Dealing With PFAS Mixtures: Modeling Approaches to Predict Mixture Effects on Molecular Initiating Events *In Vitro*

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The PFAS Mixtures Problem

- ~4,700 PFAS individual PFAS or mixtures with unique Chemical Abstract Service (CAS) numbers

https://www.defence.gov.au/environment/pfas/pfas.asp

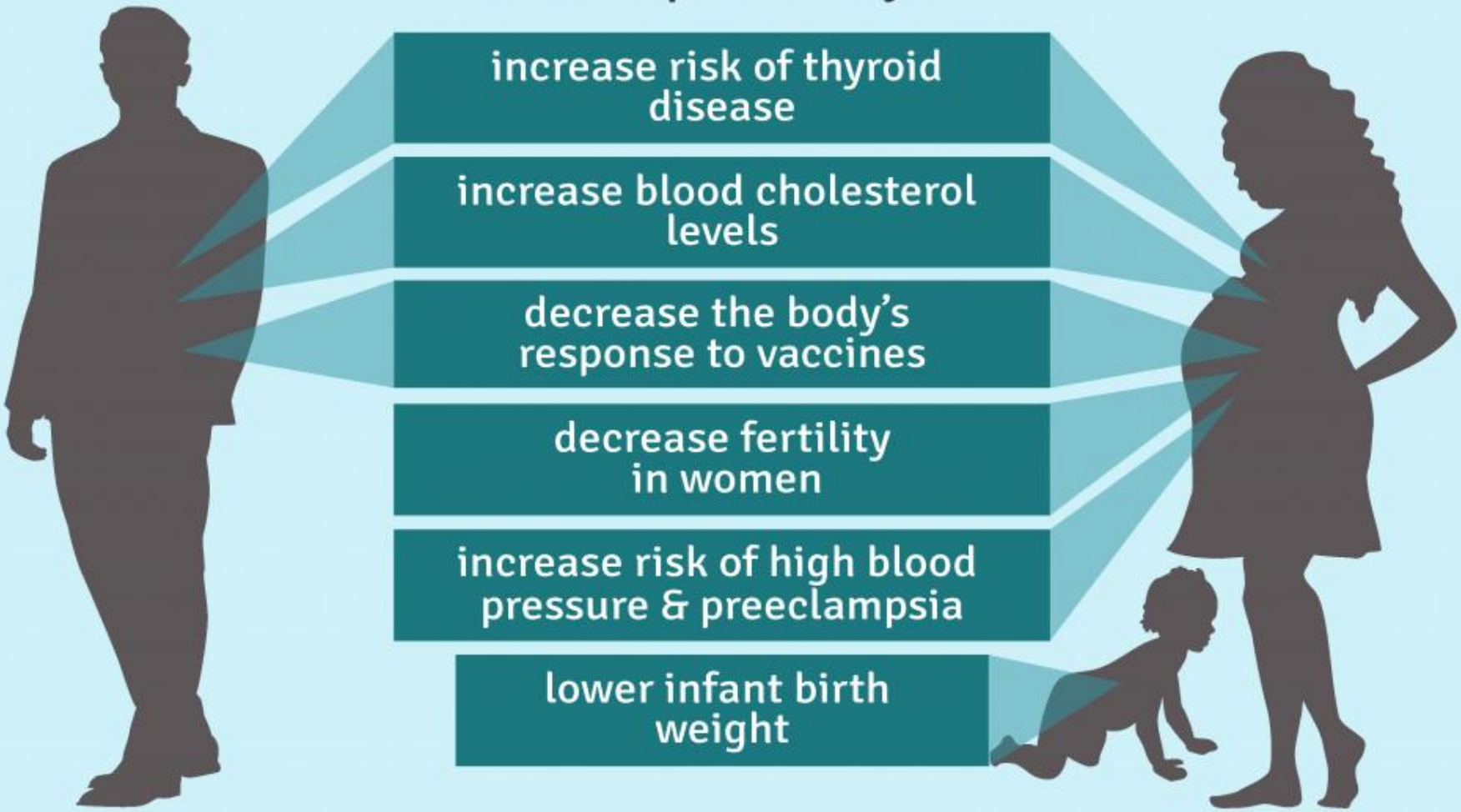
PFAS Exposure Occurs as Mixtures

- Humans are exposed to mixtures of PFAS through drinking water, food, air, household dust, soil, and consumer, and personal care products

- Multiple PFAS are found in human biological samples
  - PFOA, PFOS, PFHxS, and PFNA are consistently measured in more than 90% of the U.S. population

NORTH CAROLINA PFAS Testing Network

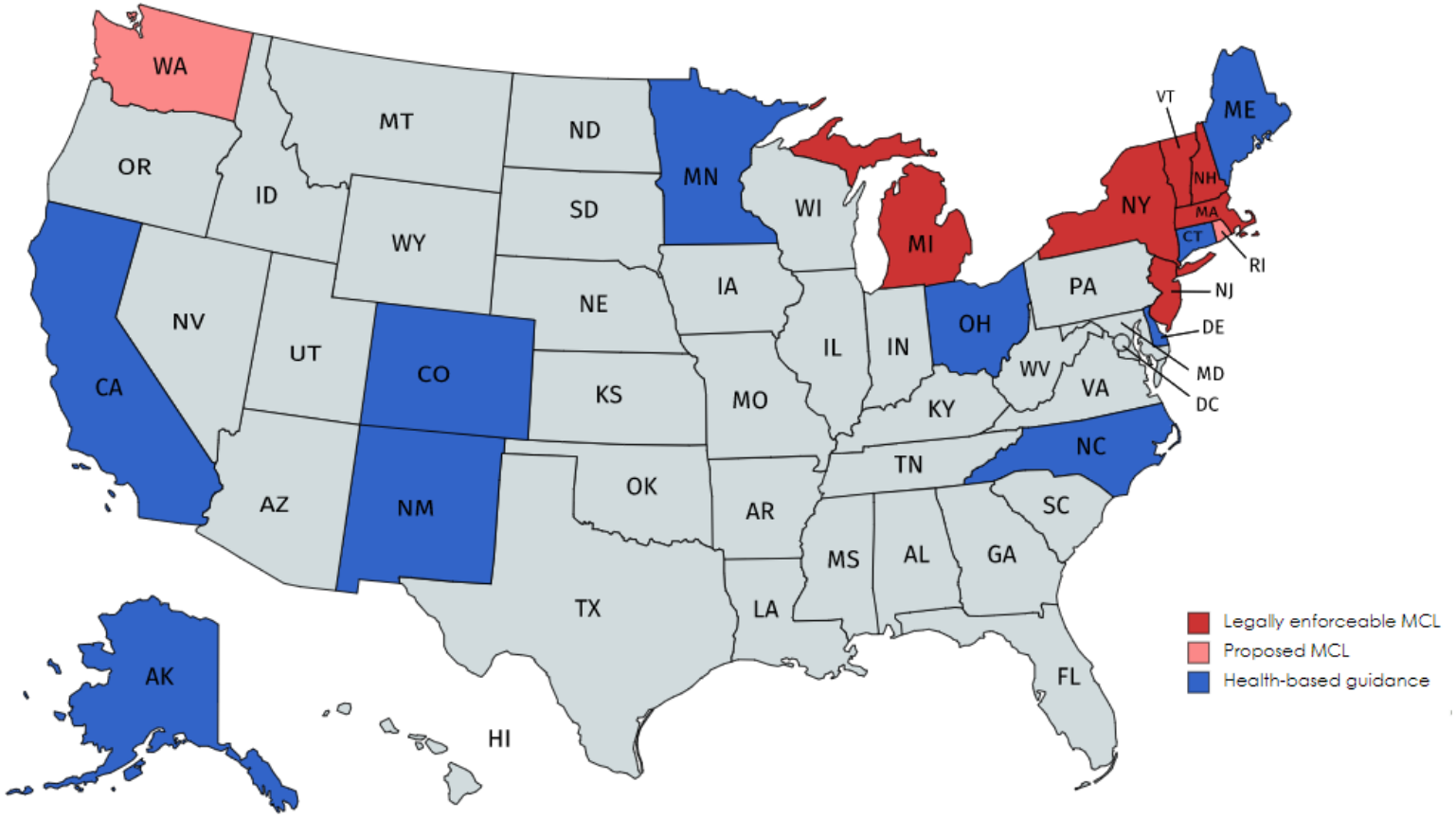
Human studies suggest PFAS exposure may...



