Dealing with PFAS Mixtures: Approaches to Predicting Joint Effects of PFAS Mixtures on Molecular Initiating Events

Greylin Nielsen, MPH
nielseng@bu.edu
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Research Team:
Jennifer Schlezinger, PhD
Thomas Webster, PhD
Wendy Heiger-Bernays, PhD

BOSTON UNIVERSITY

Boston University School of Public Health

Acknowledgements

Advisor

- Dr. Jennifer Schlezinger

BU Collaborators

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- Dr. Wendy Heiger-Bernays
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Per- and polyfluoroalkyl substance (PFAS) exposure is a mixtures problem

Humans are <u>exposed to mixtures</u> of PFAS through drinking water, food, air, household dust, soil, and consumer, personal care products, and more

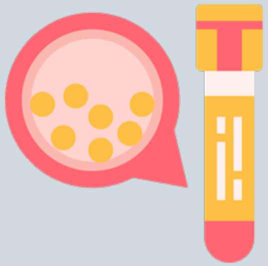
Multiple PFAS are found in humans:

PFOS, PFOA, PFHxS, and PFNA are consistently measured in more than 90% of the U.S. population

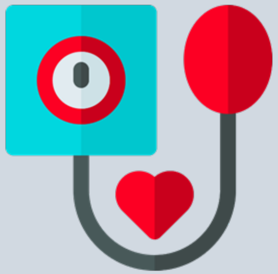
Different PFAS have similar health effects


Impaired immune response

PFAS


Increased cholesterol


Low birth weight


Preeclampsia


Thyroid disease

PFAS guidelines, advisories, and regulations are based on health risk

Drinking Water Standards and Guidance Values are Based on Reference Doses

Health Effects Research

Reference Dose = Point of Departure / Uncertainty Factors

EPA, 1993

Adverse Outcome Pathway

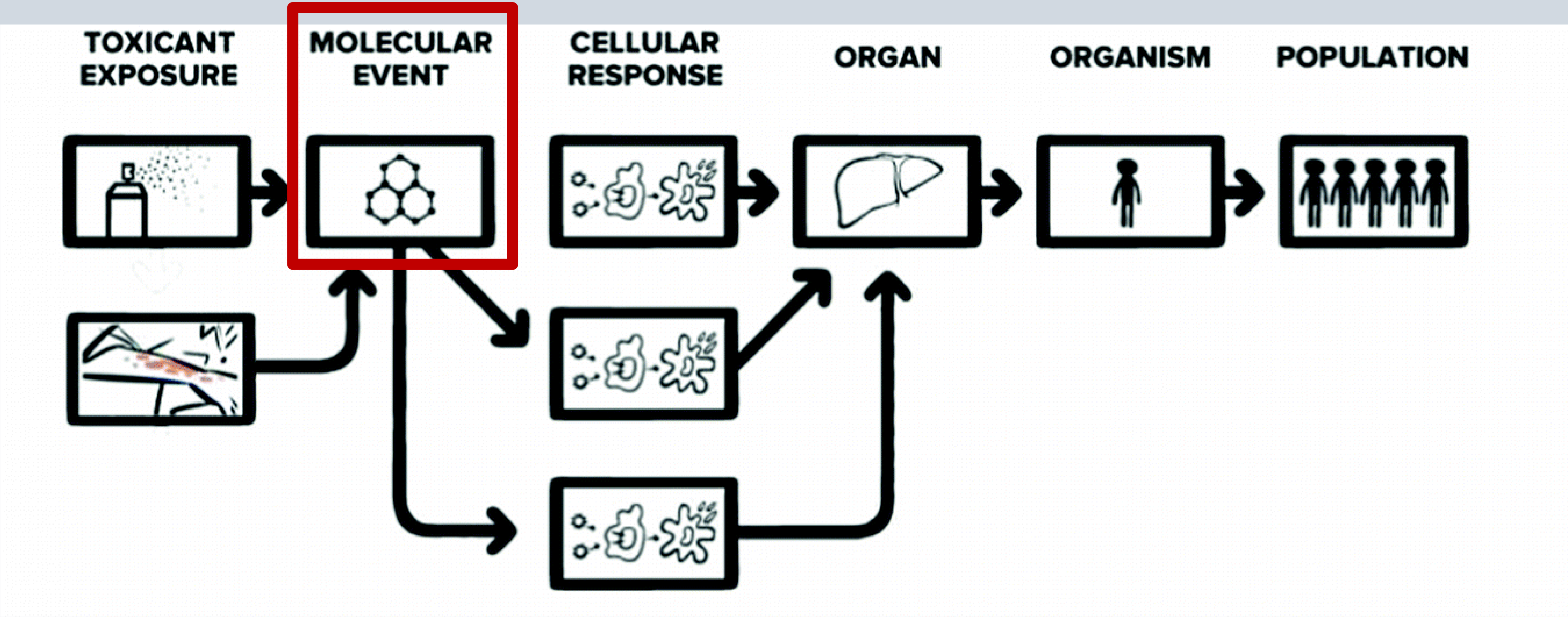
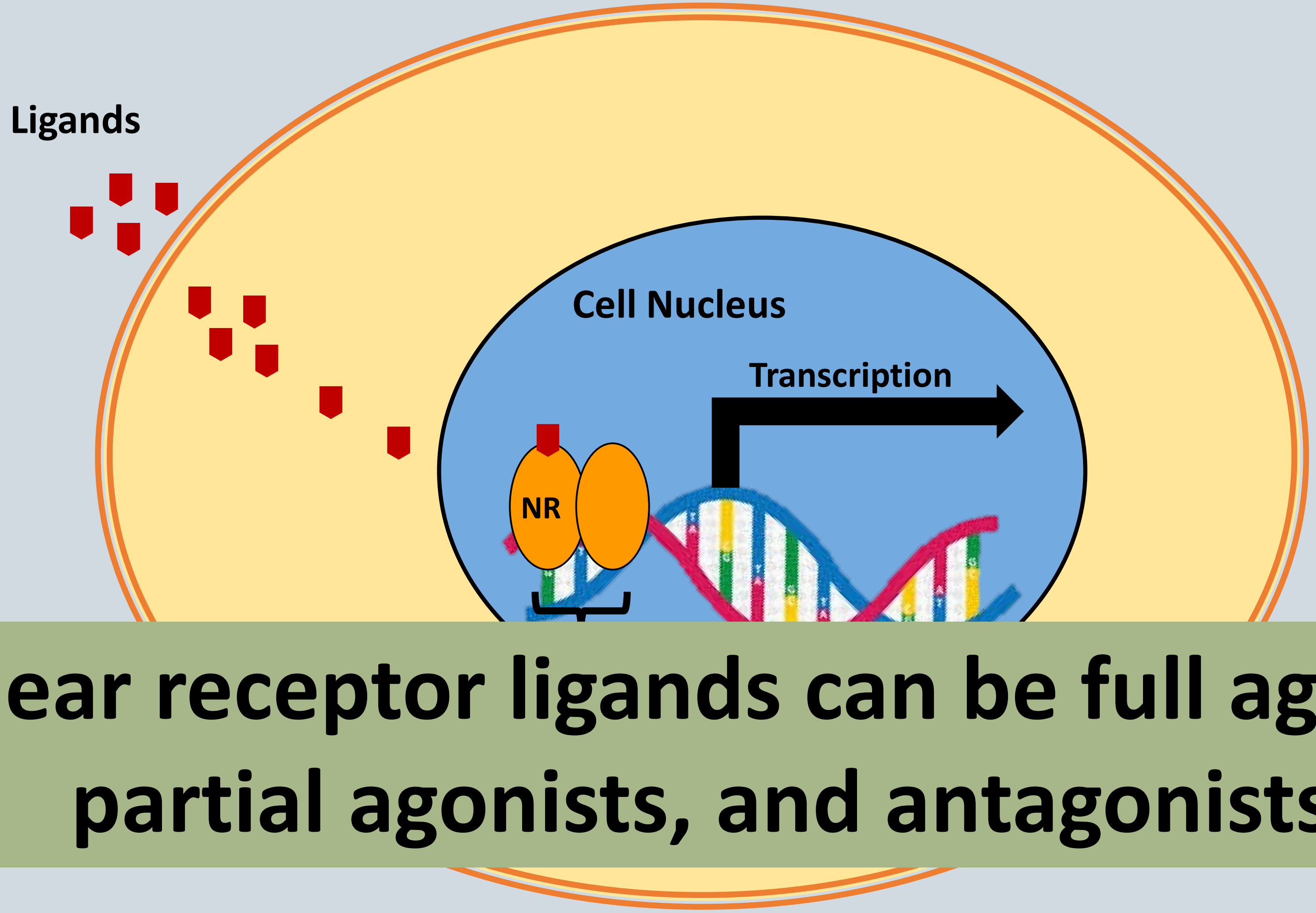


