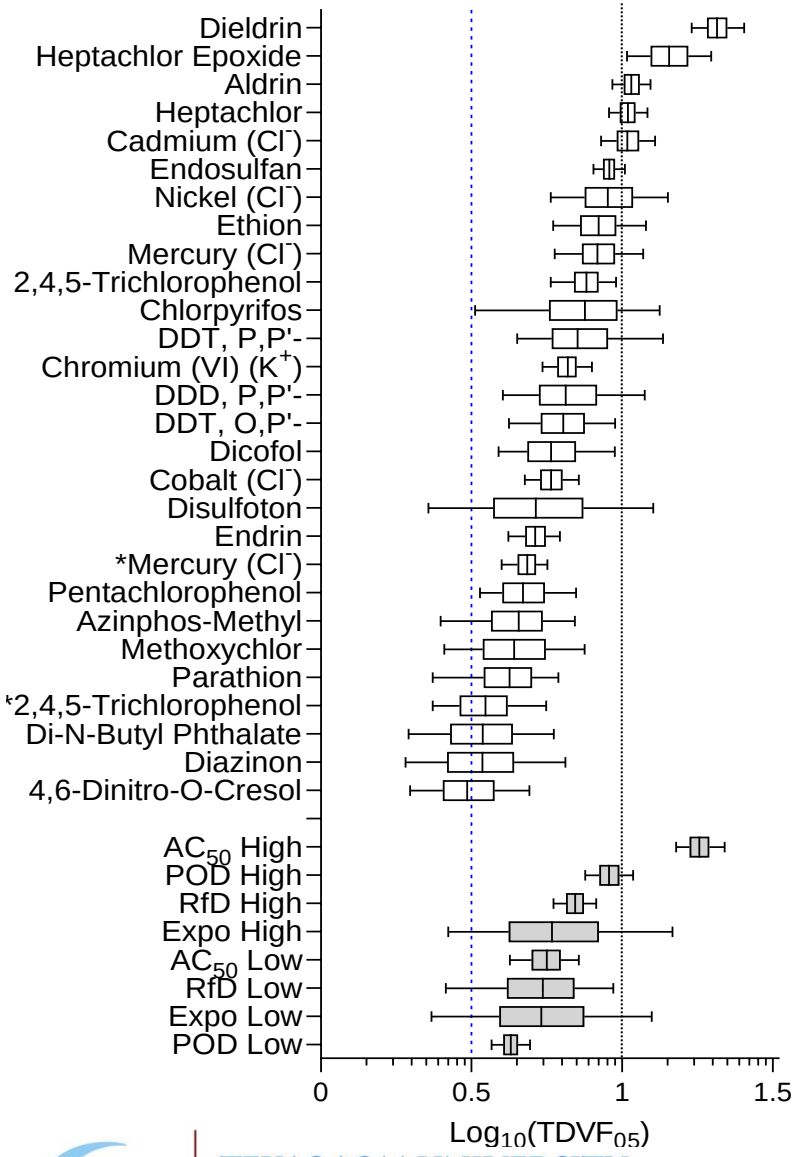
Quantifying Inter-Individual Variability

$$TDVF_{05} = \frac{EC_{10,\ median}}{EC_{10,\ 5th\ percentile}}$$



Blue Dashed line- default TDVF of $10^{1/2}$
Black Dotted line- default total variability factor of 10