Information sourced from Agency for Toxic Substances and Disease Registry | *Additional health effects have been reported and those highlighted demonstrate a range of potential effects.*

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

U.S. PFAS Drinking Water Guidelines and Regulations

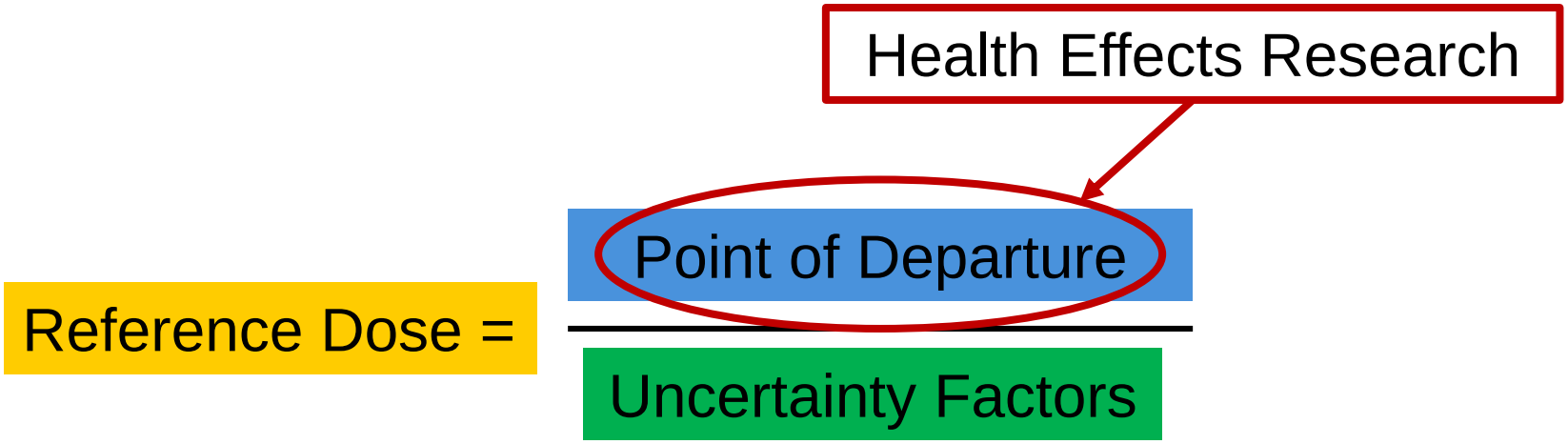


U.S. PFAS Drinking Water Guidelines and Regulations

	Long chain (ng/L)							Short chain (ng/L)			
	PFOA	PFOS	PFNA	PFHxS	PFHpA	PFDA	Total	PFBA	PFHxA	PFBS	GenX
No. of carbons	8	8	9	6	7	10		4	6	4	6
USEPA	70	70	—	—	—	—	Yes (2)[b]	—	—	—	—
CA	10	40	—	—	—	—	No[c]	—	—	—	—
CT	70	70	70	70	70	—	Yes (5)[b]	—	—	—	—
MA	20	20	20	20	20	20	Yes (6)[b]	—	—	2000	—
MI	8	16	6	51	—	—	No	—	400 000	420	370
MN	35	15	—	47	—	—	No	7000	—	2000	—
NH	12	15	11	18	—	—	No	—	—	—	—
NJ	14	13	13	—	—	—	No	—	—	—	—
NY	10	10		—	—	—	No	—	—	—	—
NC	—	—	—	—	—	—	—	—	—	—	140
OH	70	70	21	140	—	—	Yes (2)[d]	—	—	140 000	700
VT	20	20	20	20	20	—	Yes (5)[b]	—	—	—	—
WA	10	15	14	70	—	—		—	—	1300	—

Post, 2020 https://doi.org/10.1002/etc.4863

MCLs and HBVs are Based on Reference Doses



EPA, 1993

Adverse Outcome Pathway

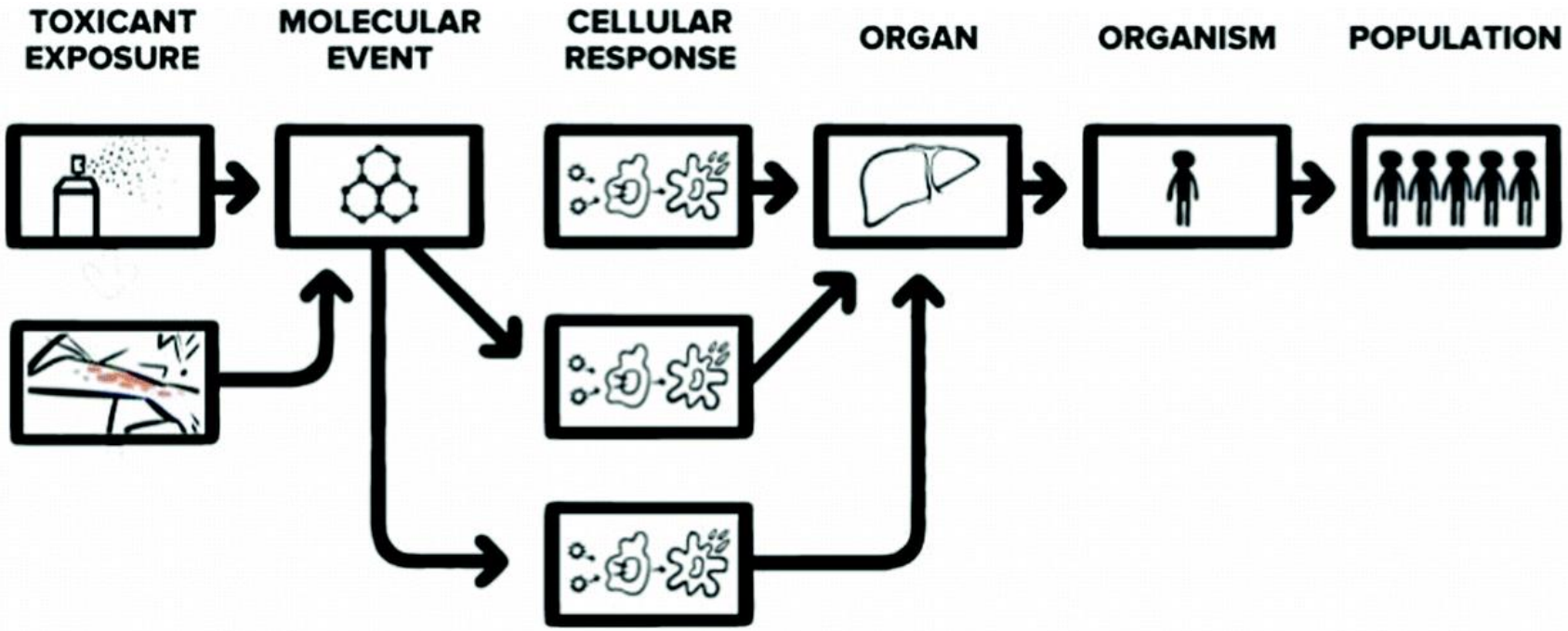
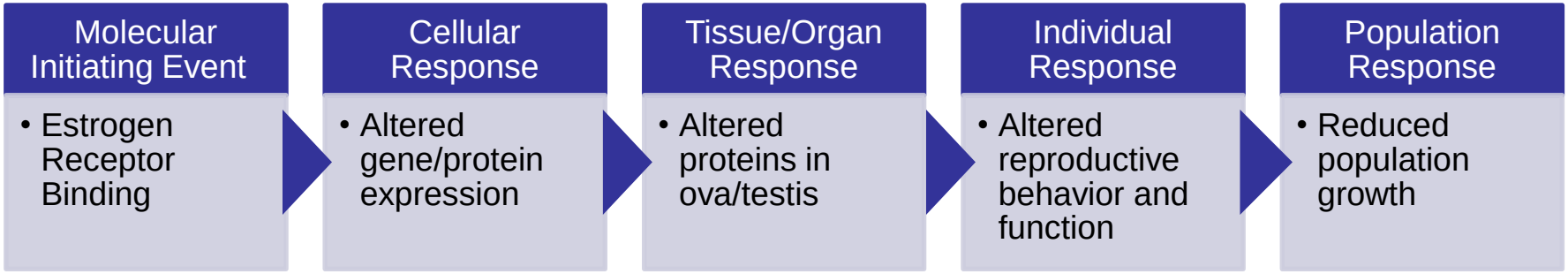


