Development of Mathematical New Approach Methods to Assess Chemical Mixtures

Rachel Broughton, Madison Feshuk, Zachary Stanfield, Kristin Isaacs, and Katie Paul Friedman

ASCCT-ESTIV Webinar Series

March 11th, 2025

The views expressed in this presentation are those of the author(s) and do not necessarily represent the views or the policies of the U.S. Environmental Protection Agency. Any mention of trade names, manufacturers or products does not imply an endorsement by the U.S. Government or the EPA. EPA and its employees do not endorse any commercial products, services, or enterprises.

Overview

Problem

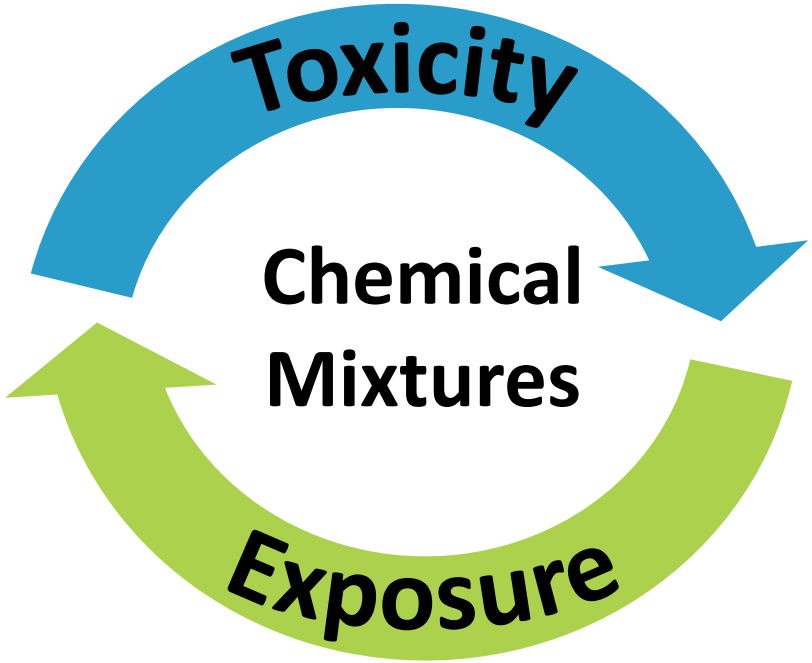
- Real-life chemical exposures are mixtures
- Screening all possible mixture combinations is impractical

Part 1: Mixture Modeling Approach

- Combine single chemical component bioactivity data using mathematical models to predict mixture response
- Evaluate with an experimental binary mixtures dataset

Part 2: MOE Case Study

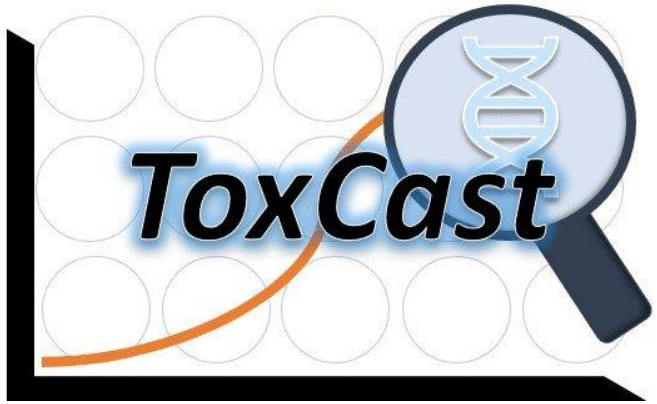
- Examine Margin of Exposure (MOE) for a PFAS subset
- Compare predicted bioactivity from mixture models (Part 1) to human biomonitoring exposure data



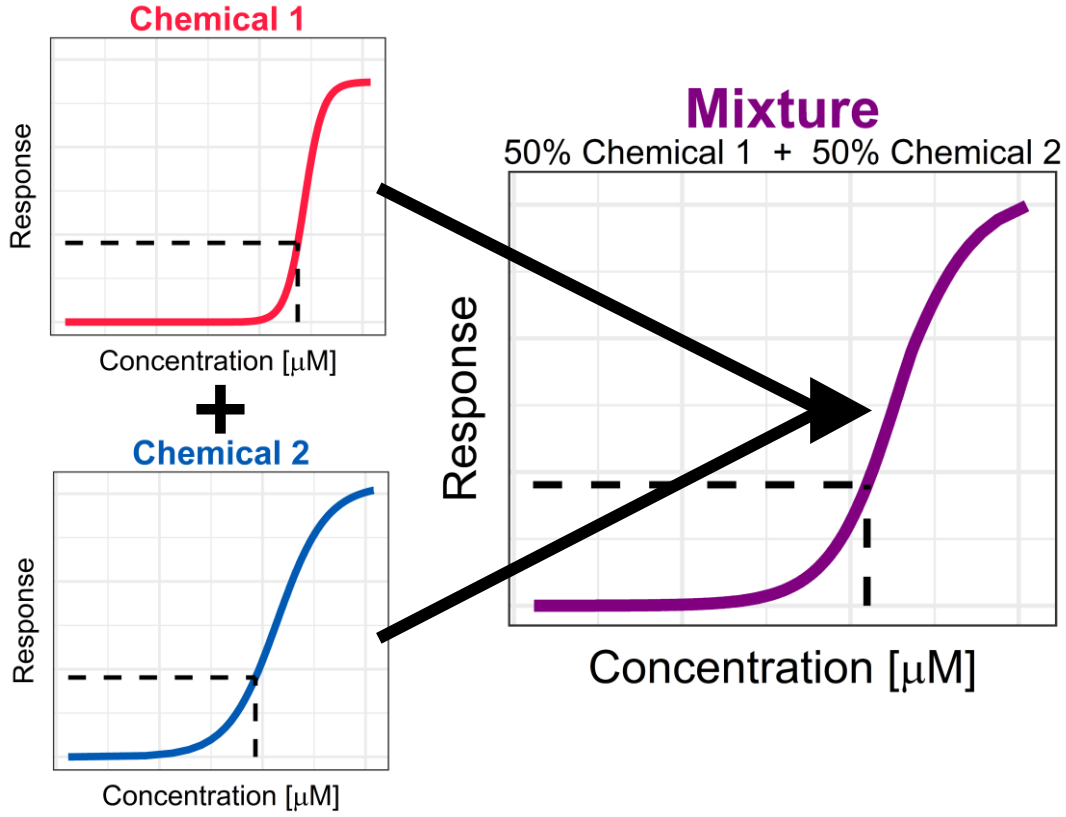
Part 1:
Mixture Modeling with Experimental Concentration-Response Data

Project Objectives

1) Use available single chemical screening assay data from the U.S. EPA's Toxicity Forecaster (ToxCast) program

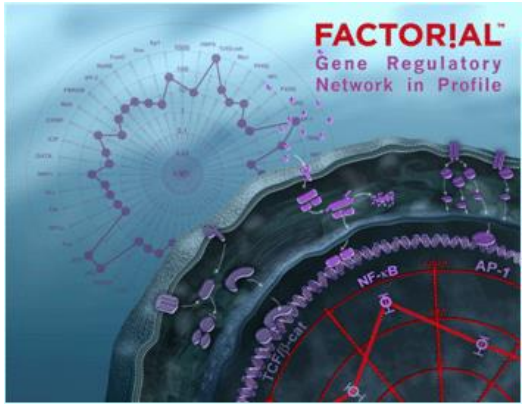


2) Predict bioactivity behavior of binary chemical mixtures with mathematical mixture models
3) Evaluate performance of predicted model fits compared to experimental data



Test Dataset

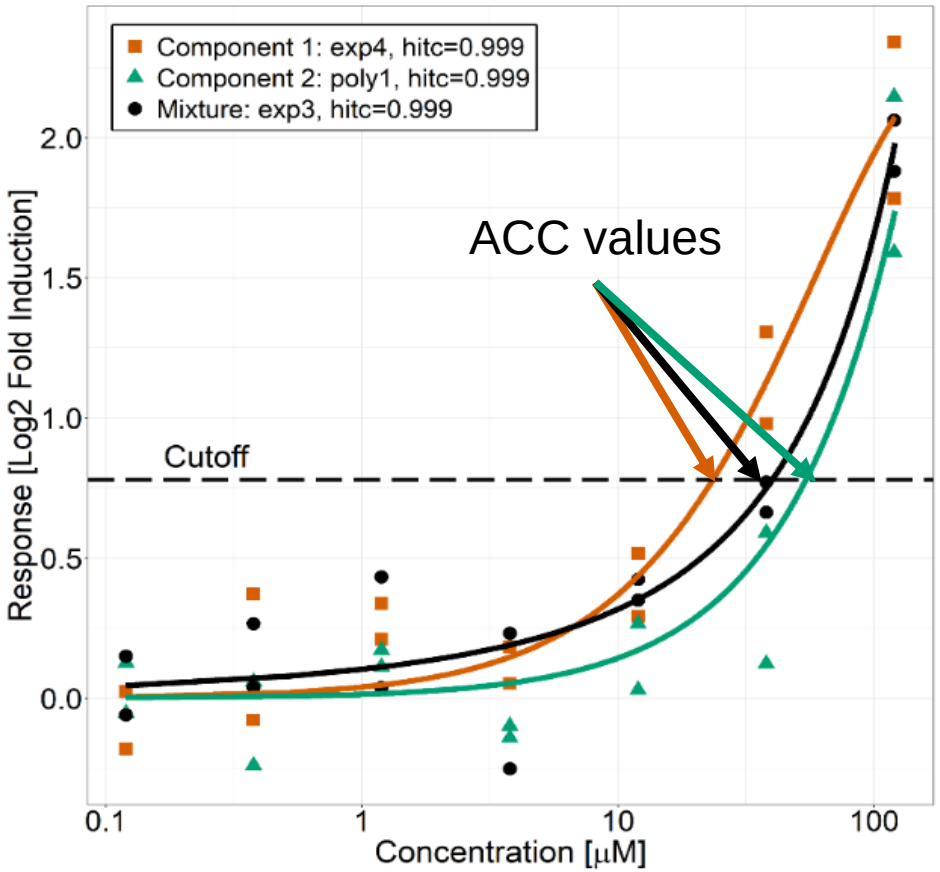
- Selected **21** binary chemical mixtures and their single components for screening
  - Inspired by consumer product ingredients
  - Included single chemical components with legacy ToxCast screening data
  - Used fixed concentration ratios (either 1:1 or 1:2)
- Mixtures and additional samples of single chemical components were screened in concentration-response in the Attagene FACTORIAL™ platform, profiling **81** transcription factors and nuclear receptor targets
- A filtered subset of **237** active mixture concentration-response curves were considered for this analysis