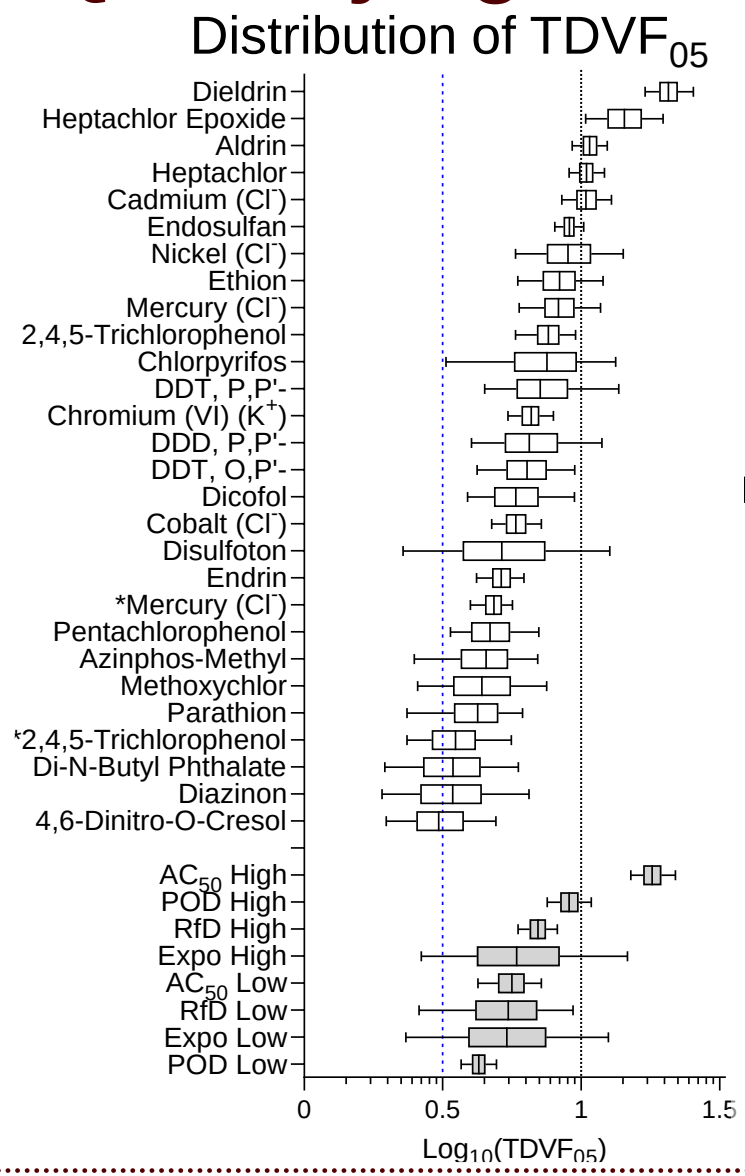
Distribution of $TDVF_{05}$

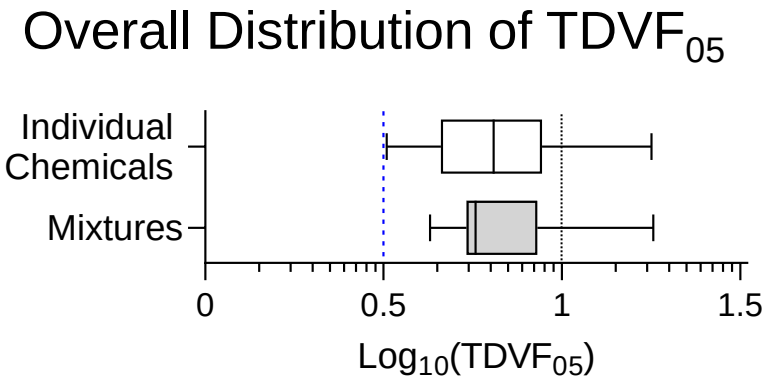


TEXAS A&M UNIVERSITY SUPERFUND RESEARCH CENTER

Figures adapted from Ford *et al.,* 2022 (PMID: 6006120)

Quantifying Inter-Individual Variability





- Pesticides had largest variability across all cells
- AC_{50} had the largest variability across all cells
- Median $TDVF_{05}$ for chemicals and mixtures were similar
- Half a log higher than default for TDVF

Blue Dashed line- default TDVF of $10^{1/2}$
Black Dotted line- default total variability factor of 10

TEXAS A&M UNIVERSITY SUPERFUND RESEARCH CENTER

Figures adapted from Ford *et al.*, 2022 (PMID: 6006120)

What are the potential molecular drivers of variability?