Diagram from “Pathways to a Better Future” video series, © The Human Toxicology Project Consortium

Estrogen Receptor AOP Example



Adapted from OECD, 2011

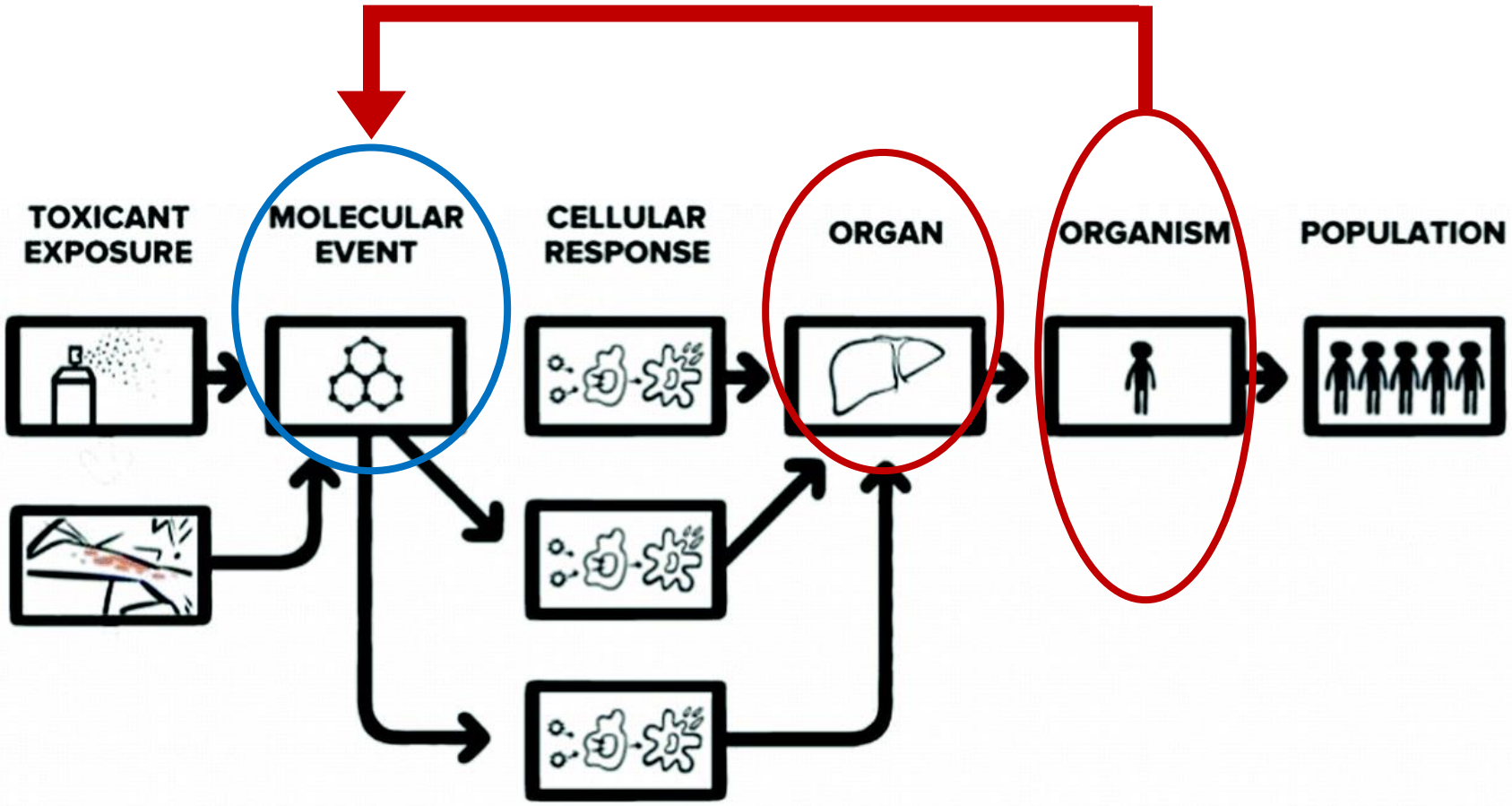


Diagram from “Pathways to a Better Future” video series, © The Human Toxicology Project Consortium

