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
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

Funding Sources

NIH NIEHS
Grant: P42 ES027704
Grant: T32 ES026568

Texas A&M University Merit Fellowship
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
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
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

- Chiu et al. ALTEX 34(3):377-388, 20172015
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
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
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 $\text{TDVF}_{0.05}$
- GWAS using cell line-specific PODs

Cell Viability (% Control)

- TSI
- GBR
- YRI
- CEU

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

**Chemical Selection and Design Mixtures**

<table>
<thead>
<tr>
<th>ID</th>
<th>SUBSTANCE NAME</th>
<th>CAS</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BENZO(A)ANTHRACENE</td>
<td>56-55-3</td>
<td>PAH</td>
</tr>
<tr>
<td>2</td>
<td>NAPHTHALENE</td>
<td>91-20-3</td>
<td>PAH</td>
</tr>
<tr>
<td>3</td>
<td>FLUORANTHENE</td>
<td>206-44-0</td>
<td>PAH</td>
</tr>
<tr>
<td>4</td>
<td>DDT, P,P'</td>
<td>50-29-3</td>
<td>Pest</td>
</tr>
<tr>
<td>5</td>
<td>DIESEL</td>
<td>60-57-1</td>
<td>Pest</td>
</tr>
<tr>
<td>6</td>
<td>ALDRIN</td>
<td>309-00-2</td>
<td>Pest</td>
</tr>
<tr>
<td>7</td>
<td>HEPTACHLOR</td>
<td>76-44-8</td>
<td>Pest</td>
</tr>
<tr>
<td>8</td>
<td>LINDANE</td>
<td>58-89-9</td>
<td>Pest</td>
</tr>
<tr>
<td>9</td>
<td>DISULFOTON</td>
<td>298-04-4</td>
<td>Pest</td>
</tr>
<tr>
<td>10</td>
<td>ENDRIN</td>
<td>72-20-8</td>
<td>Pest</td>
</tr>
<tr>
<td>11</td>
<td>DIAZINON</td>
<td>333-41-5</td>
<td>Pest</td>
</tr>
<tr>
<td>12</td>
<td>HEPTACHLOR EPoxide</td>
<td>1024-57-3</td>
<td>Pest</td>
</tr>
<tr>
<td>13</td>
<td>PENTACHLORPHENOL</td>
<td>87-86-5</td>
<td>HPV</td>
</tr>
<tr>
<td>14</td>
<td>DI-N-BUTYLPHTHALATE</td>
<td>84-74-2</td>
<td>Plastiz</td>
</tr>
<tr>
<td>15</td>
<td>CHLORPYRIFOS</td>
<td>2921-88-2</td>
<td>Pest</td>
</tr>
<tr>
<td>16</td>
<td>DI(2-EHTHXYL)PHTHALATE</td>
<td>117-81-7</td>
<td>Plastiz</td>
</tr>
<tr>
<td>17</td>
<td>2,4,6-TRICHLORPHENOL</td>
<td>88-06-2</td>
<td>HPV</td>
</tr>
<tr>
<td>18</td>
<td>ETHION</td>
<td>563-12-2</td>
<td>Pest</td>
</tr>
<tr>
<td>19</td>
<td>AZINPHOS-METHYL</td>
<td>86-50-0</td>
<td>Pest</td>
</tr>
<tr>
<td>20</td>
<td>2,4,5-TRICHLORPHENOL</td>
<td>95-95-4</td>
<td>HPV</td>
</tr>
<tr>
<td>21</td>
<td>PARATHION</td>
<td>56-38-2</td>
<td>Pest</td>
</tr>
<tr>
<td>22</td>
<td>BENZO(B)FLUORANTHENE</td>
<td>205-99-2</td>
<td>PAH</td>
</tr>
<tr>
<td>23</td>
<td>TRIFLURALIN</td>
<td>1582-09-8</td>
<td>Pest</td>
</tr>
<tr>
<td>24</td>
<td>ACENAPHTENE</td>
<td>83-32-9</td>
<td>PAH</td>
</tr>
<tr>
<td>25</td>
<td>DDD, P,P'</td>
<td>72-54-8</td>
<td>Pest</td>
</tr>
<tr>
<td>26</td>
<td>BENZIDINE</td>
<td>92-87-5</td>
<td>HPV</td>
</tr>
<tr>
<td>27</td>
<td>ENDOSULFAN</td>
<td>115-29-7</td>
<td>Pest</td>
</tr>
<tr>
<td>28</td>
<td>METHOXYCHLOR</td>
<td>72-43-5</td>
<td>Pest</td>
</tr>
<tr>
<td>29</td>
<td>2,4-DINITROPHENOL</td>
<td>51-28-5</td>
<td>Pest</td>
</tr>
<tr>
<td>30</td>
<td>2,4-DINITROTOLUENE</td>
<td>121-14-2</td>
<td>HPV</td>
</tr>
<tr>
<td>31</td>
<td>DICOFOL</td>
<td>115-32-2</td>
<td>Pest</td>
</tr>
<tr>
<td>32</td>
<td>CRESOL, PARA-</td>
<td>106-44-5</td>
<td>HPV</td>
</tr>
<tr>
<td>33</td>
<td>DDT, O,P'</td>
<td>789-02-6</td>
<td>Pesticide</td>
</tr>
<tr>
<td>34</td>
<td>4,6-DINITRO-D-CRESOL</td>
<td>534-52-1</td>
<td>HPV</td>
</tr>
<tr>
<td>35</td>
<td>1,2,3-TRICHLOROBENZENE</td>
<td>87-61-6</td>
<td>HPV</td>
</tr>
<tr>
<td>36</td>
<td>LEAD NITRATE</td>
<td>10099-74-8</td>
<td>Metal</td>
</tr>
<tr>
<td>37</td>
<td>CADMIUM CHLORIDE</td>
<td>10108-64-2</td>
<td>Metal</td>
</tr>
<tr>
<td>38</td>
<td>ZINC CHLORIDE</td>
<td>7646-85-7</td>
<td>Metal</td>
</tr>
<tr>
<td>39</td>
<td>MERCURIC CHLORIDE</td>
<td>7487-94-7</td>
<td>Metal</td>
</tr>
<tr>
<td>40</td>
<td>POTASSIUM CHROMATE</td>
<td>7789-00-6</td>
<td>Metal</td>
</tr>
<tr>
<td>41</td>
<td>COBALT CHLORIDE</td>
<td>7646-79-9</td>
<td>Metal</td>
</tr>
<tr>
<td>42</td>
<td>NICKEL CHLORIDE</td>
<td>7718-54-9</td>
<td>Metal</td>
</tr>
</tbody>
</table>