GWAS Work Flow

Run GWAS analysis for top 28 chemicals and mixtures

↓

Identify top gene hits for each chemical/mixture

↓

Genes functions

↓

Gene expression in LCL

↓

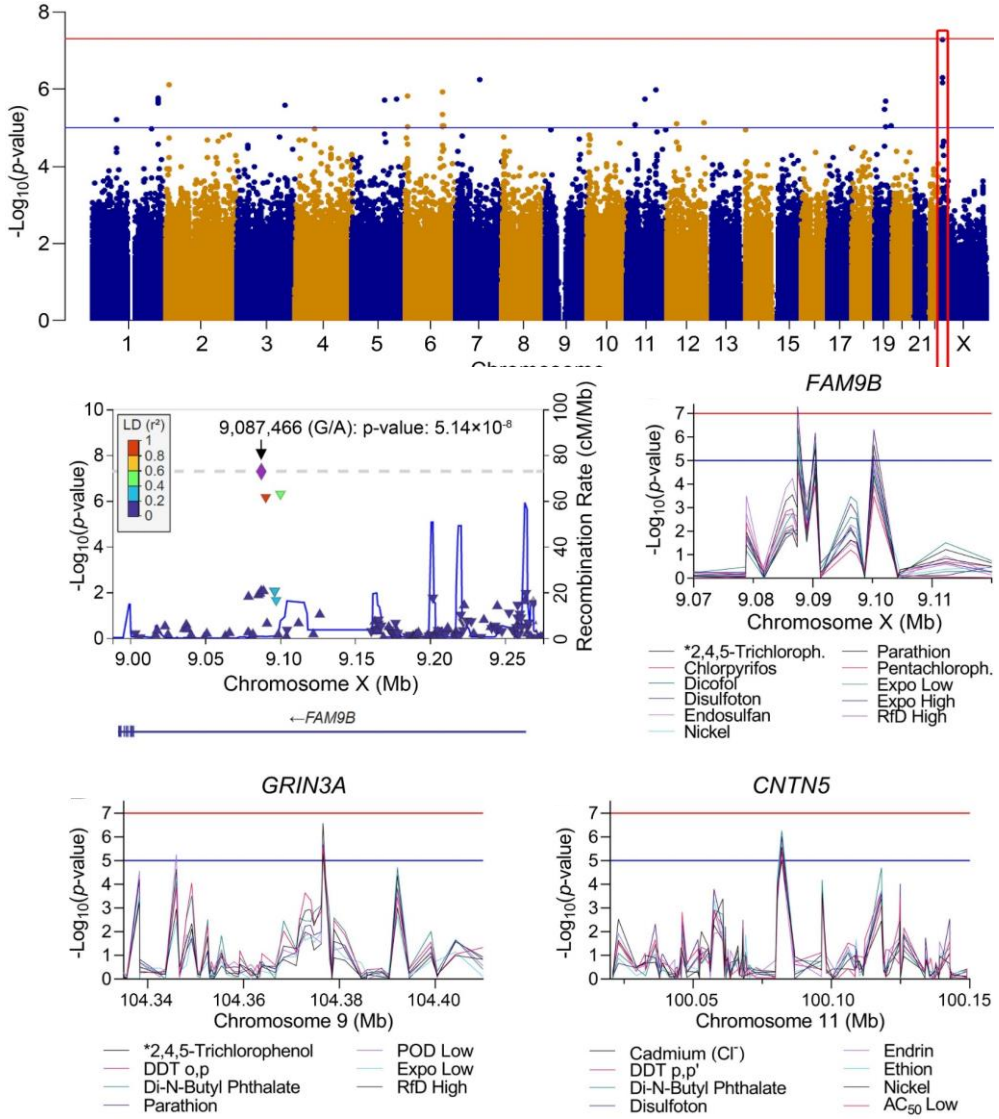
Correlation across chemicals

↓

Gene hits in mixtures and chemicals

↓

Research chemical exposure and gene of interest



TEXAS A&M UNIVERSITY SUPERFUND RESEARCH CENTER

Figures adapted from Ford *et al.,* 2022 (PMID: 6006120)

So what?