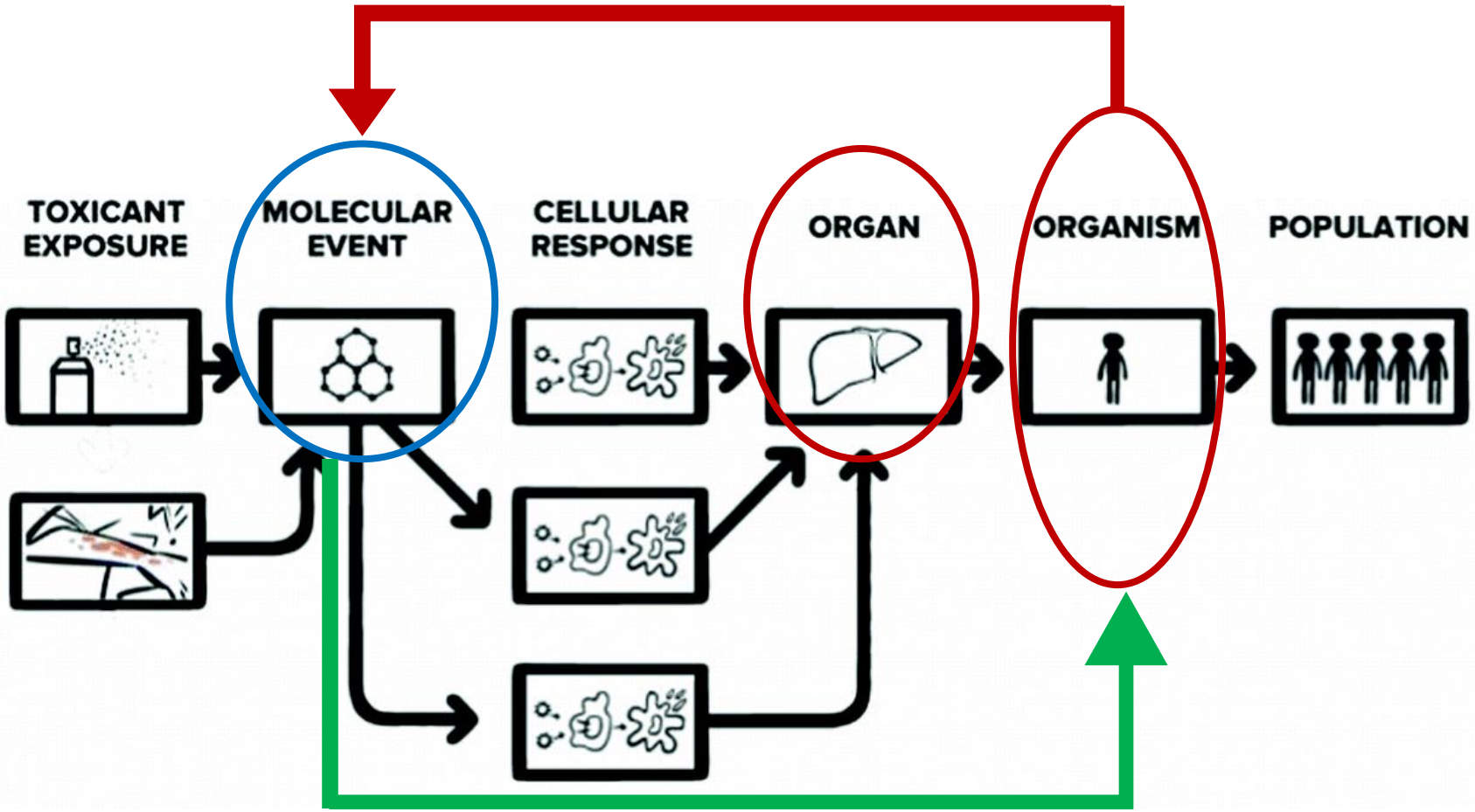
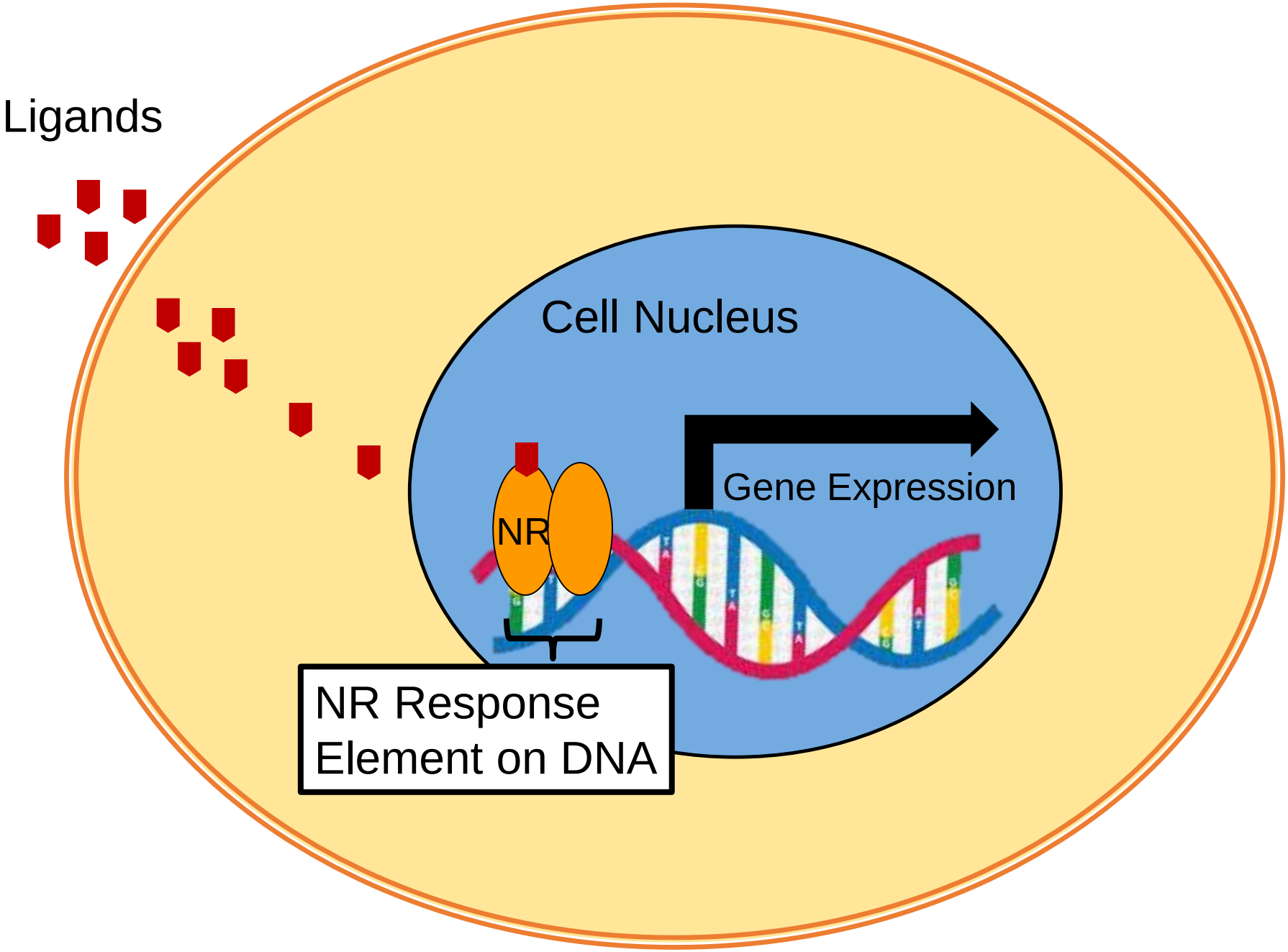


Diagram from “Pathways to a Better Future” video series, © The Human Toxicology Project Consortium

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

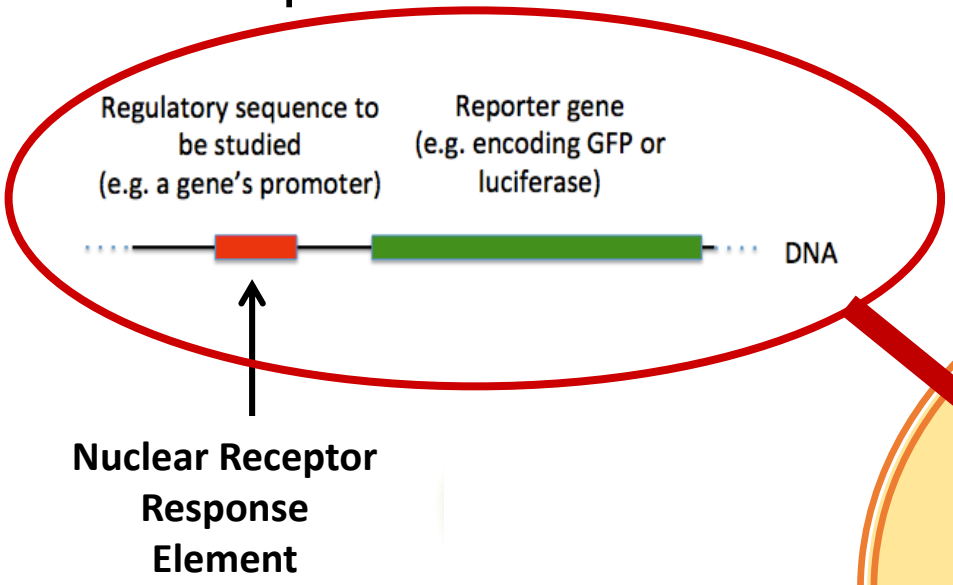

Ligands
Cell Nucleus
Gene Expression
NR
NR Response
Element on DNA

Some Nuclear Receptor Examples

RECEPTOR	FUNCTION	ENVIRONMENTAL LIGANDS
Aryl Hydrocarbon Receptor	Cell determination, cell cycle regulation, stress response	Dioxin-like chemicals, halogenated and polycyclic aromatic hydrocarbons
Estrogen Receptor	Female reproductive development and fertility, cardiovascular health	Bisphenols, organophosphate-based flame retardants (OPFRs)
Peroxisome proliferator-activated receptors (PPARα, PPARγ, PPARβ/δ)	Fatty acid and lipid homeostasis	Phthalates, organotins, OPFRs, PFAS