Binary Mixtures (Concentration Ratio)
1) Propylparaben; 2-(Phenylmethylene)octanal (33:67)
2) Propylparaben; 2-(Phenylmethylene)octanal (67:33)
3) Propylparaben; Butylated hydroxytoluene (33:67)
4) Propylparaben; Butylated hydroxytoluene (67:33)
5) 2-(Phenylmethylene)octanal; Butylated hydroxytoluene (33:67)
6) 2-(Phenylmethylene)octanal; Butylated hydroxytoluene (67:33)
7) Citric acid; Butylated hydroxytoluene (33:67)
8) Citric acid; Butylated hydroxytoluene (67:33)
9) Dodecanoic acid; Propylparaben (67:33)
10) Dodecanoic acid; Propylparaben (33:67)
11) 1-Phenyl-3-methyl-5-pyrazolone; Propylparaben (33:67)
12) 1-Phenyl-3-methyl-5-pyrazolone; Propylparaben (67:33)
13) Propylparaben; Bisphenol A (50:50)
14) Propylparaben; Triclosan (50:50)
15) Propylparaben; Rifampicin (50:50)
16) 2-(Phenylmethylene)octanal; Bisphenol A (50:50)
17) 2-(Phenylmethylene)octanal; Triclosan (50:50)
18) 2-(Phenylmethylene)octanal; Pioglitazone hydrochloride (50:50)
19) 2-(Phenylmethylene)octanal; Bexarotene (50:50)
20) Citric acid; Bexarotene (50:50)
21) Butylated hydroxytoluene; Bexarotene (50:50)

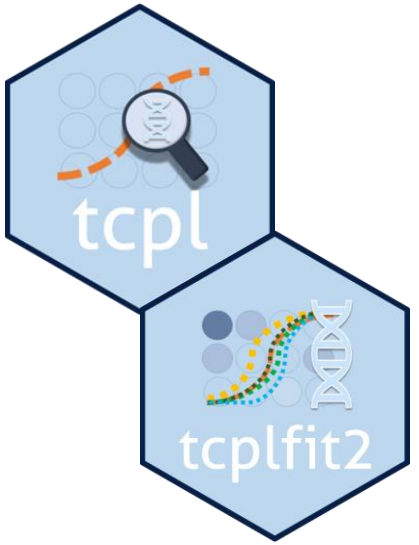
Stanfield, Z. et al. Mining of consumer product ingredient and purchasing data to identify potential chemical coexposures. *Environmental Health Perspectives*. 2021 Jun; 129(6). PMID: 34160298

Concentration-Response Data



Mixture: 0.67 2-(Phenylmethylene)octanal + 0.33 Butylated hydroxytoluene
Endpoint: Nuclear factor (erythroid-derived 2)-like 2 (ATG NRF2 ARE CIS)

- Example **Binary Mixture** consists of
  67% Chemical Component 1 and
  33% Chemical Component 2
- Key Point of Departure (POD) used was the **Activity Concentration at the Cutoff (ACC)** since it allows comparability across endpoints
- Data is available in ToxCast's invitrodb v4.2, fit with ToxCast Pipeline software *tcpl* v3.2.0 and *tcplfit2* v0.1.7.



U.S. EPA. 2024. ToxCast from invitrodb_v4.2. Retrieved from https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data on Oct. 1, 2024.

Mathematical Mixture Models

Model Inputs:
1) Data for single components
   - Compare component data from test dataset vs. from legacy ToxCast database
2) Concentration fractions of the mixture

Extrapolated Concentration Addition (CA)

- Assumes chemicals act in the same way on the same target
- Input = concentrations at given response levels [x(y)]

$for\ y < 0.7 * \mathrm{top}_2$:

$$AC_{mix,y} = \left(\sum_{j=1}^{J} \frac{q_j}{AC_{j,y}}\right)^{-1}$$

$for\ 0.7 * \mathrm{top}_2 < y < \mathrm{top}_1$:

$$AC_{mix} = \frac{AC_{1,y}}{q_1} \cdot (1 - TU_2)$$

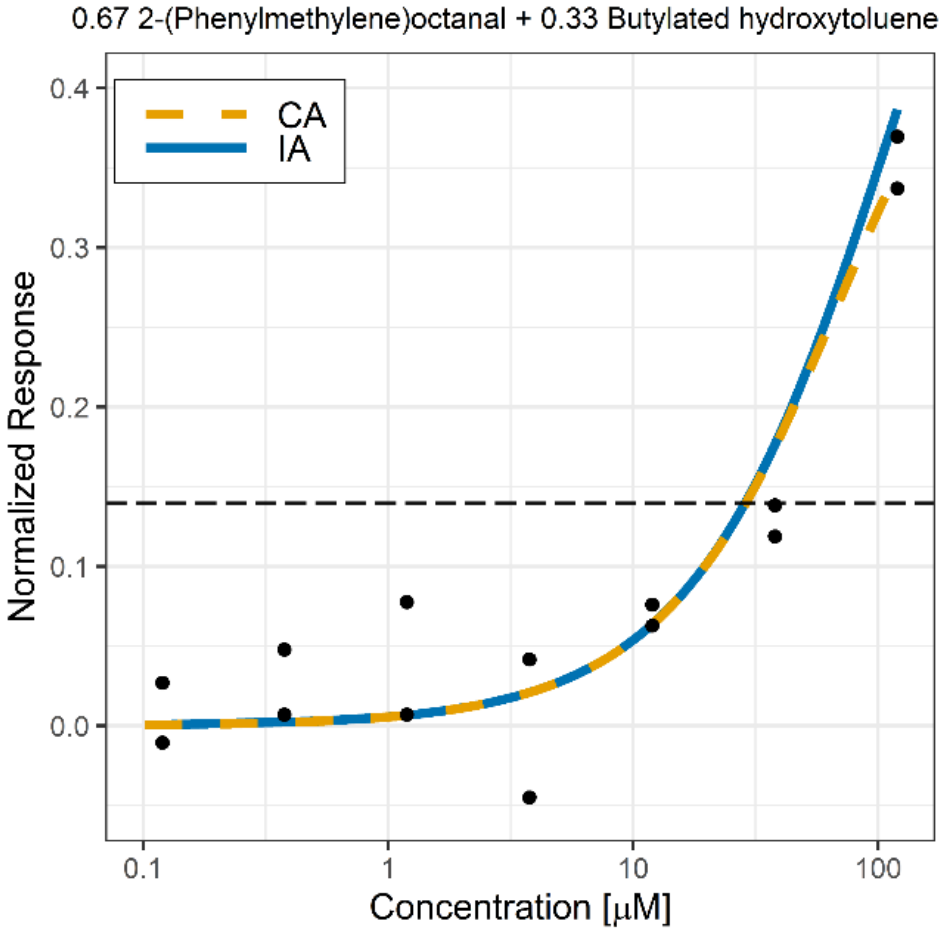
Concentration fraction

$$q_j = \frac{c_{j,y}}{\sum_{j=1}^{J} c_{j,y}}$$

Independent Action (IA)

- Assumes chemicals act independently through different pathways to reach an apical response
- Treats responses as probabilities
- Input = response curves for given concentrations [y(x)]

$$E_{mix}(c_{mix}) = 1 - \prod_{j=1}^{J}\left(1 - E_j\left(q_j \cdot c_{mix}\right)\right)$$



Scholze, Martin et al. "Extending the applicability of the dose addition model to the assessment of chemical mixtures of partial agonists by using a novel toxic unit extrapolation method." *PloS one* vol. 9,2 (2014).

Backhaus, Thomas et al. "Predictability of the toxicity of a multiple mixture of dissimilarly acting chemicals to *Vibrio fischeri." Environmental Toxicology and Chemistry* vol. 19,9 (2000).