Diagram from “Pathways to a Better Future” video series, © The Human Toxicology Project Consortium

Nuclear receptor activation is an important molecular initiating event for PFAS

Nuclear receptors = proteins in cells that **recognize** and **respond** to molecules in the body (like hormones), therapeutic drugs, and environmental chemicals

Nuclear receptor activation is an important molecular initiating event for PFAS



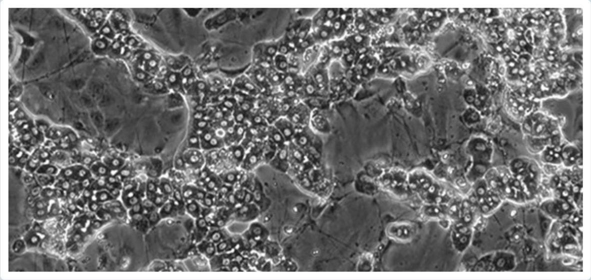
Nuclear receptor ligands can be full agonists, partial agonists, and antagonists

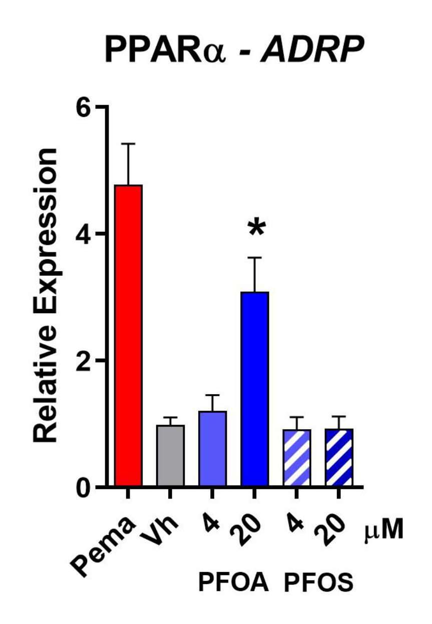
PFAS Engage <u>Multiple Nuclear Receptor Pathways</u>

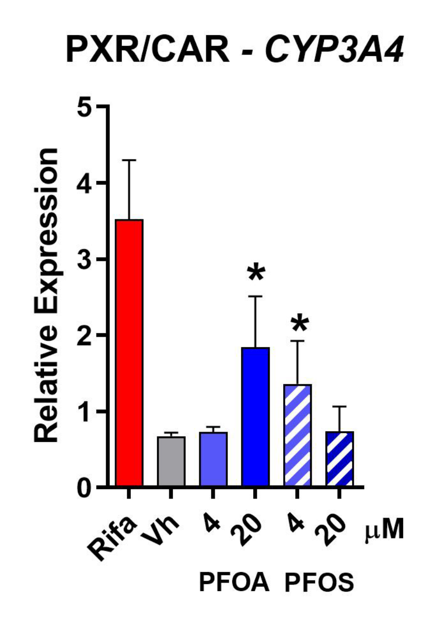
PFOA and PFOS upregulated target gene expression of:

- **Peroxisome Proliferator-Activated Receptor α (PPARα)**
- **Pregnane X Receptor (PXR)**
- **Constitutive androstane receptor (CAR)**
- **Liver X receptor**
- **Farnesoid X receptor**
- Receptor affinity studies have shown that PFOA binds to human **ERα**