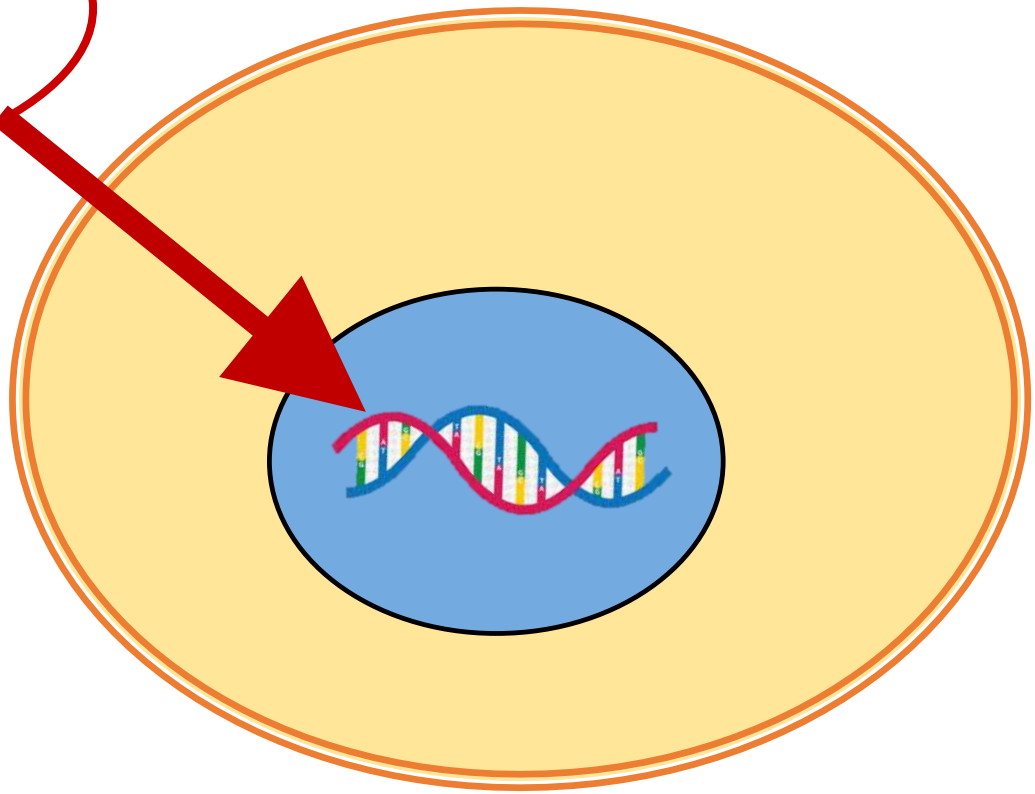
How do we study nuclear receptor activity?