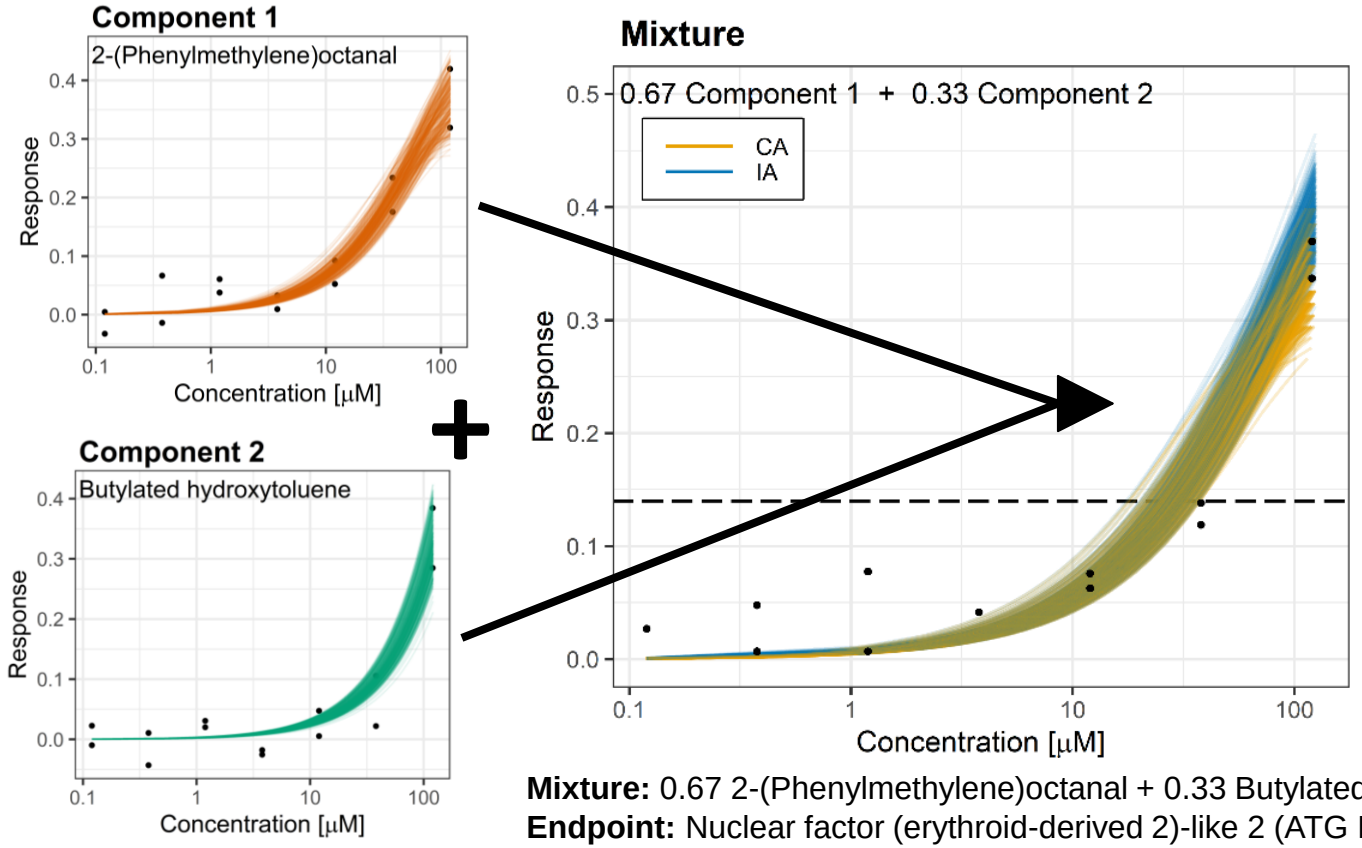
Capturing Uncertainty

Uncertainty from experimental mixture concentration-response data

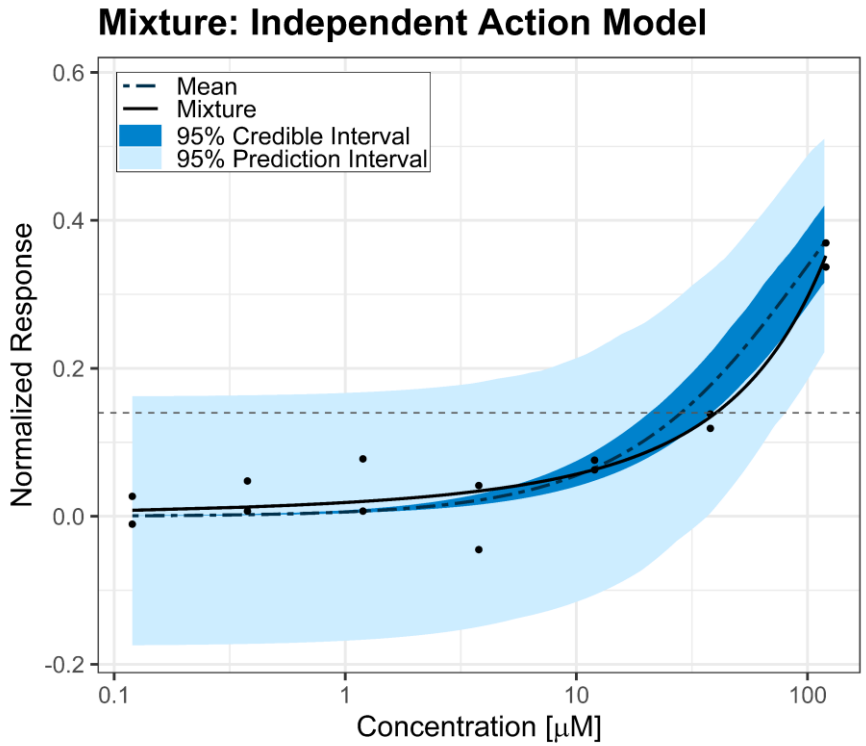
+

Uncertainty from single chemical concentration-response curves input to the models

Bootstrap Resampling



Bayesian Statistical Framework



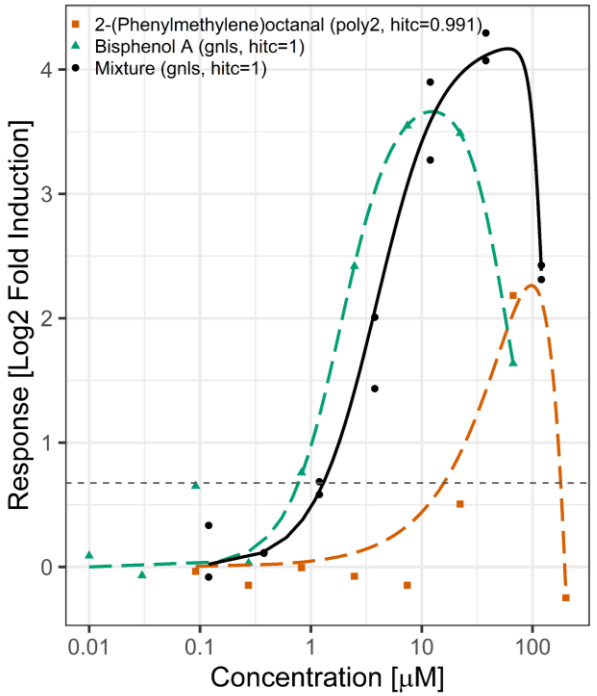
Mixture: 0.67 2-(Phenylmethylene)octanal + 0.33 Butylated hydroxytoluene
Endpoint: Nuclear factor (erythroid-derived 2)-like 2 (ATG NRF2 ARE CIS)

Watt E.D., Judson R.S. (2018) Uncertainty quantification in ToxCast high throughput screening. *PLoS ONE* 13(7): e0196963.

Example Result

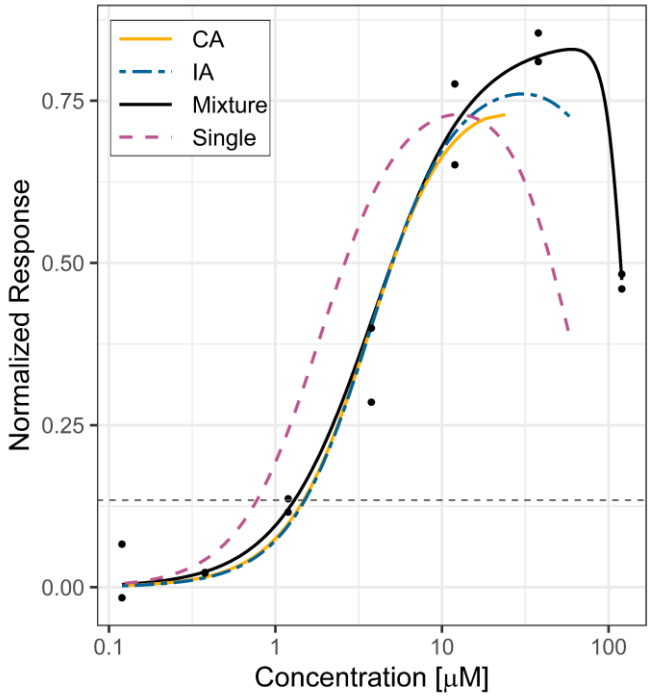
Mixture: 0.5 2-(Phenylmethylene)octanal + 0.5 Bisphenol A
Endpoint: Pregnane X receptor (ATG PXRE CIS)

Data



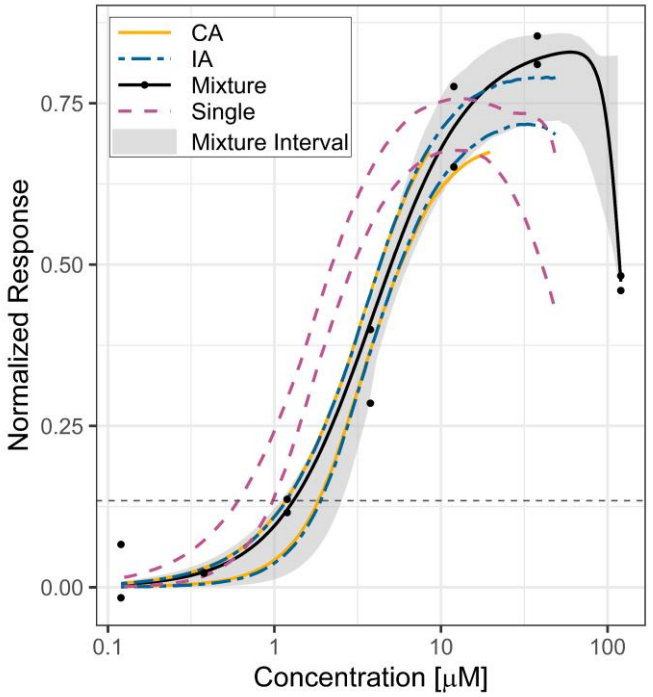
- Observed mixture from test dataset
- Modeling performed with single component data from legacy ToxCast screening