Human Liver Cell Model





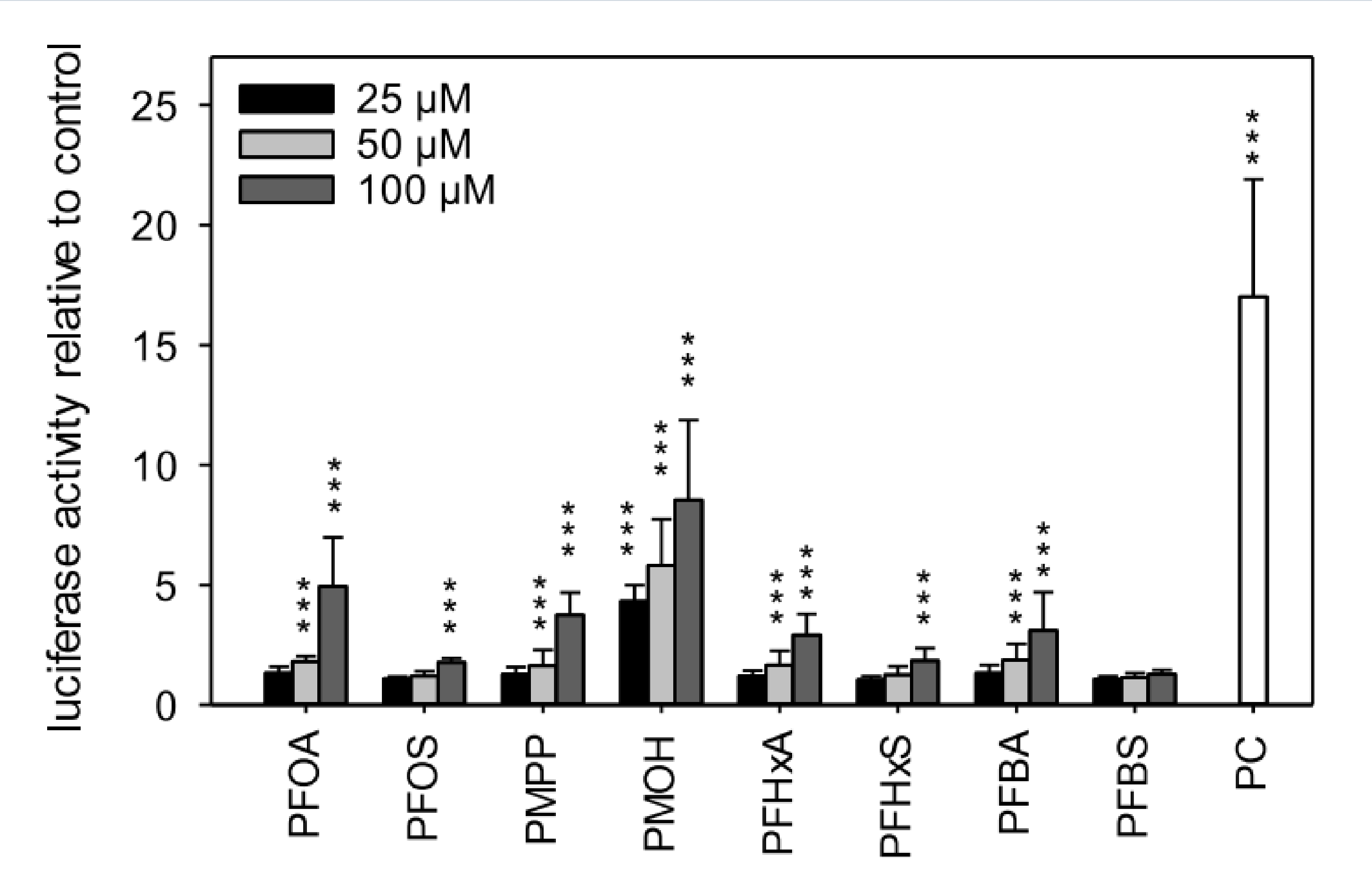


PFAS Engage Multiple Nuclear Receptor Pathways

PPARα accounts 80-90% of PFAS regulated genes in WT mice[1] but only ~55-60% in mice expressing human PPARα

1. PMIDs: 18281256, 28558994

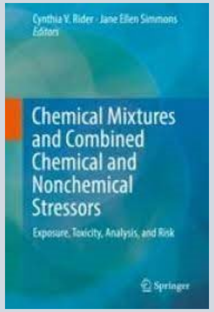
Multiple PFAS Activate PPARα



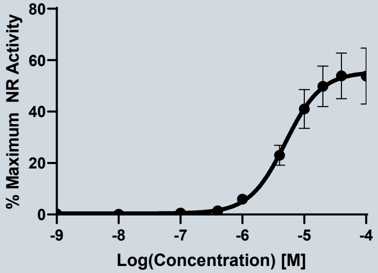
Behr et al., 2020 PMID: 31676336

1. Can we model the effects of multiple PFAS on a single molecular initiating event (PPARα activity)?

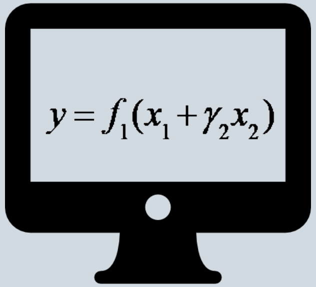
Can we model the effects of multiple PFAS on PPARα activity?



1. Define null hypothesis mixtures models



2. Generate data on individual PFAS and PFAS mixtures



3. Compare activity predicted by models to empirical PFAS mixtures activity

Models of Additivity

Concentration/Dose Addition

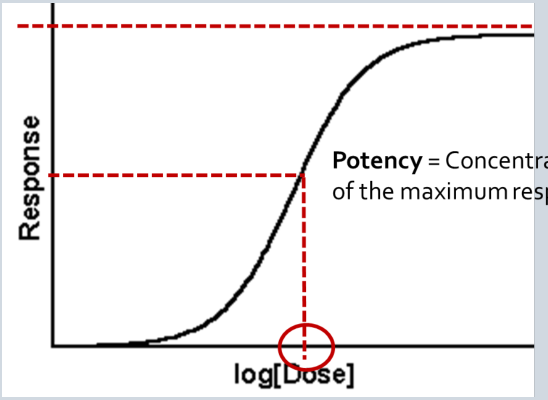
Applied to chemicals with "similar" mechanisms of action

Relative Potency Factor (RPF)

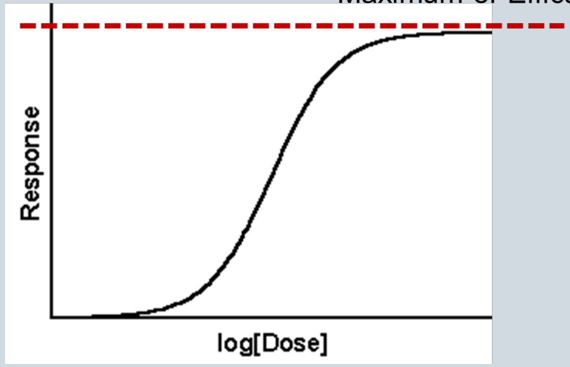
Sums: doses as dilutions of a reference compound

Assumes: equal efficacy

$$y = f_1(x_1 + \gamma_2 x_2)$$



Maximum or Efficacy



Models of Additivity

Concentration/Dose Addition
Applied to chemicals with "similar" mechanisms of action

Relative Potency Factor (RPF)

Sums: doses as dilutions of a reference compound
Assumes: equal efficacy

$$y = f_1(x_1 + \gamma_2 x_2)$$

Generalized Concentration Addition (GCA)

Sums: doses
Assumes: equal or unequal efficacy

$$y = \frac{\alpha_1 \frac{x_1}{K_1} + \alpha_2 \frac{x_2}{K_2}}{1 + \frac{x_1}{K_1} + \frac{x_2}{K_2}}$$