Reporter Construct

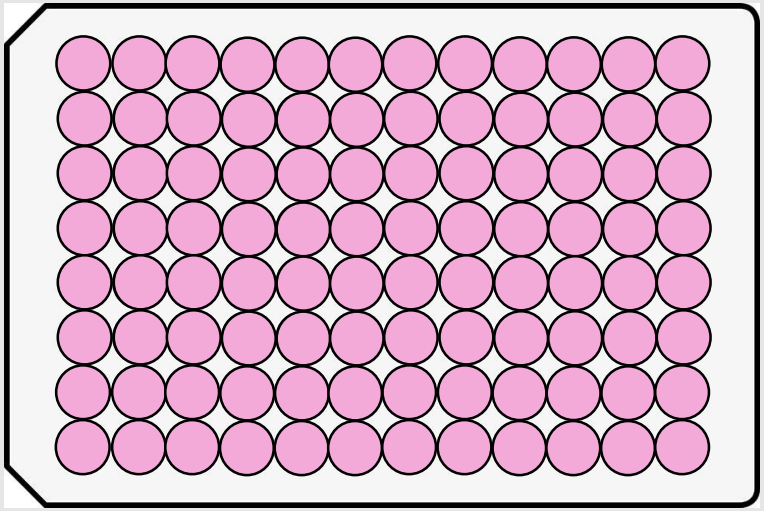


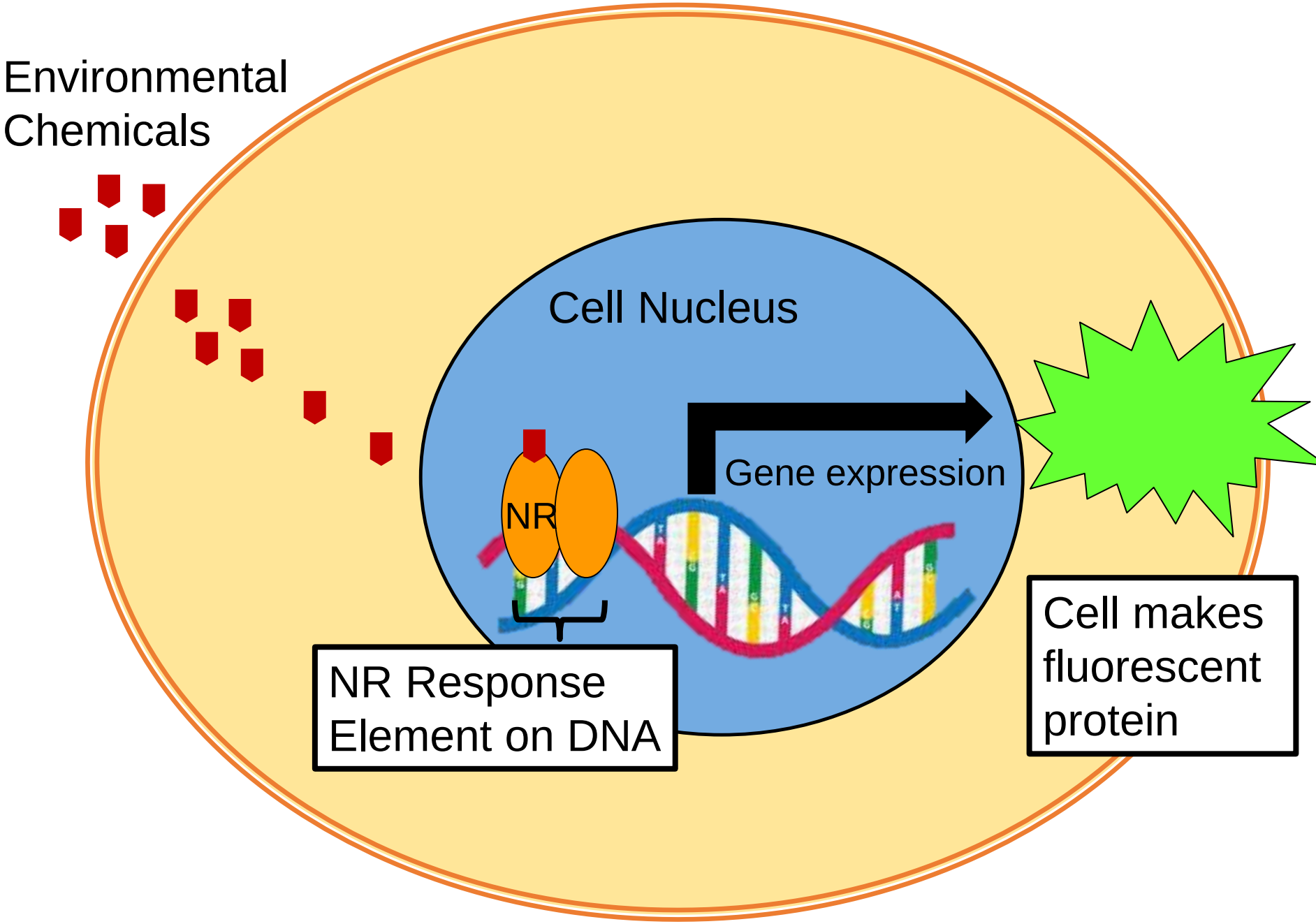
Nuclear Receptor Response Element

Cells we can grow easily in the lab

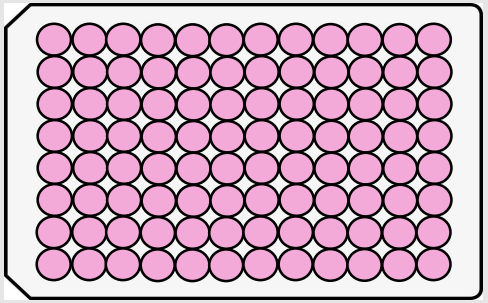


Treat cells with chemicals or chemical mixtures

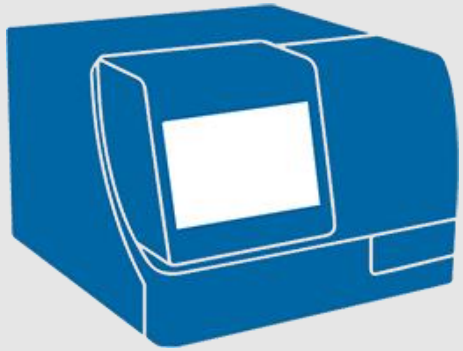


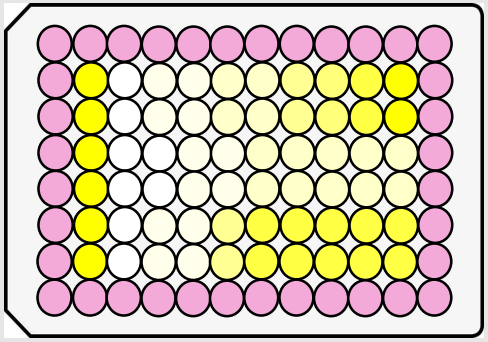

Environmental Chemicals
Cell Nucleus
Gene expression
NR
NR Response Element on DNA
Cell makes fluorescent protein