Model Curve



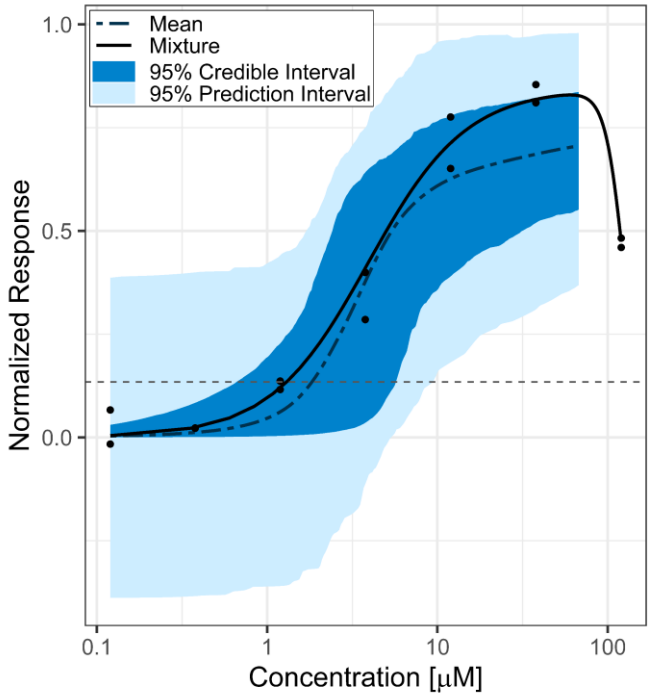
1) CA
2) IA
3) 100% most potent single component
4) **Observed mixture**

Bootstrap Intervals



95% bootstrap resampled confidence intervals

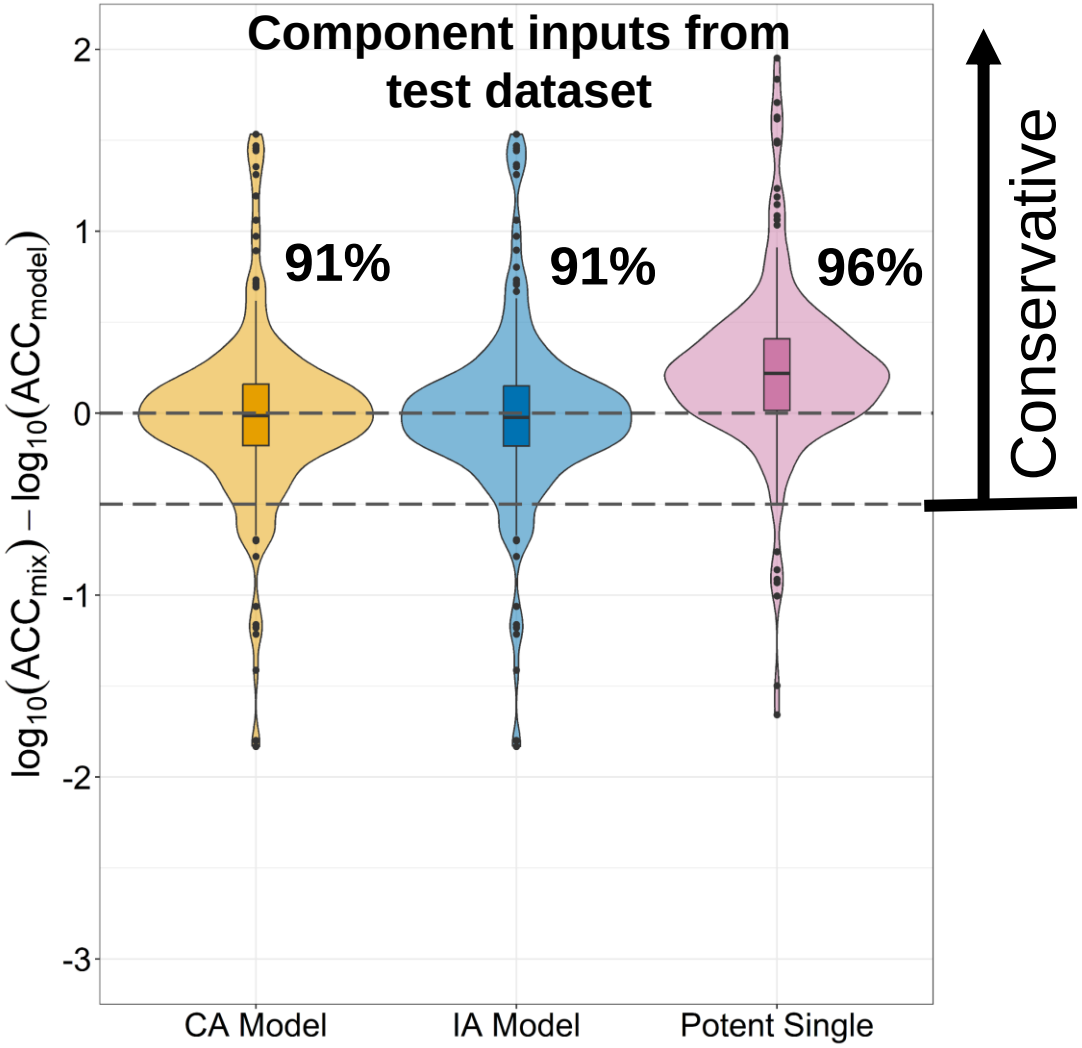
Bayesian Intervals

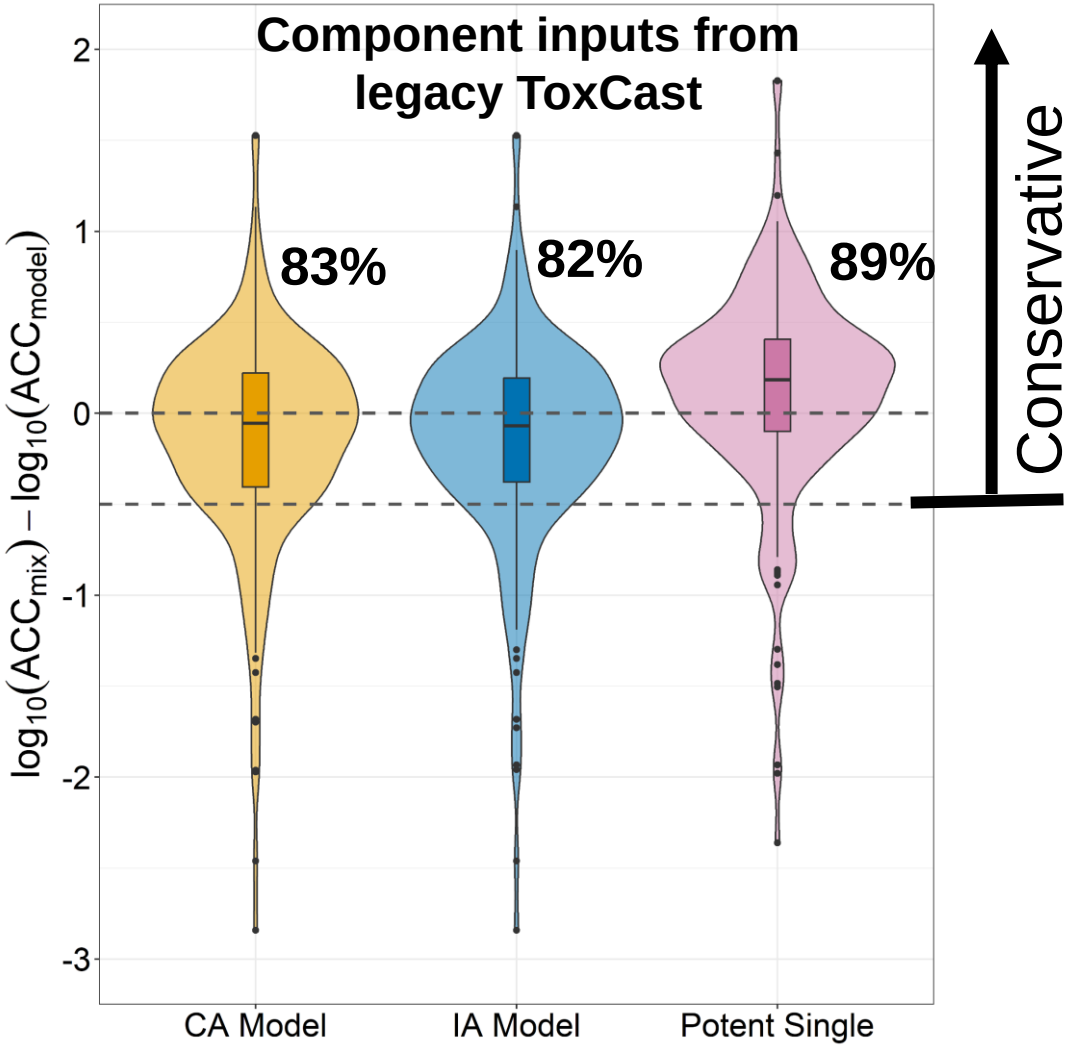


IA 95% credible interval and 95% prediction interval

Performance Metrics

Comparison of ACC predictions from each model to the experimentally-derived ACC for the binary mixture