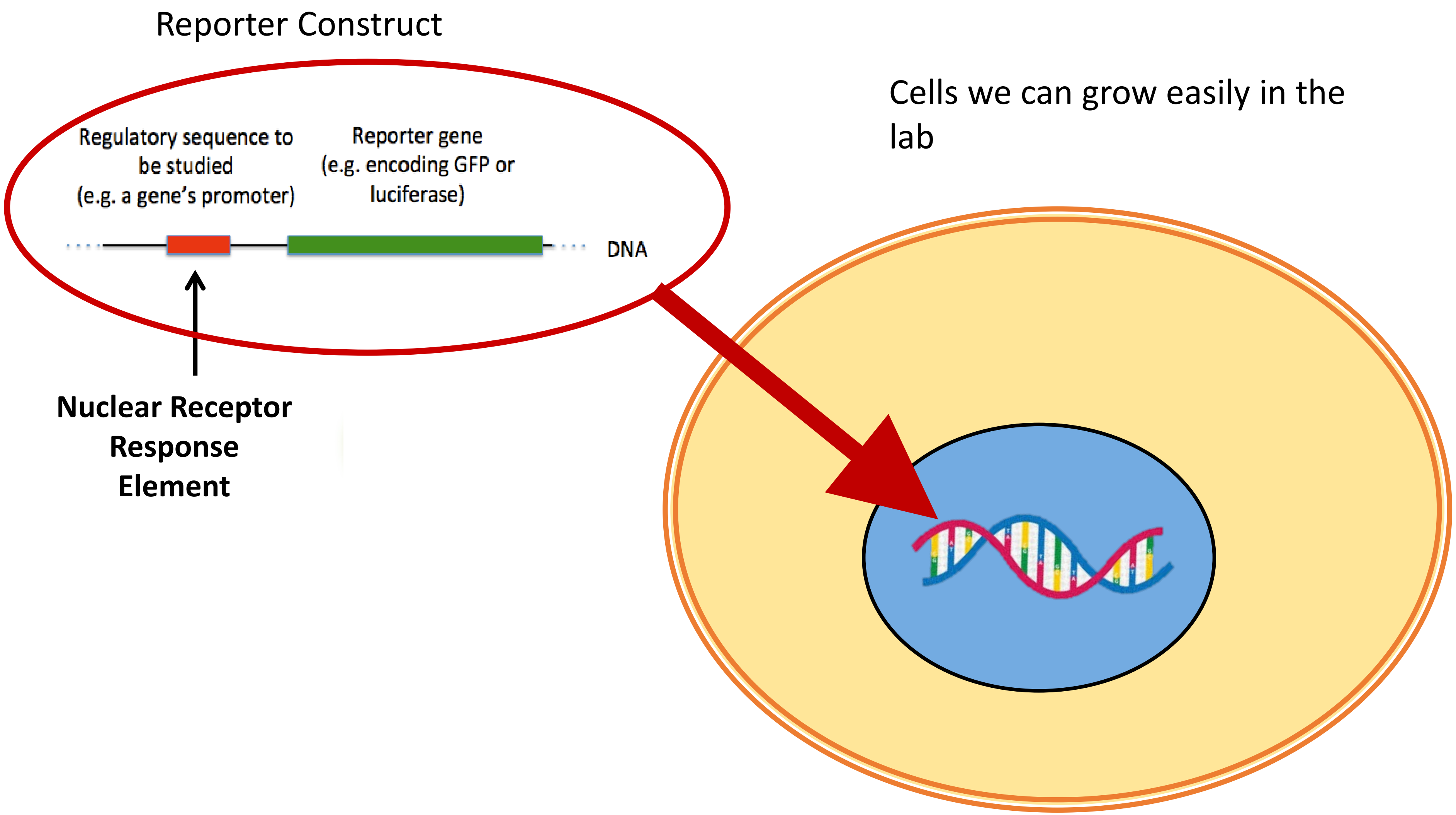
Response Addition
Applied to chemicals with "dissimilar" mechanisms of action

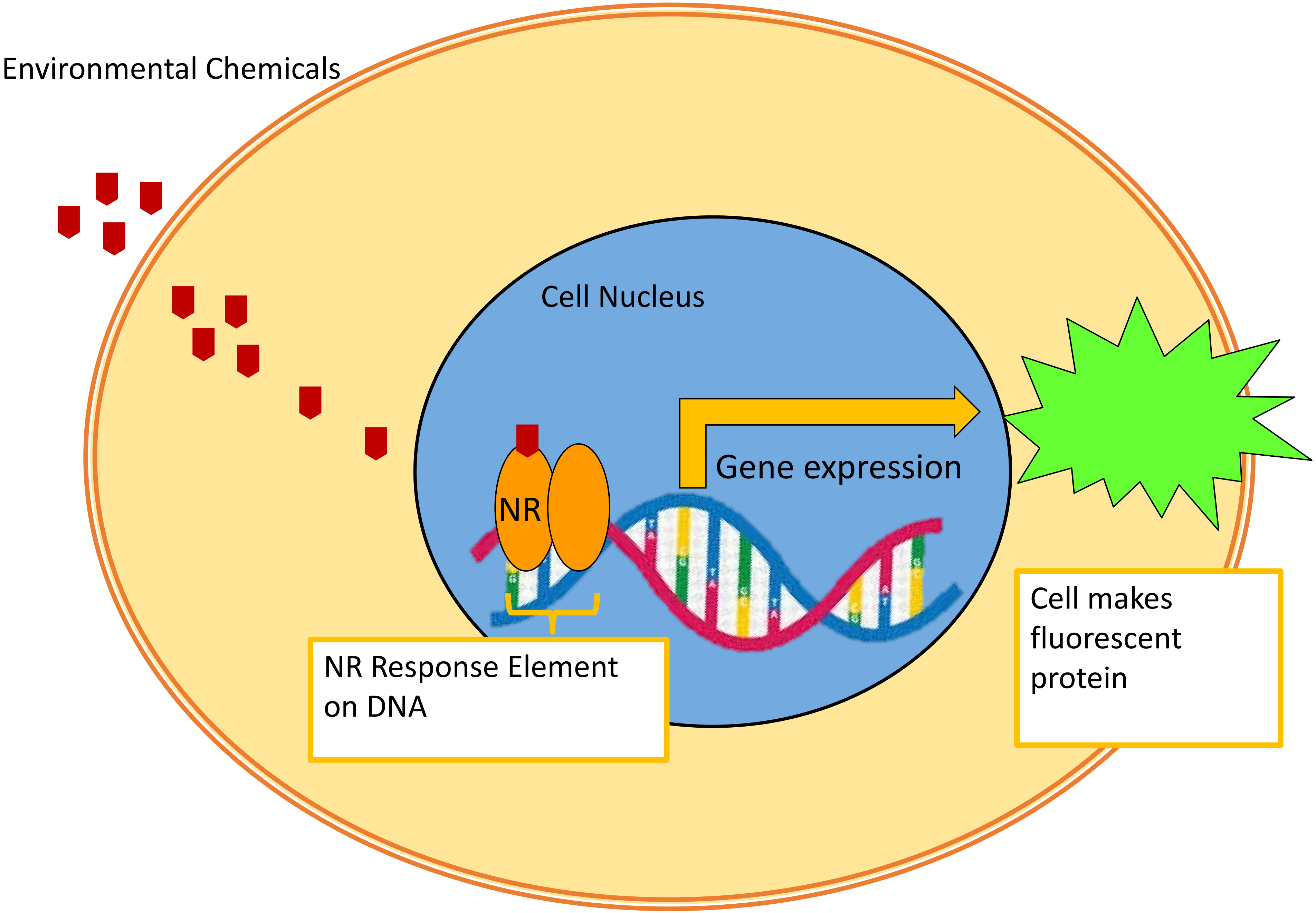
Effect Summation (ES)

Sums: Effect levels
Assumes: Linear dose response curves

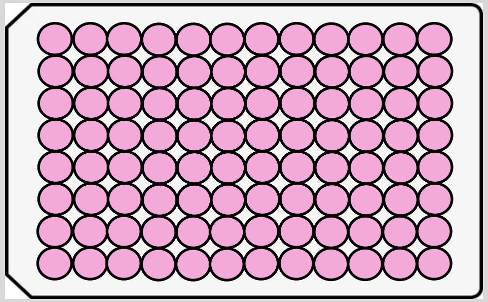
$$y = f_1(x_1) + f_2(x_2)$$

How do we study nuclear receptor activity?