Treat cells with chemicals or chemical mixtures

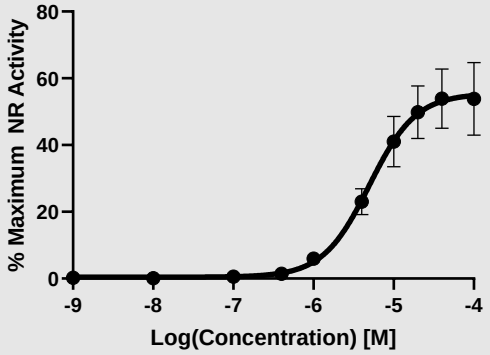


Measure light intensity

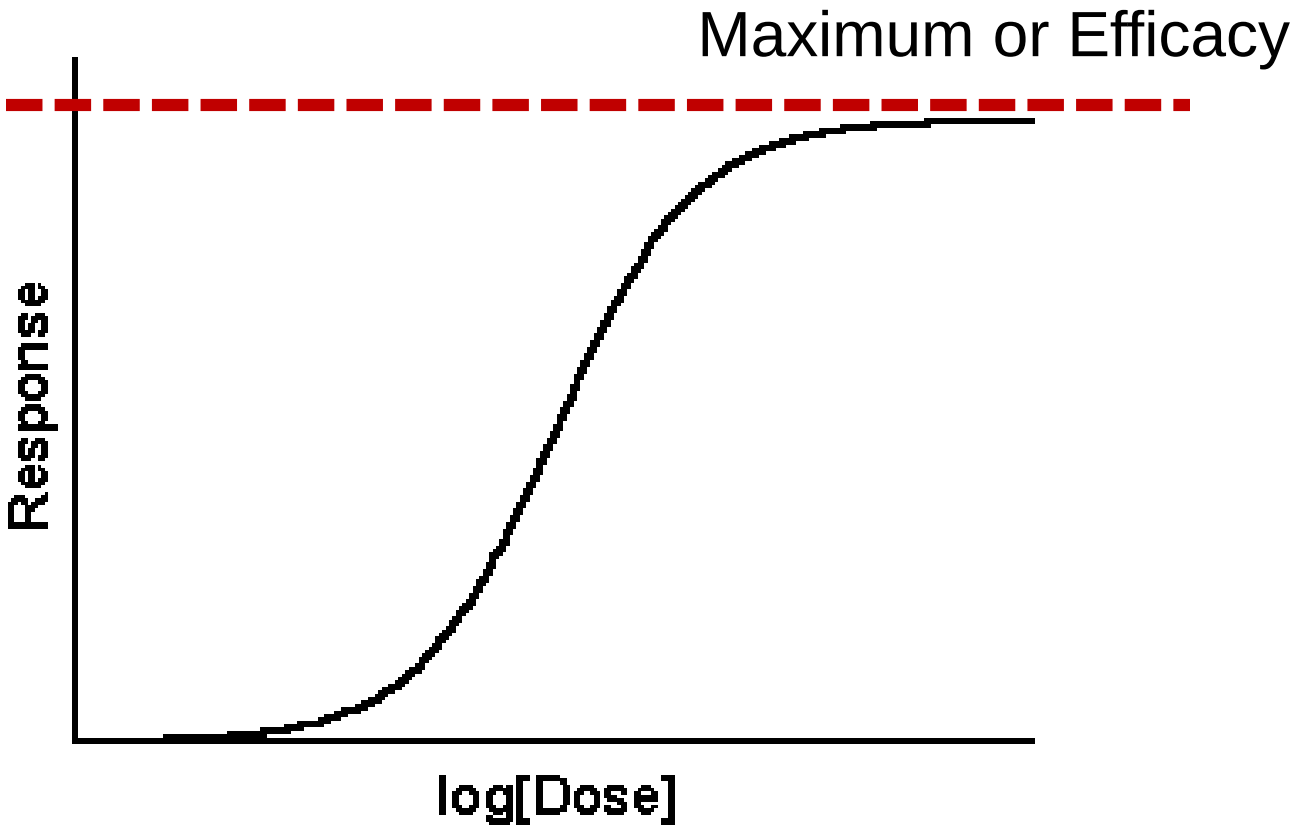




Analyze Results

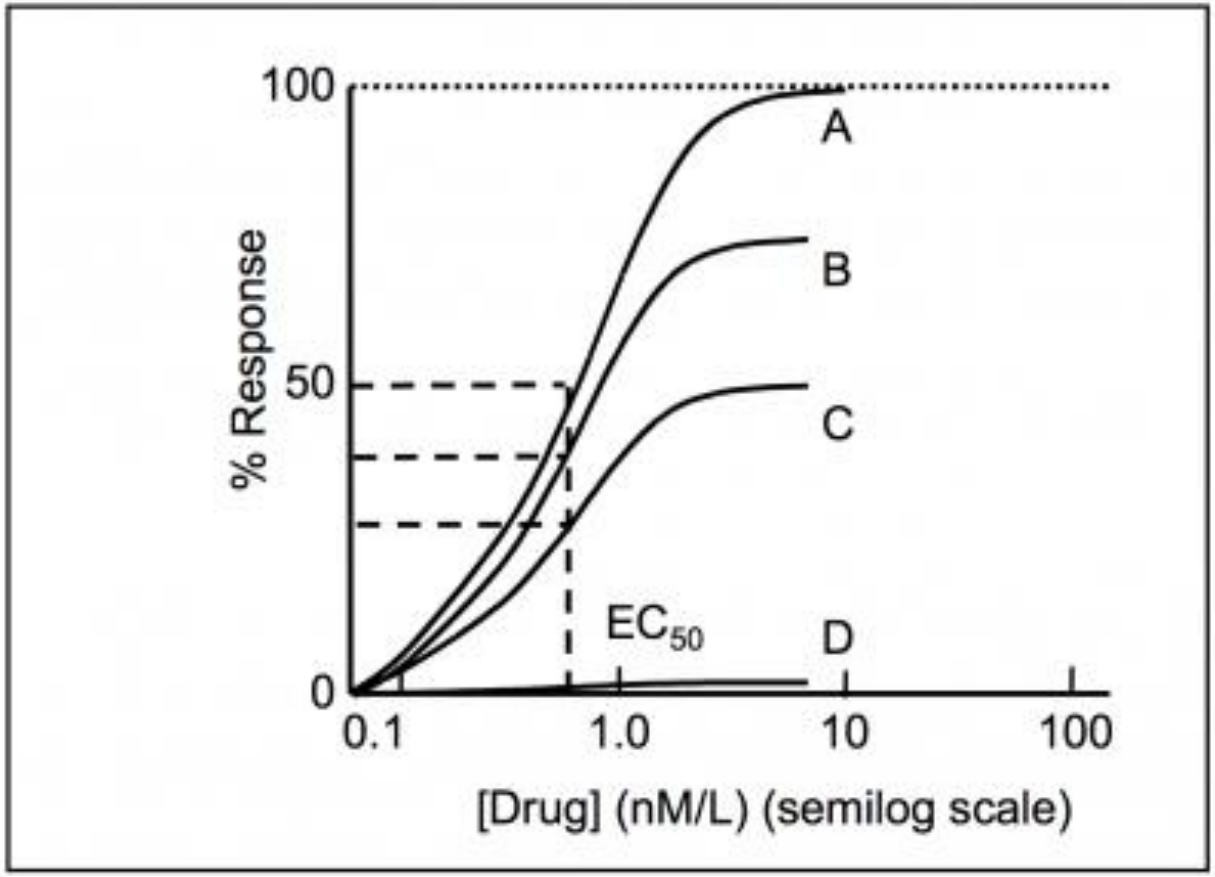


Dose Response Curve Properties: Maximum or Efficacy

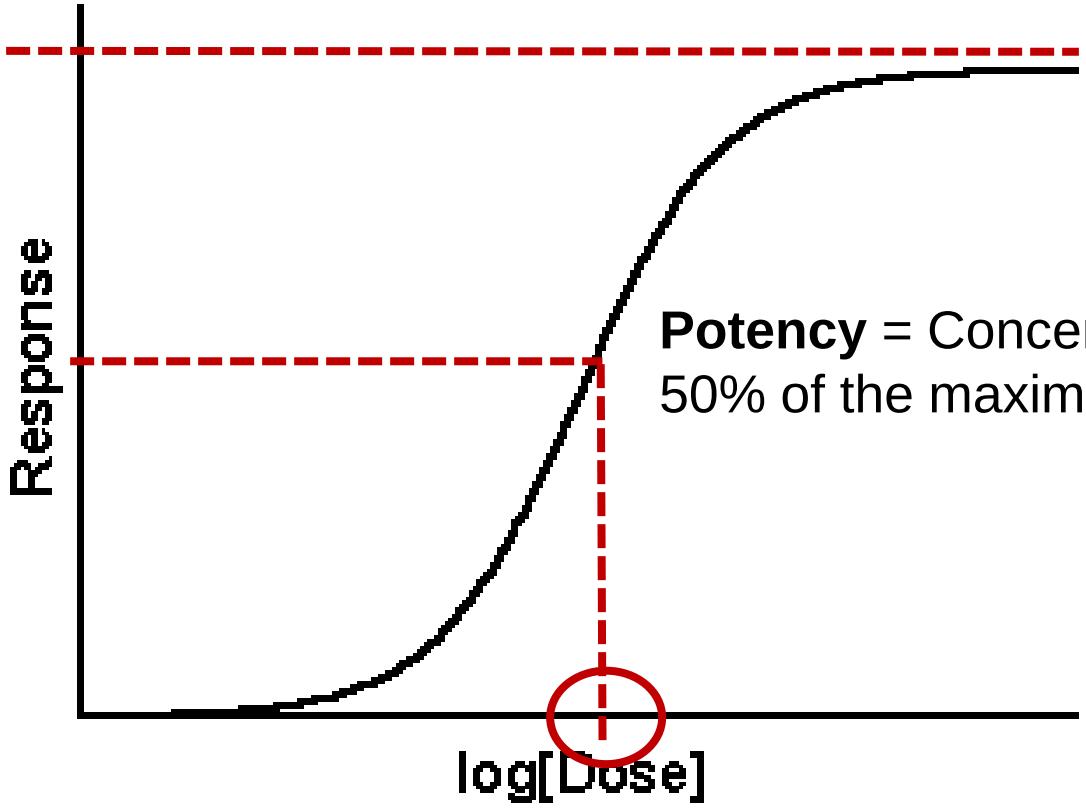


Dose Response Curve Properties: Maximum or Efficacy

A = Full Agonist
B, C, D = Partial Agonist



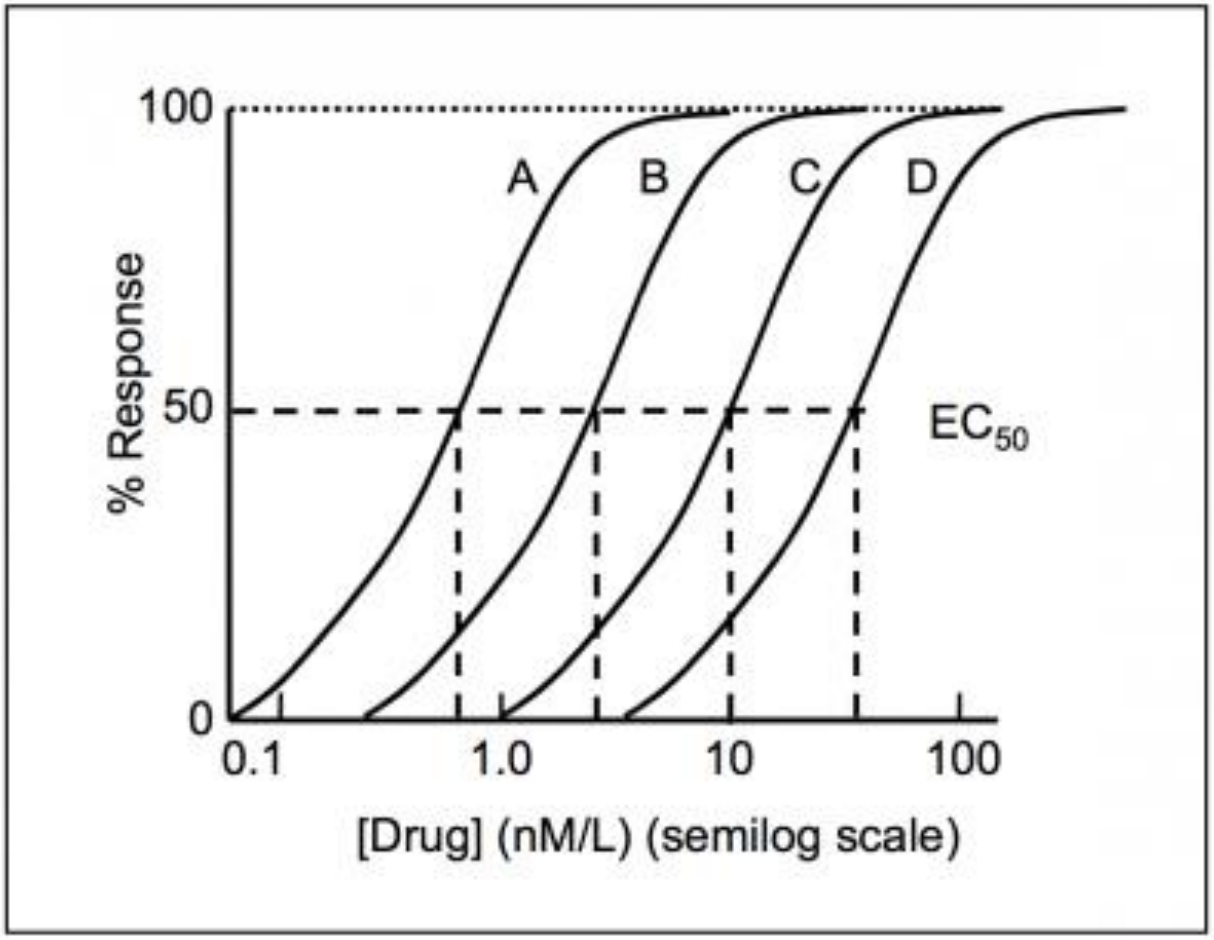
Dose Response Curve Properties: Potency



Potency = Concentration producing 50% of the maximum response

Dose Response Curve Properties: Potency

Potency: A>B>C>D



http://tmedweb.tulane.edu/pharmwiki/doku.php/drug_receptor_theory

We can use potency and efficacy of individual chemicals to predict the effects of chemical mixtures on nuclear receptor activity with mathematical models