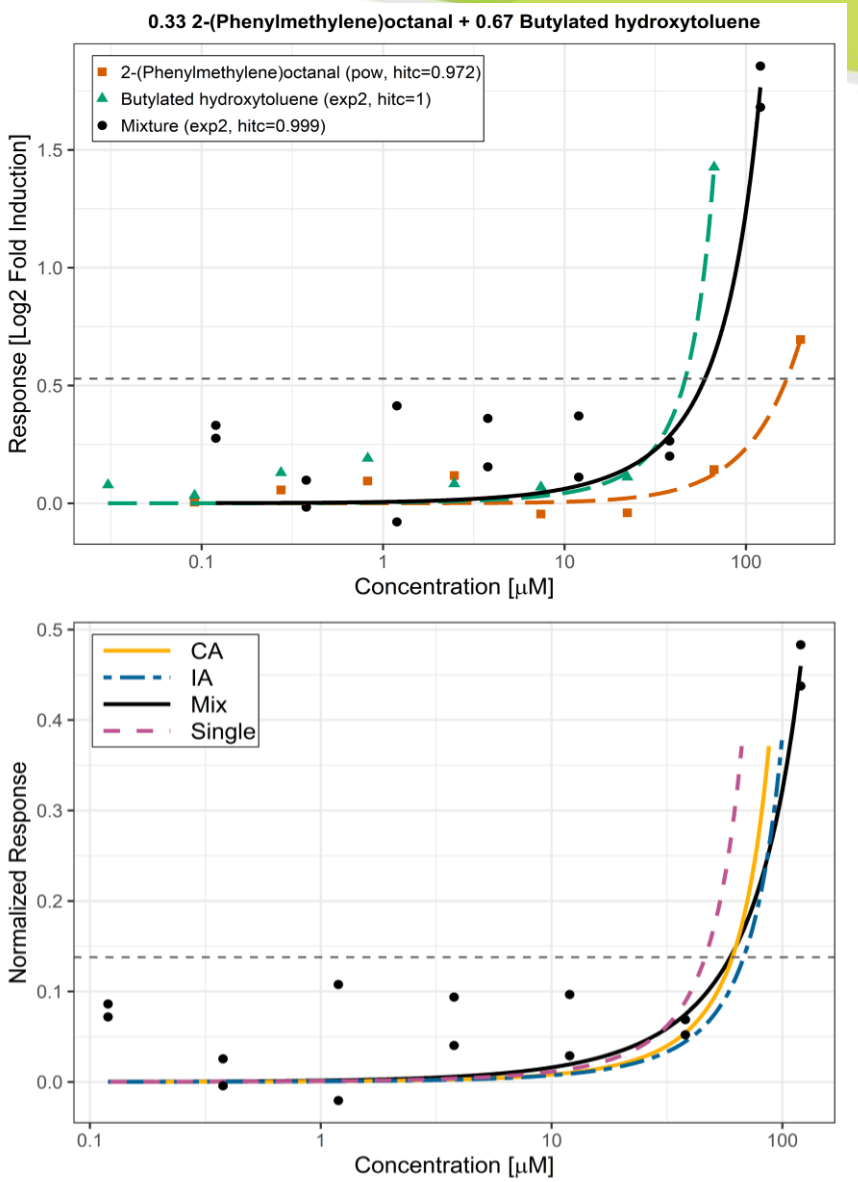




Mixture models performed well for both test and legacy datasets, although use of legacy ToxCast data demonstrated slightly poorer results

Part 1 Summary

- Concentration Addition (CA), Independent Action (IA), and 100% single component models were evaluated for prediction of bioactivity of binary mixtures
- For this chemical set, the CA and IA models performed similarly and captured the mixture behavior, especially at the ACC
  - Models do not include non-additive behaviors
  - Most potent single chemical model was usually conservative, but not as accurate
- Model inputs of single component data from the test dataset versus legacy ToxCast data were compared to predict the same mixtures
  - Both approaches performed well, but using legacy data provided slightly poorer predictions due to more sources of uncertainty



Part 2:
Margin of Exposure Case Study

Exposure Dataset

- Examined the 2009 – 2010 CDC National Health and Nutrition Examination Survey (NHANES) for exposure data
  - Included laboratory blood serum samples from human subjects, specifically polyfluoroalkyl (PFAS) chemical concentrations for 2233 subjects
  - Analyzed via the *survey* package in R

Chemicals Evaluated:

PFOS = Perfluorooctane sulfonic acid

PFNA = Perfluorononanoic acid

PFOA = Perfluorooctanoic acid

PFHxS = Perfluorohexane sulfonic acid

Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Data. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, (2009-2010) https://wwwn.cdc.gov/Nchs/Nhanes/.

MOE Calculation

Margin of Exposure (MOE) for an individual was computed as:

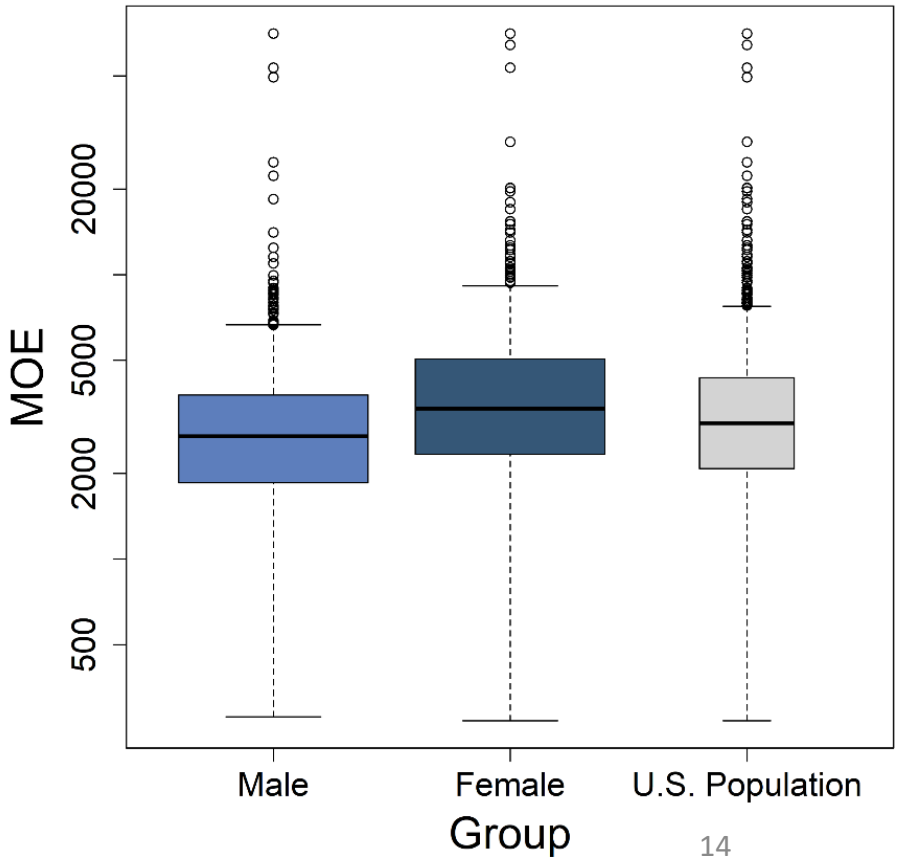
$$\frac{\textbf{Activity Concentration at the Cutoff (ACC)}}{\textbf{Exposure Concentration}}$$

Simulated from **Concentration Addition** mixture model with single component inputs from ToxCast [µmol/L]

NHANES reported concentration in subject's blood serum [µmol/L]

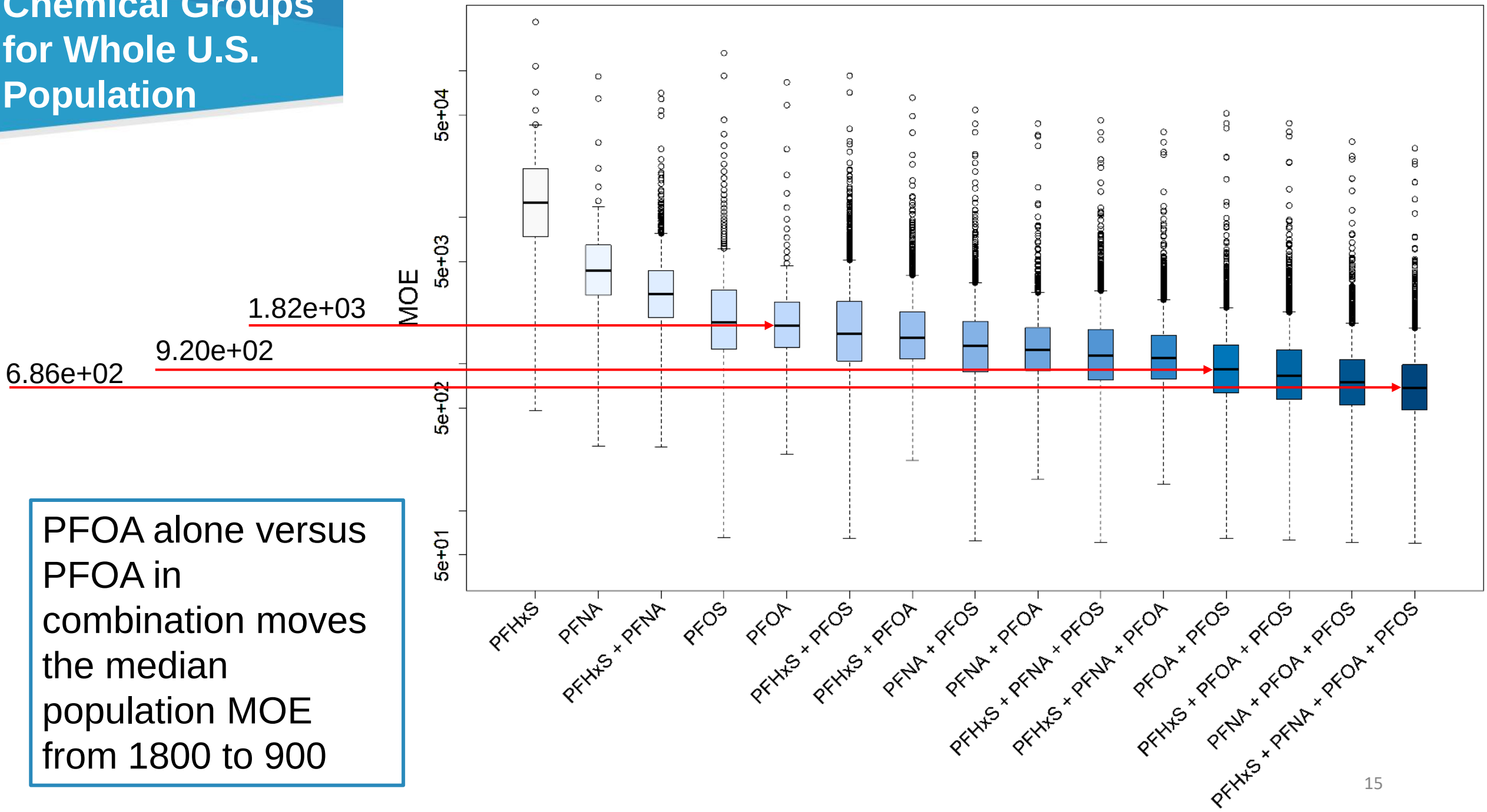
Values may be interpreted as follows:

↓ACC + ↑Exposure → ↓MOE → ↑Bioactivity-based estimate of risk



Addicks, Gregory C. et al. "Per- and polyfluoroalkyl substances (PFAS) in mixtures show additive effects on transcriptomic points of departure in human liver spheroids", *Toxicological Sciences*, vol. 194, 1, (2023)

Chemical Groups for Whole U.S. Population

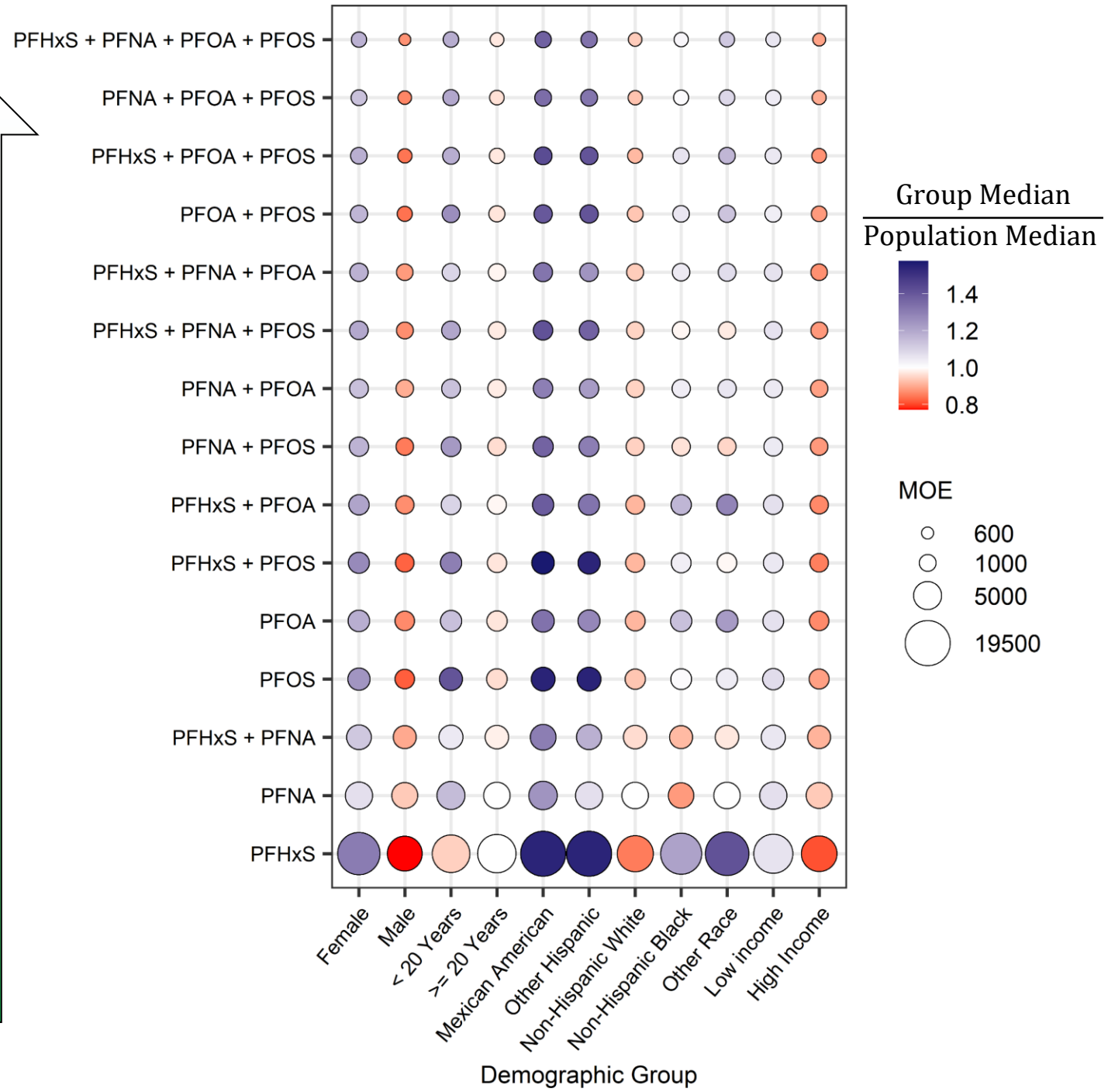


PFOA alone versus PFOA in combination moves the median population MOE from 1800 to 900

MOE Analysis

- MOE's derived considering the bioactivity ACC for one endpoint, peroxisome proliferator-activated receptor-α (ATG_PPARa_TRANS)
- **Red Circles** may indicate where population median MOE may not be protective for that group
- *Note: Demographic groups overlap subjects*

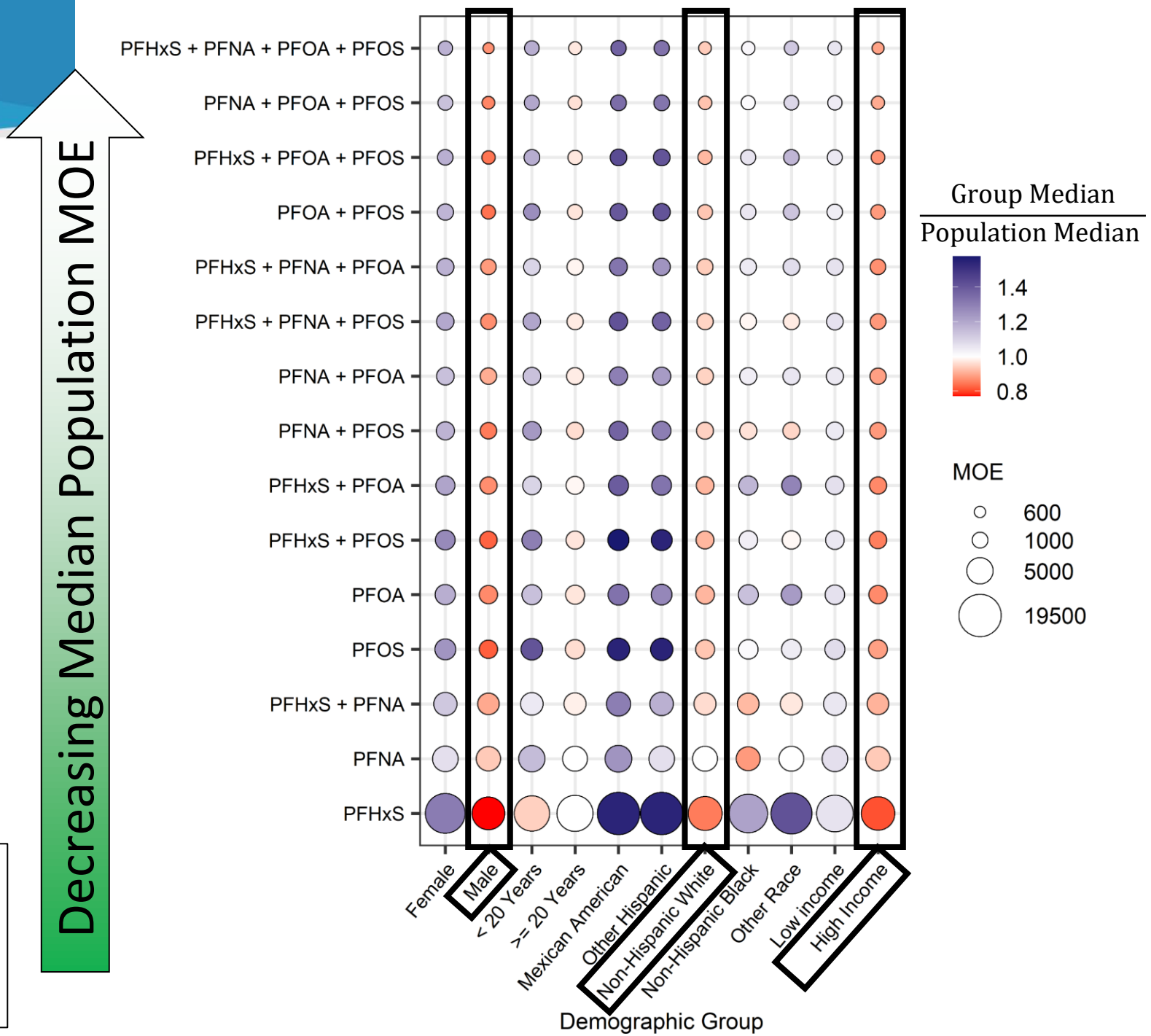
Decreasing Median Population MOE



Legend
Circle Size: Median MOE
Color**:** Ratio of demographic group median to population median