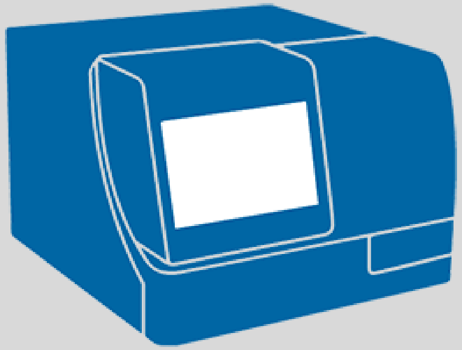


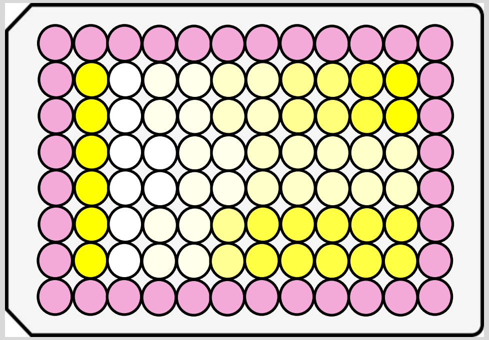

Environmental Chemicals
Cell Nucleus
Gene expression
NR
NR Response Element on DNA
Cell makes fluorescent protein

Treat cells with chemicals or chemical mixtures

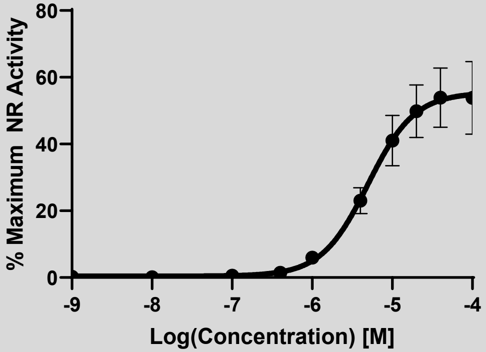


Measure light intensity





Analyze Results



Data Analysis

I. Fit individual dose response curves normalized to PC (GW7647)

II. Extract **potency** and **efficacy** for modeling

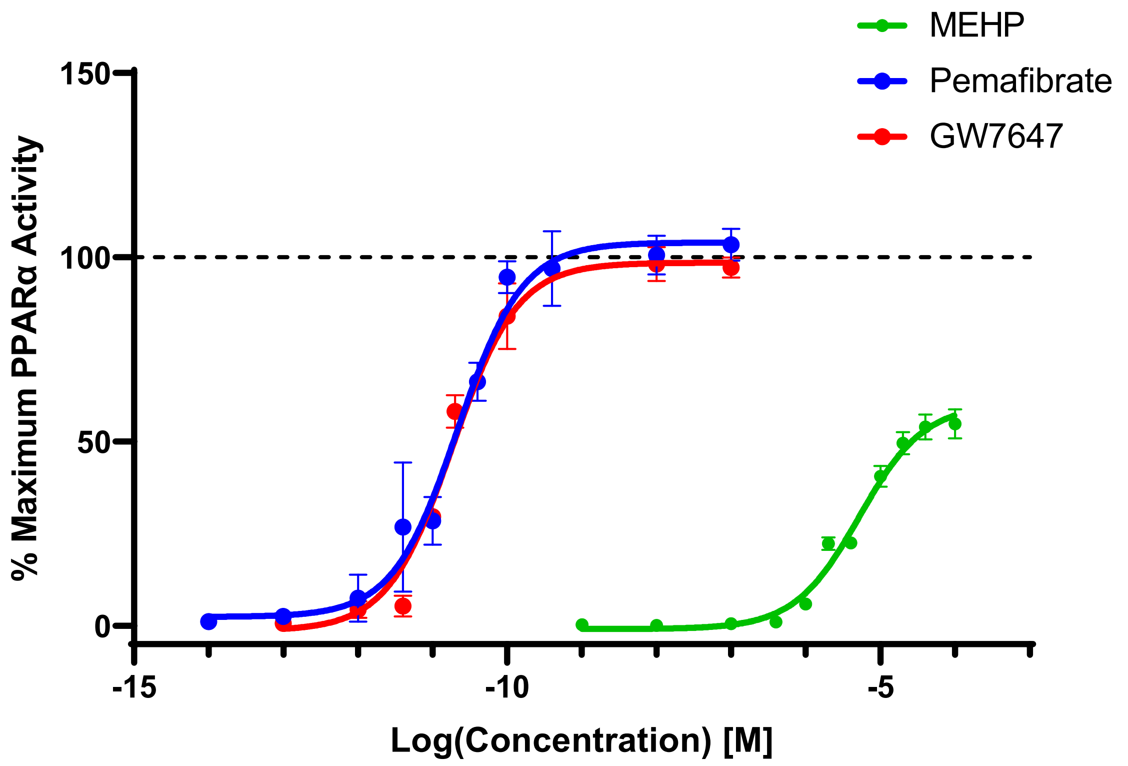
III. Create and test binary and complex mixtures with known concentrations of each component

IV. Employ individual dose-response data to predict mixture activity with different models of additivity

V. Statistically compare predicted activity to experimental activity

Ligand	Ligand Type	Potency (EC_{50}) M	Efficacy (% Max. Activity)
GW7647	**Full Agonist**	1.8×10^{-11}	99
Pemafibrate	**Full Agonist**	2.2×10^{-11}	104
MEHP	**Partial Agonist**	5.2×10^{-6}	60.1
GW6471	**Antagonist**	$^*7.3 \times 10^{-9}$	0

Only Generalized Concentration Addition (GCA) predicts mixture effects for all different ligand types