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

**Chemical Selection and Design Mixtures**

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

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

### Preparation of 8 Defined Mixtures

- **Active Concentration 50% (AC₅₀)**
- **Point of Departure (POD)**
- **Exposure (Expo)**
- **Reference Dose (RfD)**

**LOW:** median values for each assumption

**HIGH:** 95th percentile for each assumption

**In vitro toxicity data from ToxCast/Tox21**

**POD derived from experimental animal studies**

**Exposure estimates from ExpoCast**

**Experimental animal data converted to oral equivalent dose in humans**
Donor-Specific Concentration-Response Profiling

- **Chemical PODs**
  - YRI: Yellow
  - GBR: Orange
  - TSI: Red
  - CEU: Blue

- **Mixture PODs**
  - YRI: Yellow
  - GBR: Orange
  - TSI: Red

Overall Distribution of PODs

- 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

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

Figures adapted from Ford et al., 2022 (PMID: 6006120)
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
Quantifying Inter-Individual Variability

\[ TDVF_{05} = \frac{EC_{10 \text{ median}}}{EC_{10 \text{ 5th percentile}}} \]

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

Blue Dashed line- default TDVF of 10^{1/2}
Black Dotted line- default total variability factor of 10
Quantifying Inter-Individual Variability

Distribution of TDVF\(_{05}\)

- 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

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

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

In Vitro-to-In Vivo Extrapolation

Oral equivalent dose

\[ OEDs = PODs \times \frac{1 \text{ mg/kg/day}}{Css} \]

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

Experimental Data
- Characterizing inter-individual variability in cytotoxicity across 146 lymphoblast cell lines in 42 priority chemicals and 8 mixtures (Ford et al., 2022)

Bayesian Dose-Response Modeling for Each Chemical and Mixture
- POD = EC_{10} (concentration for 10% decline in viability) for each cell line, population GM, and population GSD
- Toxicodynamic variability factor (TDVF_{01} = EC_{10,median} / EC_{10,1%}) for 1% sensitive individual

Mixture Toxicity Prediction using Concentration Addition (CA) Methods
- CA_{indiv}: Apply CA to each individual (i) separately, then combine into population
- CA_{cumsum}: Apply CA to overall lognormal population distribution (uncorrelated individuals)
- CA_{median}: Apply CA separately to median and sensitive individuals

Comparisons Between Predicted and Measured Mixture POD_{median}, POD_{1%}, and TDVF_{01}
- Loewe Additivity Index (LAI): Ratio of experimentally measured mixture PODs to predicted mixture PODs using different CA methods (LAI<1: synergy, LAI >1: antagonism)
- Toxicodynamic Variability Factor at 1% (TDVF_{01}) for Inter-individual variability

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

---

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
Thank you!

Questions?