Demographic Trends

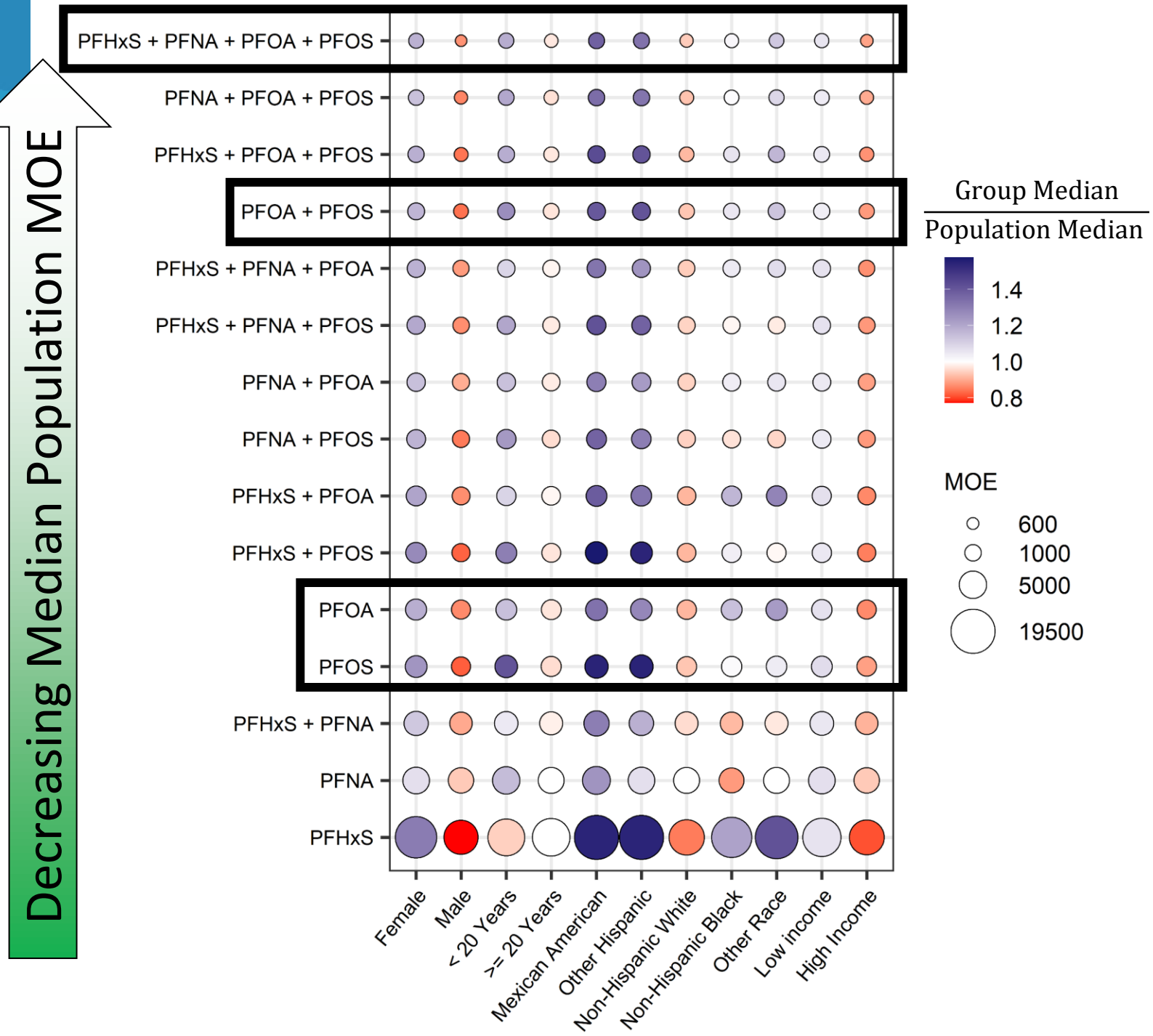
- MOEs derived considering the bioactivity ACC for one endpoint, peroxisome proliferator-activated receptor-α (ATG_PPARa_TRANS)
- **Red Circles** may indicate where population median MOE may not be protective for that group
- *Note: Demographic groups overlap subjects*



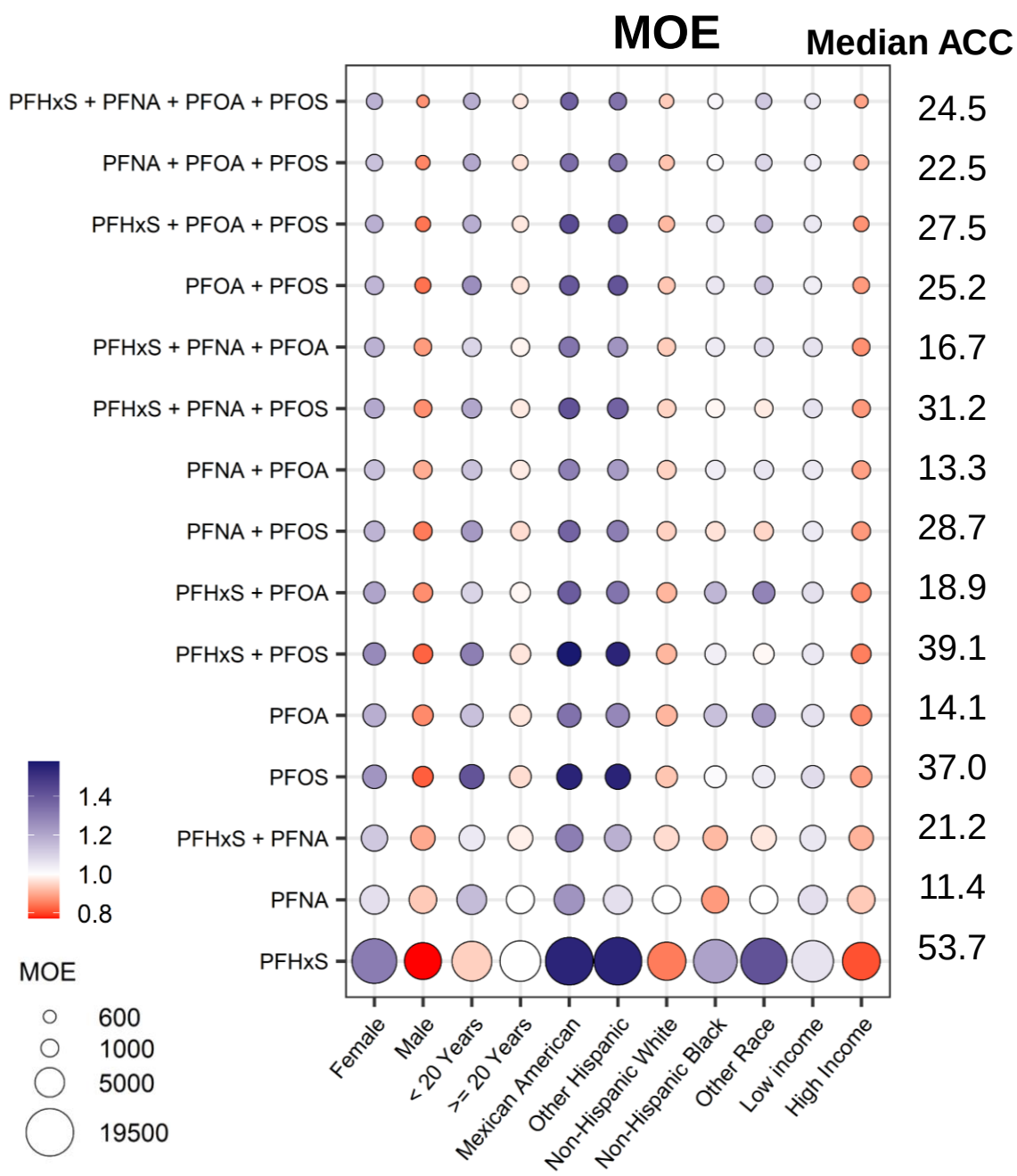
Legend
Circle Size: Median MOE
Color: Ratio of demographic group median to population median

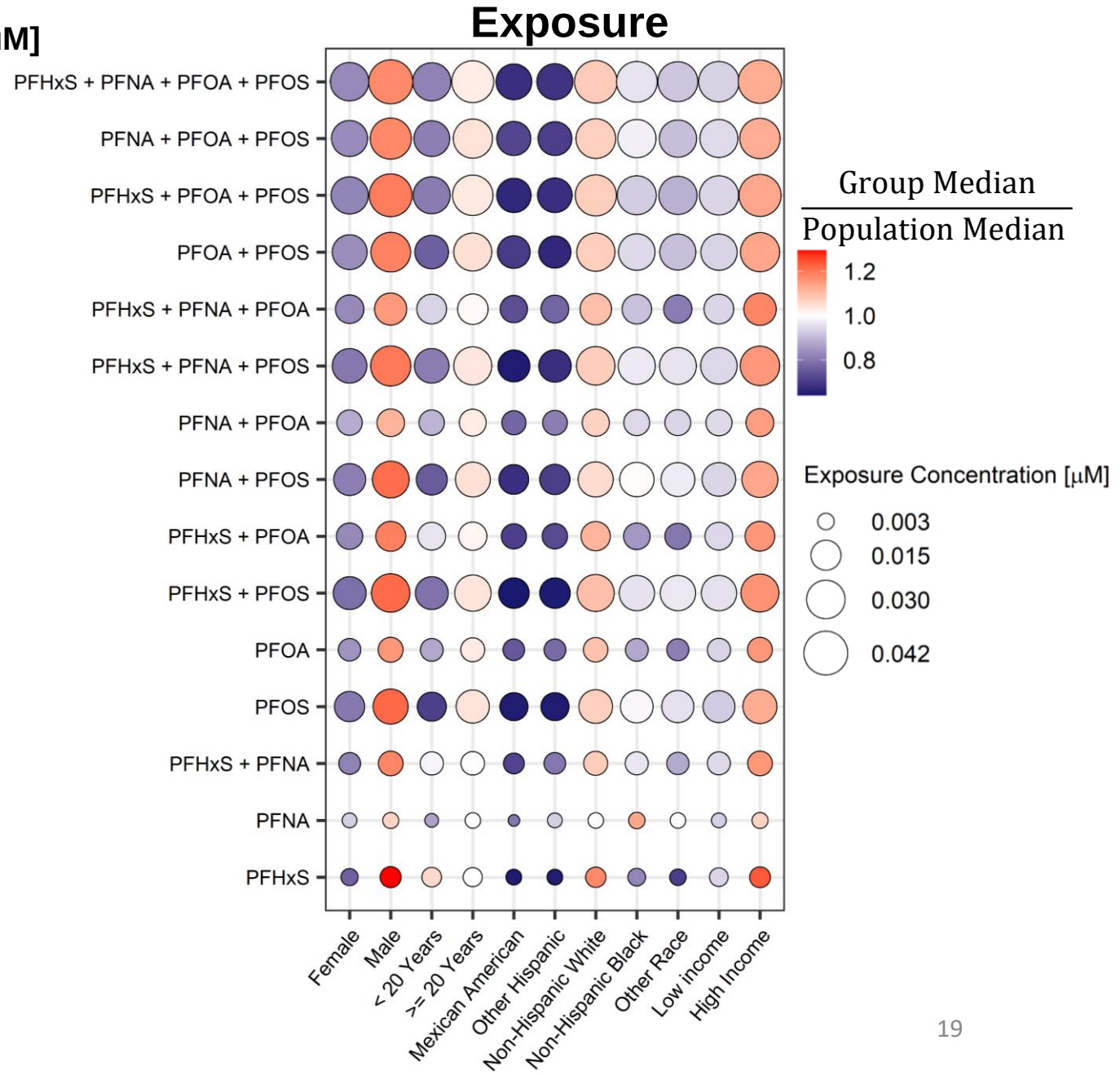
Chemical Groups

- PFOA and PFOS have lower MOE values
- Beyond single chemicals, the MOE values are similar
- Mixtures with both PFOA and PFOS have lowest MOE values, with the combination of all 4 chemicals being the lowest