*equilibrium dissociation constant

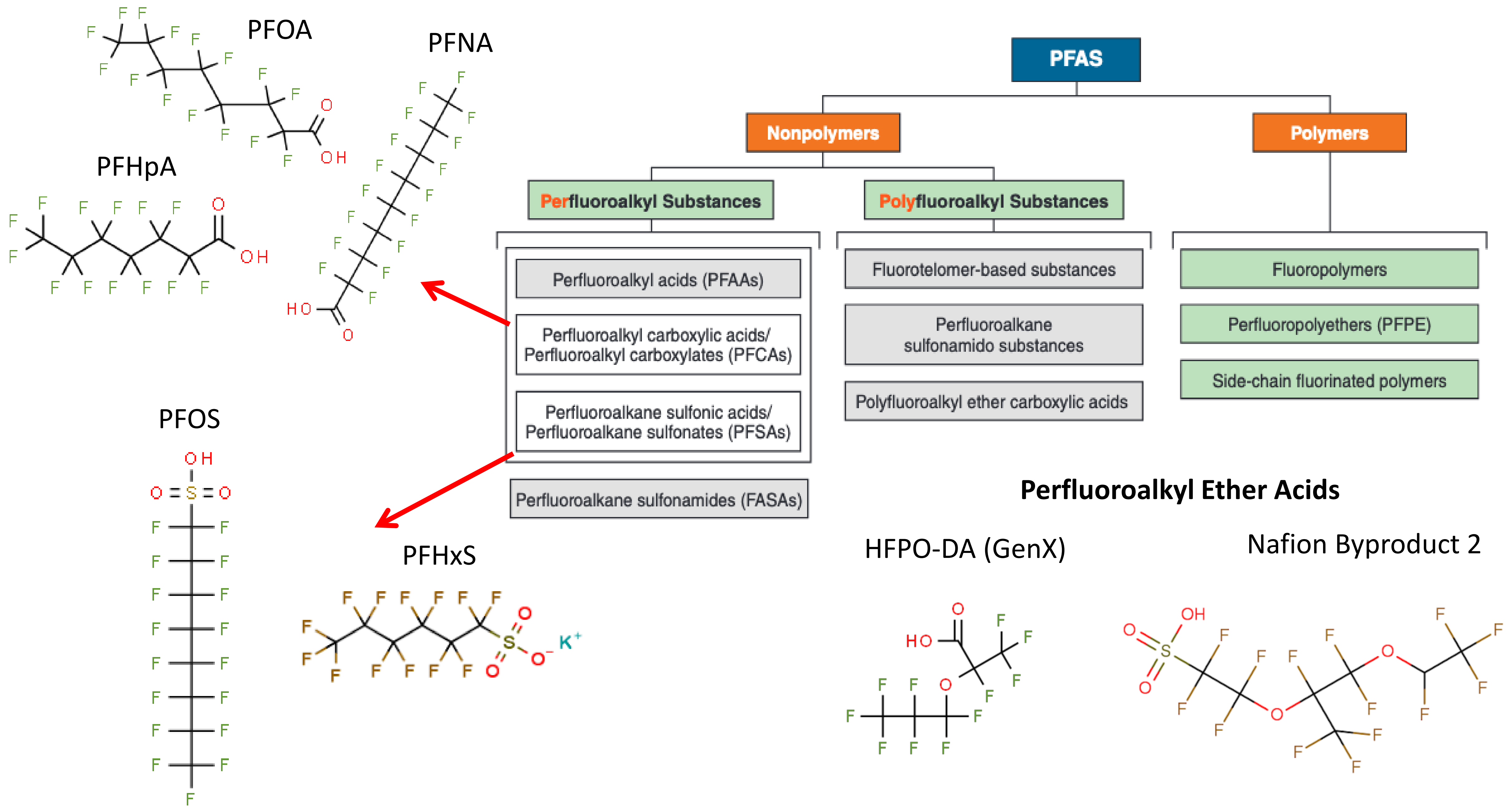
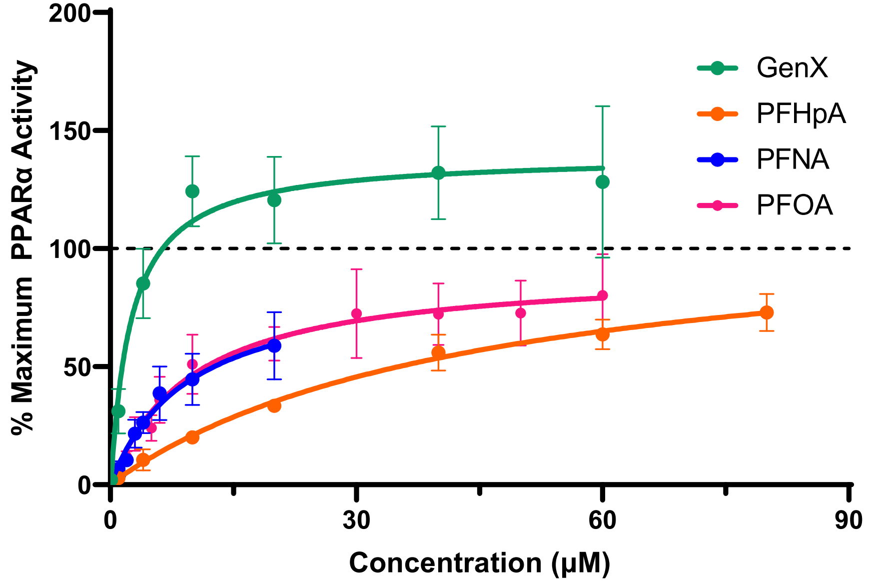


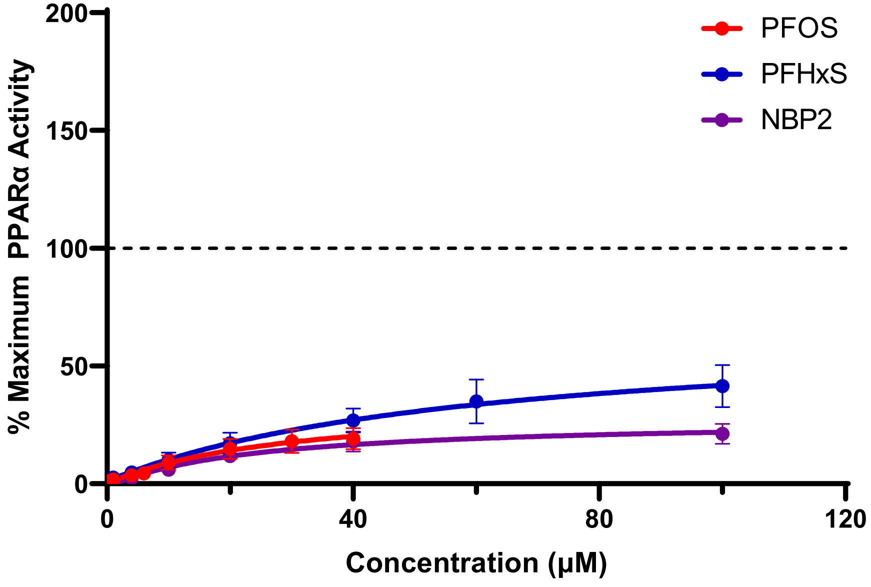
Image from: https://pfas-1.itrcweb.org/fact_sheets_page/PFAS_Fact_Sheet_Naming_Conventions_April2020.pdf (Thanks to Jamie DeWitt)