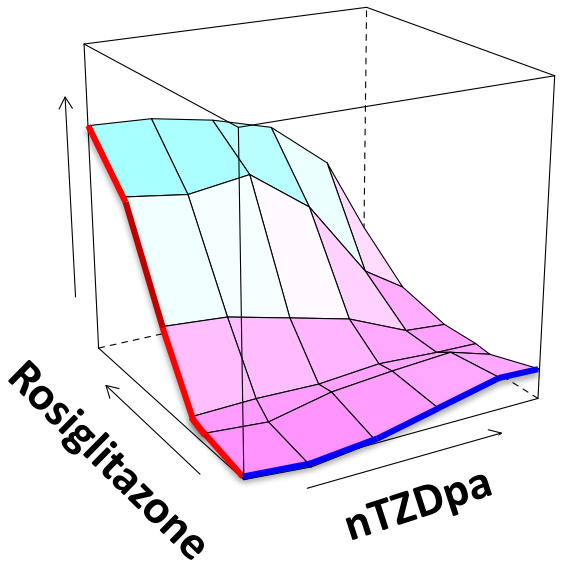
Modeling Approaches to Predict Mixture Activity

Concentration Additive models	**Sums:**	**Assumes:**
Relative Potency Factor **(RPF)**	Doses, as dilutions of a reference compound	Equal efficacies
Generalized Concentration Addition **(GCA)**	Doses	Equal or unequal efficacies

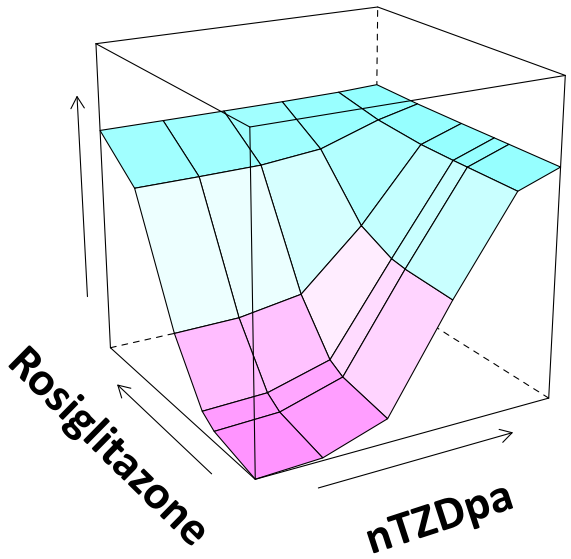
Predicting Effects of Full and Partial PPARγ Agonists

Rosiglitazone (full agonist) +nTZDpa (partial agonist)

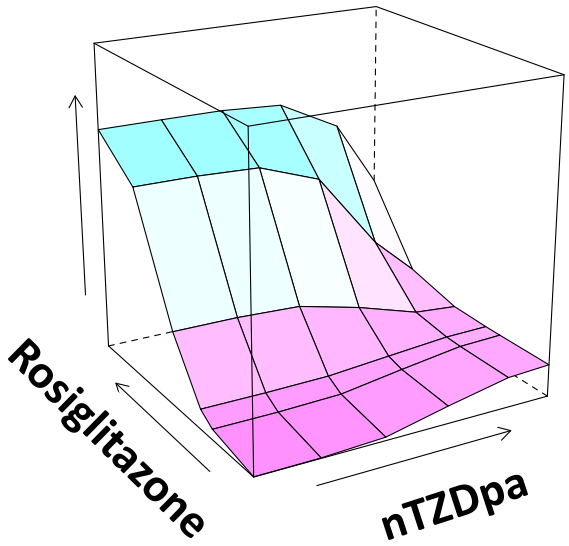
Experimental



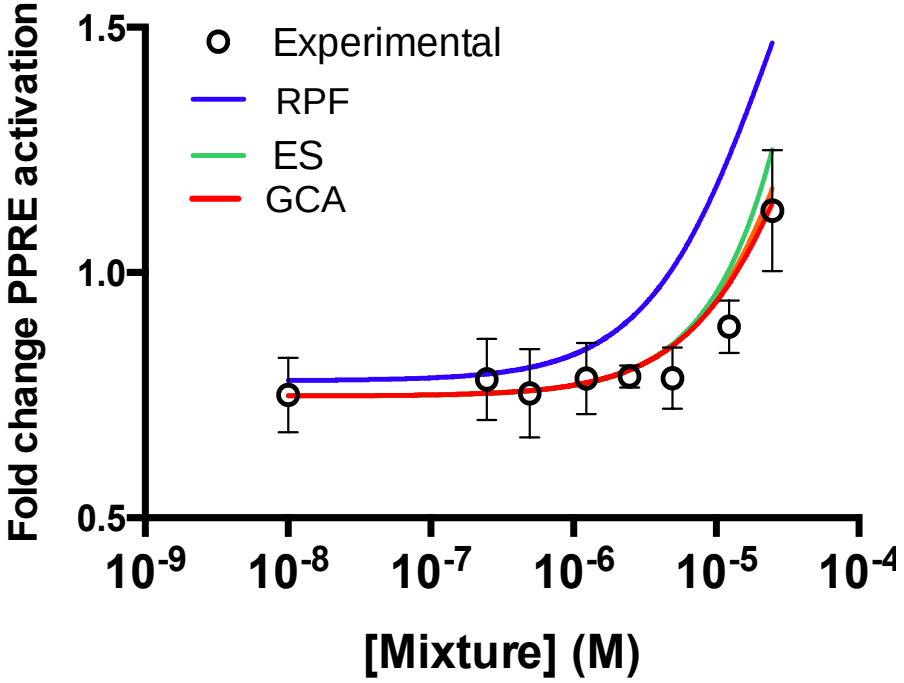
Relative Potency



Generalized Concentration Addition



GCA Models A Complex Mixture of Phthalate Compounds



RPF: Relative Potency
GCA: Generalized Concentration Addition
ES: Effect Summation (a third modeling approach)

Watt et al., 2016 PMC5013877

Generalized Concentration Addition Also Predicts Effects of Mixtures on:

- Aryl Hydrocarbon Receptor
  - Howard et al., PMID: 20435555)
- Androgen Receptor
  - Schlezinger et al., 2020 PMID: 32726424

PFAS are full and partial human PPARα agonists with varying potency

GCA predicts the effect of full (Pemafibrate) and partial (MEHP) PPARα agonists

Conclusions

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

II. Modeling techniques that incorporate efficacy and potency can predict the effects of mixtures on nuclear receptor activity, including PPARα

III. PFAS are full and partial PPARα agonists and mixtures will have **complicated but predictable** effects on PPARα activity

This is just the first step…

Acknowledgements

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  - Dr. Jennifer Schlezinger

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