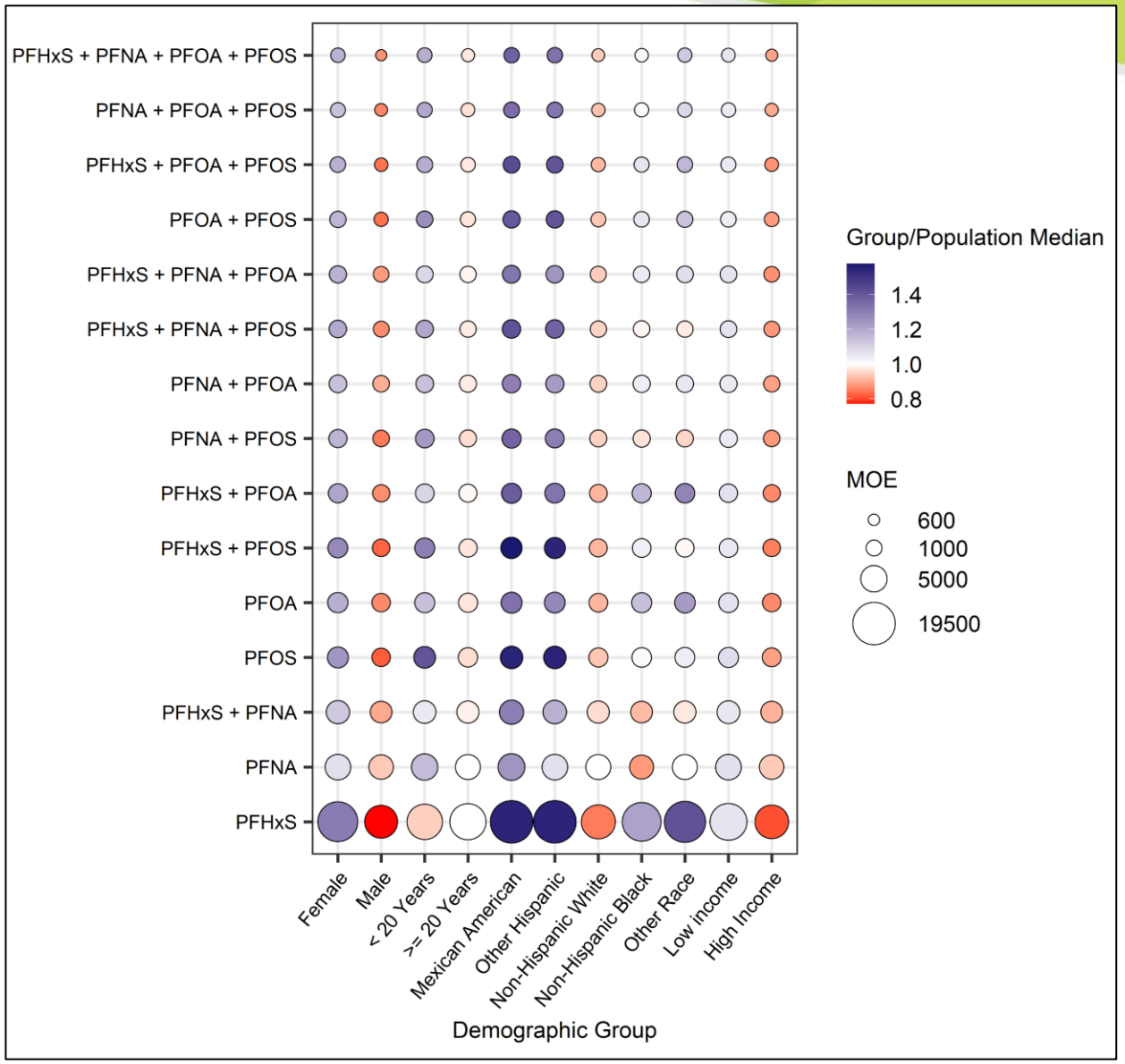
Trends appear to be related to exposure levels, not the potencies of the chemical components





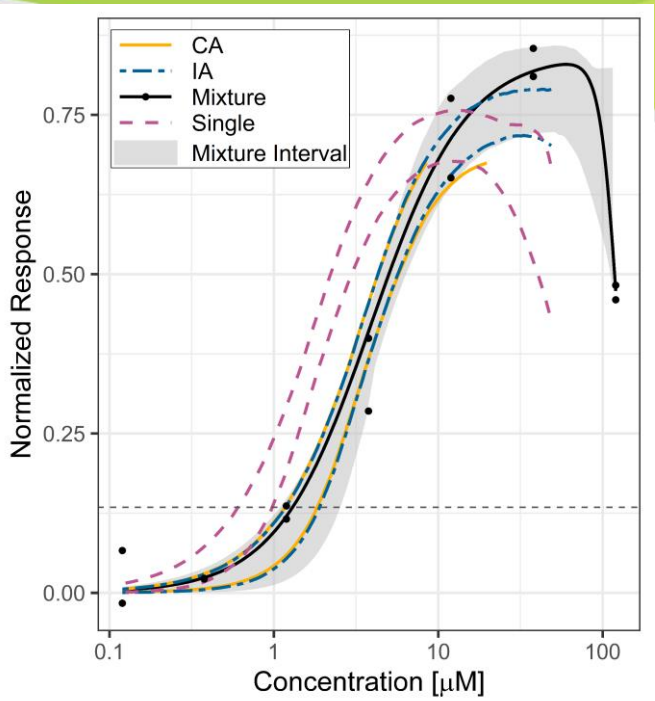
Part 2 Summary

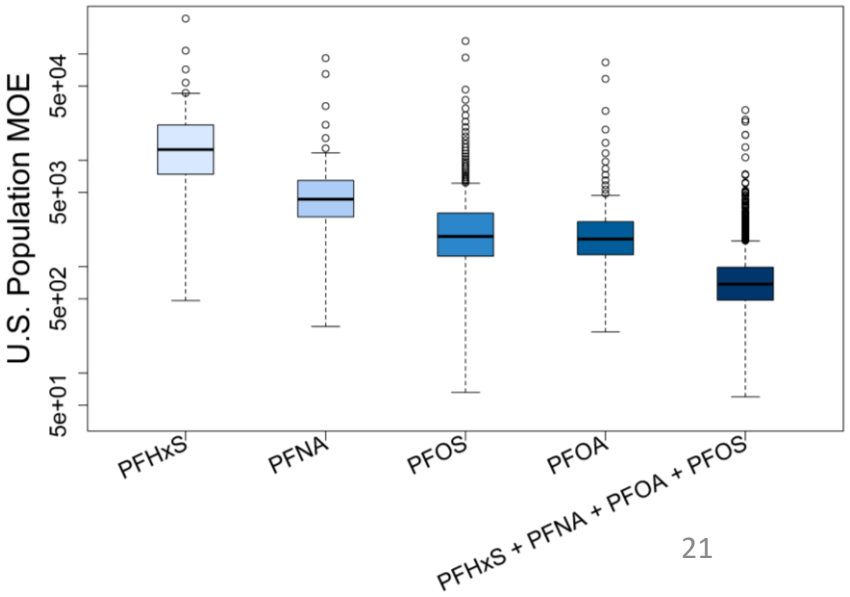
- Examined the median margin of exposures (MOE) of 4 PFAS chemicals and mixture combinations, as detected in CDC 2009-2010 NHANES blood serum samples
  - Considered the predicted mixture's bioactivity ACC to calculate median MOE for U.S. population, comparing different demographic subsets
- Differences in MOE across demographic groups appear to be driven by differences in exposure levels
- More information needed to make conclusive observations about exposure patterns



Conclusions

- Demonstrated ability to determine mixture bioactivity from additive mathematical mixture models for an experimental set of binary chemical mixtures in ToxCast
  - Able to make conservative ACC predictions
  - Represents progress towards predictive analysis of chemical mixtures
- Similar methods can be applied to other chemical mixture combinations
  - Requires components to have been tested in ToxCast and knowledge of mixture concentration ratios
- Case study of simulated mixtures to compute margins of exposure highlights possible future applications for mixtures informed by relevant co-exposures
  - Additional investigation of exposure sources may be informative





Thank You

- Mentors: Katie Paul Friedman and Kristin Isaacs
- Collaborators: Madison Feshuk and Zachary Stanfield
- Thank you to the ToxCast team
- This project was supported in part by an appointment to the Research Participation Program at the Office of Research and Development, U.S. Environmental Protection Agency, administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy and EPA.