PFCAs more efficaciously activate PPARα

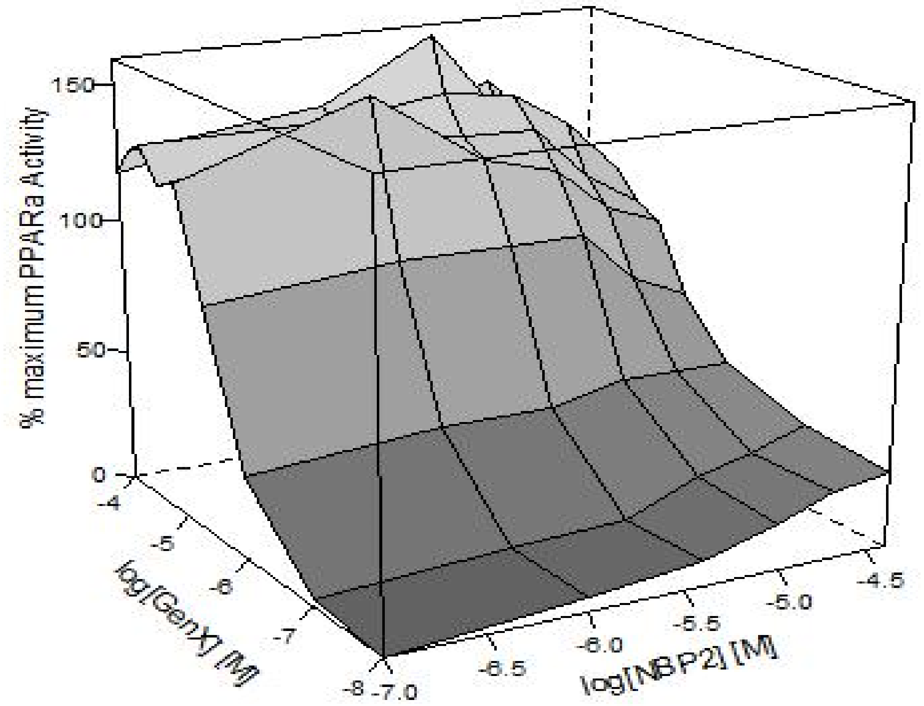
Perfluorinated Carboxylic Acids



Perfluorinated Sulfonic Acids

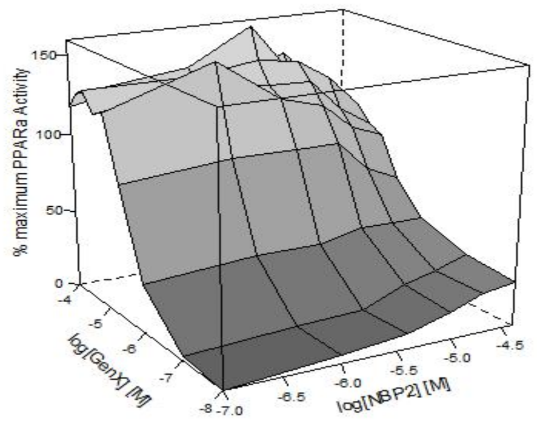


GCA predicts the effects of binary PFAS mixtures: GenX and NBP2

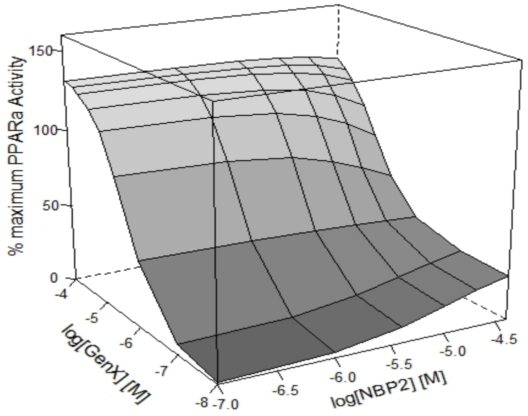


Empirical

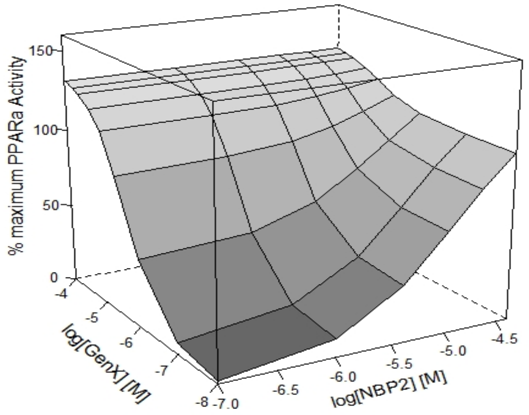
GCA predicts PPARα activation by binary PFAS mixtures: GenX and NBP2



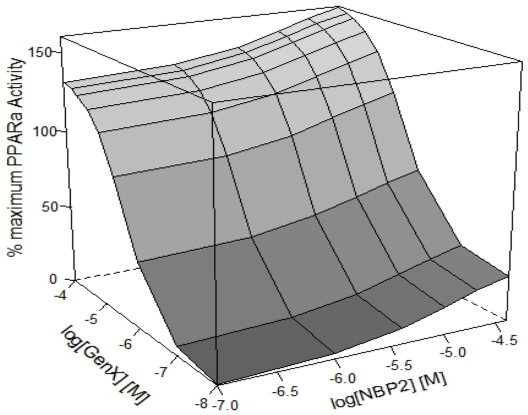
Empirical



GCA (RMSE = 10.9)

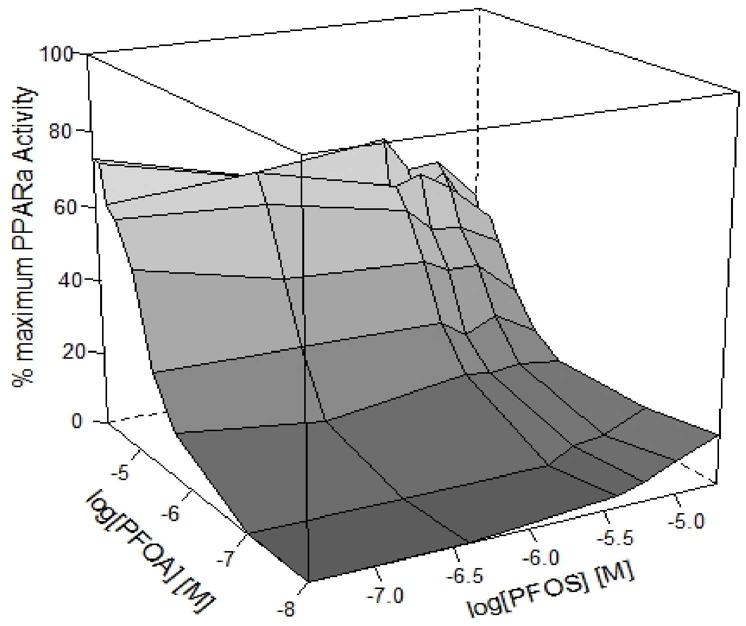


RPF (RMSE = 30.0)

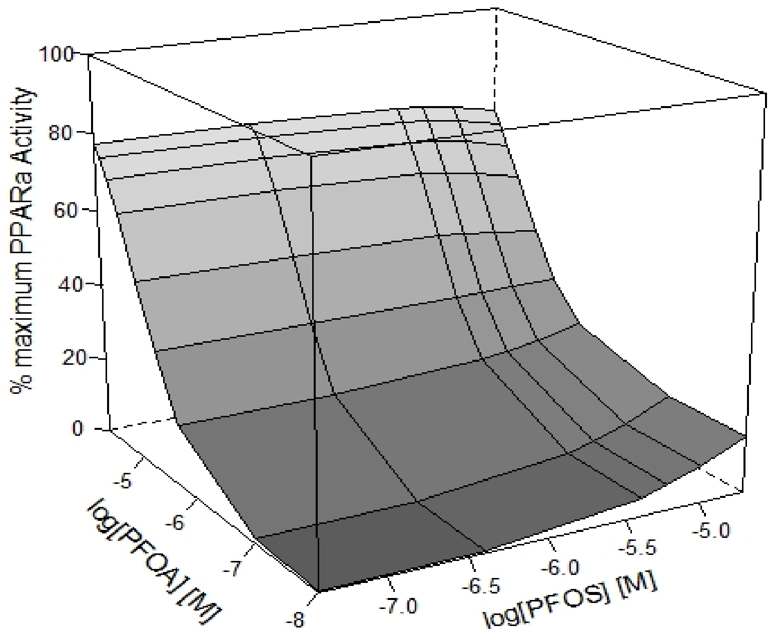


ES (RMSE = 25.7)

GCA predicts PPARα activation by binary PFAS mixtures: PFOA and PFOS



Empirical

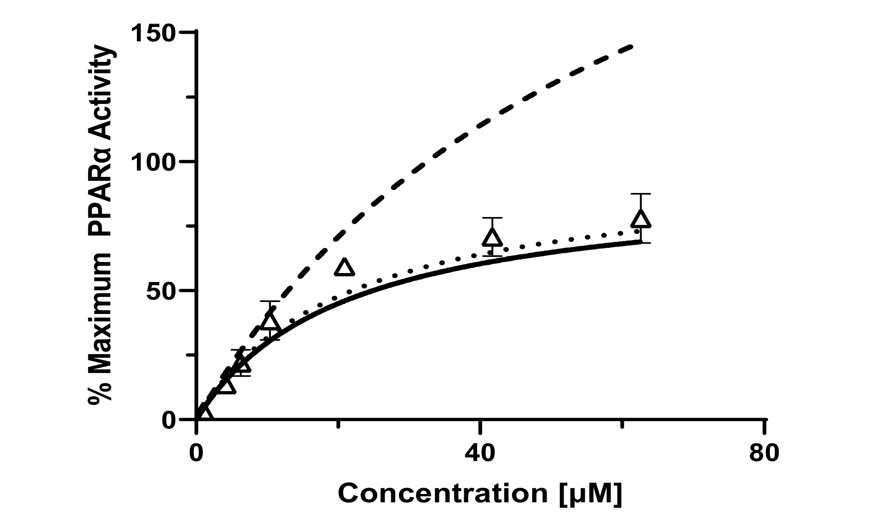


GCA (RMSE = 11.9)