- Lymphoblasts were in range with human iPSC-derived models
- Quantified inter-individual variability for chemicals and mixtures
  - Population variability of mixtures does NOT exceed that of the most variable component
  - Similar $TDVF_{05}$ for chemicals and mixtures, BUT higher median than the default uncertainty factor of $10^{1/2}$
- Genome-wide associations among chemicals may be used to group constituents in a mixture

This model is a reasonable approach to quantify inter-individual variability and can be used to reduce uncertainties with complex exposure scenarios

Study Limitations

- Model lacks metabolic function
- Reflecting acute high-dose treatments
- Realistic routes of exposure
- Limited chemical classes

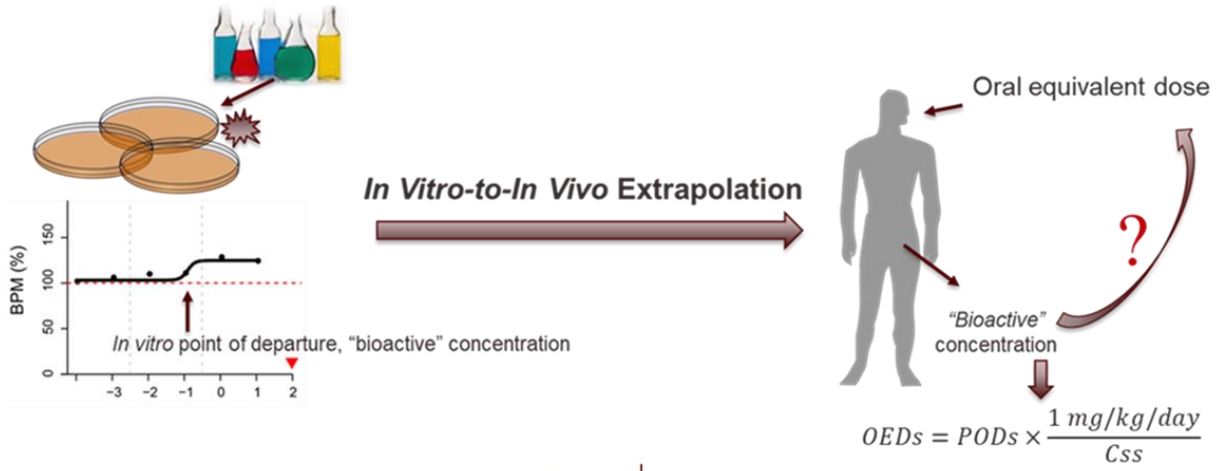
Quantitative *in vitro* to *in vivo* extrapolation (QIVIVE)