RPF RMSE = 19.1, ES RMSE = 19.8

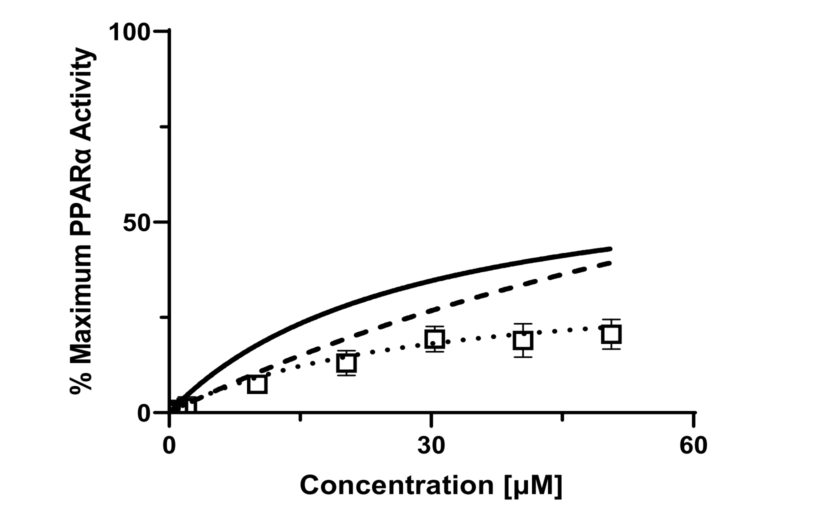
RPF and GCA Predict PPARα Activation by Mixtures of PFCAs
GCA Predicts PPARα Activation by Mixtures of PFSAs

PFOA*, PFHpA, PFNA
(equipotent mixture)



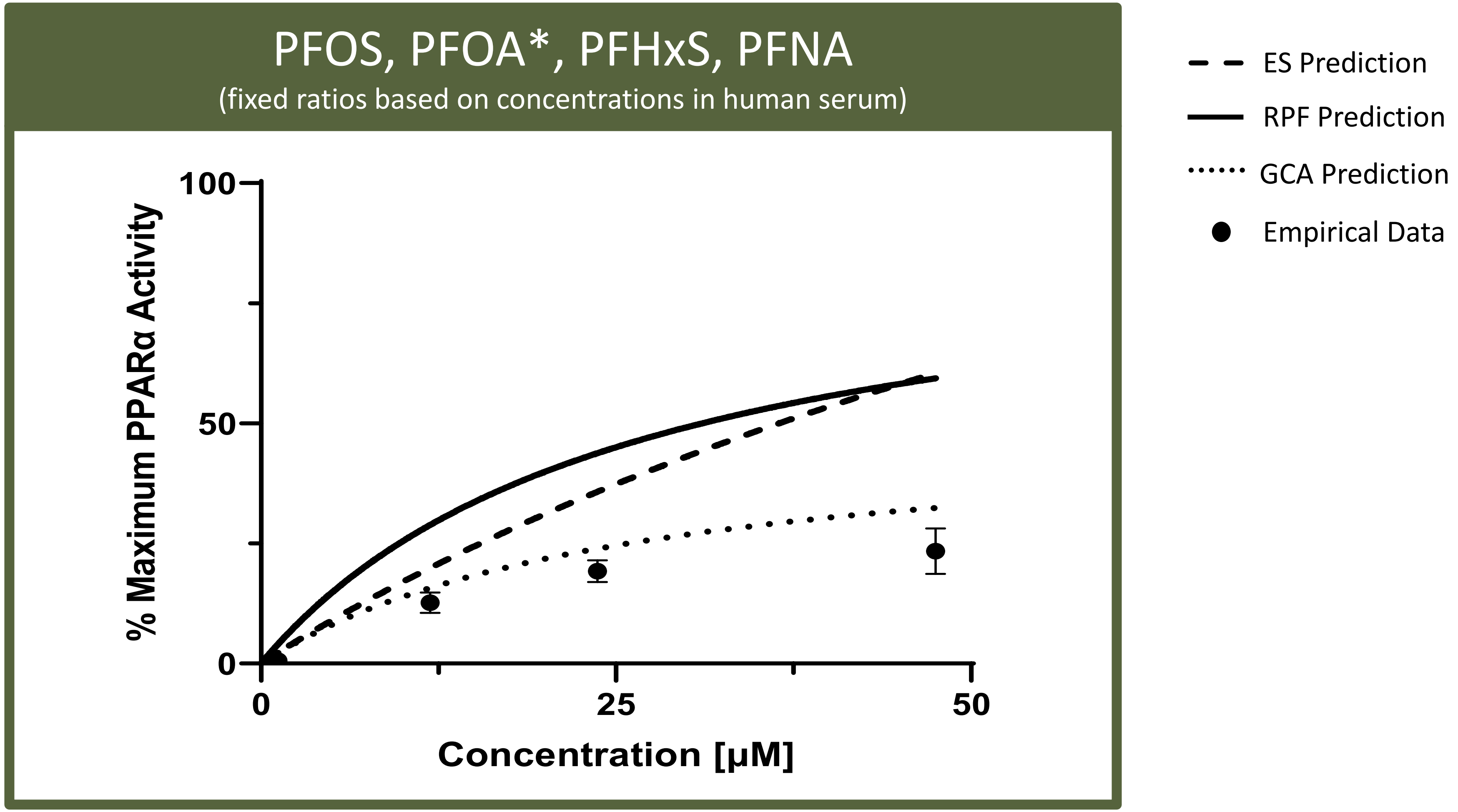
ES Prediction
RPF Prediction
GCA Prediction
Empirical PFCA Data
Empirical PFSA Data

PFHxS, PFOS*, NBP2
(equipotent mixture)



* Reference compound for RPF modeling

GCA Predicts PPARα Activation by Human-Relevant Mixtures



* Reference compound for RPF modeling

Conclusions

I. **Human relevant biological systems** provide insight into the interaction between environmental chemicals and key molecular initiating events

II. PFAS are human PPARα agonists that vary in potency and efficacy

III. Modeling approaches that incorporate both potency and efficacy provide the most accurate predictions of PPARα activity by diverse ligands

IV. Generalized Concentration Addition accurately predicts the effects of PFAS mixtures on human PPARα activity *in vitro*

1. We can model the effects of multiple PFAS on single molecular initiating event.
2. Can we use these modeling approaches to support regulatory efforts to group PFAS?

More about this project:

Follow up with any questions
Greylin Nielsen
nielseng@bu.edu

Thanks again to the team:

Dr. Wendy Heiger-Bernays

Dr. Jennifer Schlezinger

Dr. Tom Webster