In Vitro Data + Exposure Data

↓

Oral Equivalent Dose in Humans

Chronic exposures + Routes of exposure



TEXAS A&M UNIVERSITY SUPERFUND RESEARCH CENTER

Image adapted from Yu-Syuan Luo

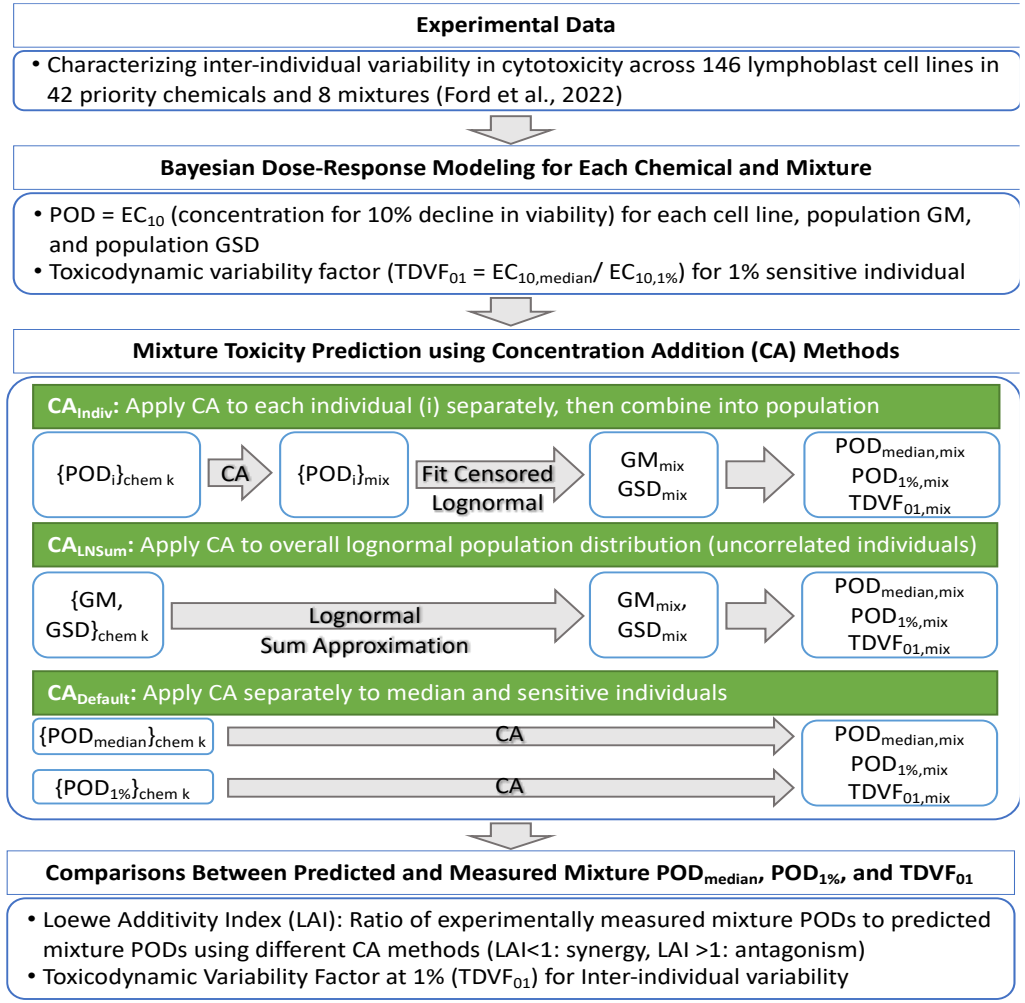
Where do we go from here?

- Apply study design to evaluate toxicity of other defined and environmental mixtures
  - Screening realistic exposure scenarios using available biomonitoring data
  - Use environmental samples to conduct region-specific exposure assessments

- Complimentary work has been done with additivity models to reconstruct the variability using the chemical data (Jang et al., 2022, under review)

Probabilistic Concentration Addition of Defined Mixture Exposures in a Population-Based Human *In Vitro* Model

Can we use concentration additivity approaches to predict inter-individual variability in responses to mixtures?



TEXAS A&M UNIVERSITY SUPERFUND RESEARCH CENTER

Figures adapted from Jang *et al.*, 2022 (under review)

Probabilistic Concentration Addition of Defined Mixture Exposures in a Population-Based Human *In Vitro* Model

Can we use concentration additivity approaches to predict inter-individual variability in responses to mixtures?

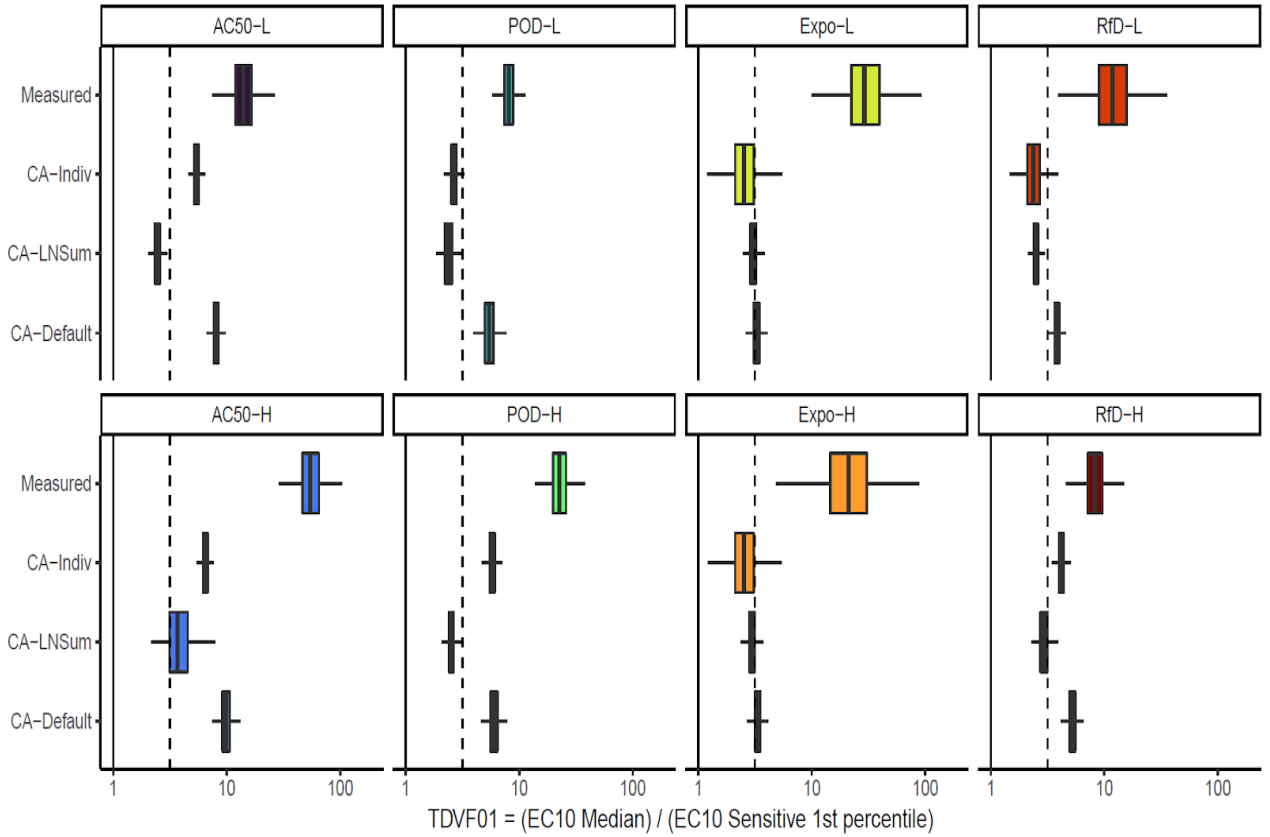
Concentration additivity models may underestimate potency

⬇

Continue to use *in vitro* to test whole mixtures
OR
Implement more stringent risk indices
(ex: lower hazard index)

⬇

Ensure public health protection from combined exposures



TEXAS A&M UNIVERSITY SUPERFUND RESEARCH CENTER

Figures adapted from Jang *et al.*, 2022 (under review)

Overall Conclusions

Do we need *in vitro* systems to assess population-variability in responses to mixtures?

- Demonstrates feasibility of using population-based *in vitro* model that can be used in mixtures risk assessment
- Understand differences in inter-individual variability in responses to chemicals and mixtures
- Provides chemical and mixture-specific variability estimates that can be used to replace default assumptions
- Various concentration addition (CA) approaches demonstrate inter-individual variability, but tend to underestimate both the *in vitro* experimental POD and TDVF values
- Results from CA predictions supports continuation of *in vitro* toxicity testing for mixtures

TEXAS A&M UNIVERSITY
SUPERFUND
RESEARCH CENTER

Thank you!

Questions?