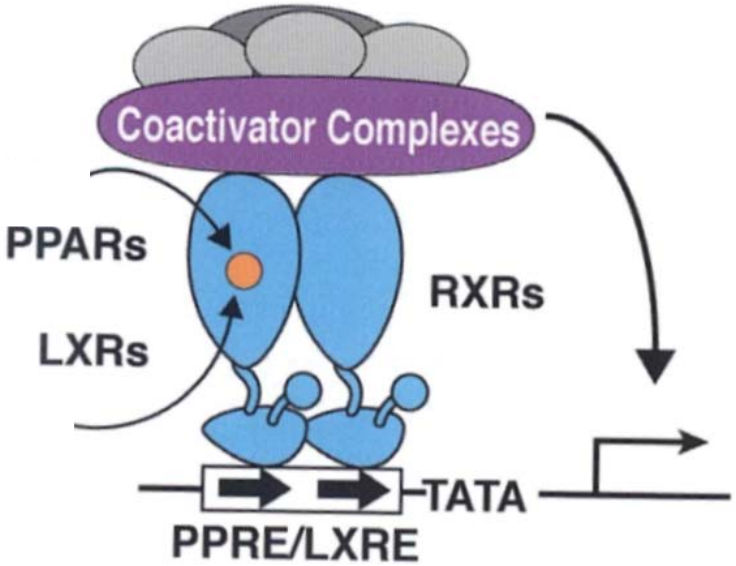
Developing a "Molecular Context" for the Biological Effects of PFOA

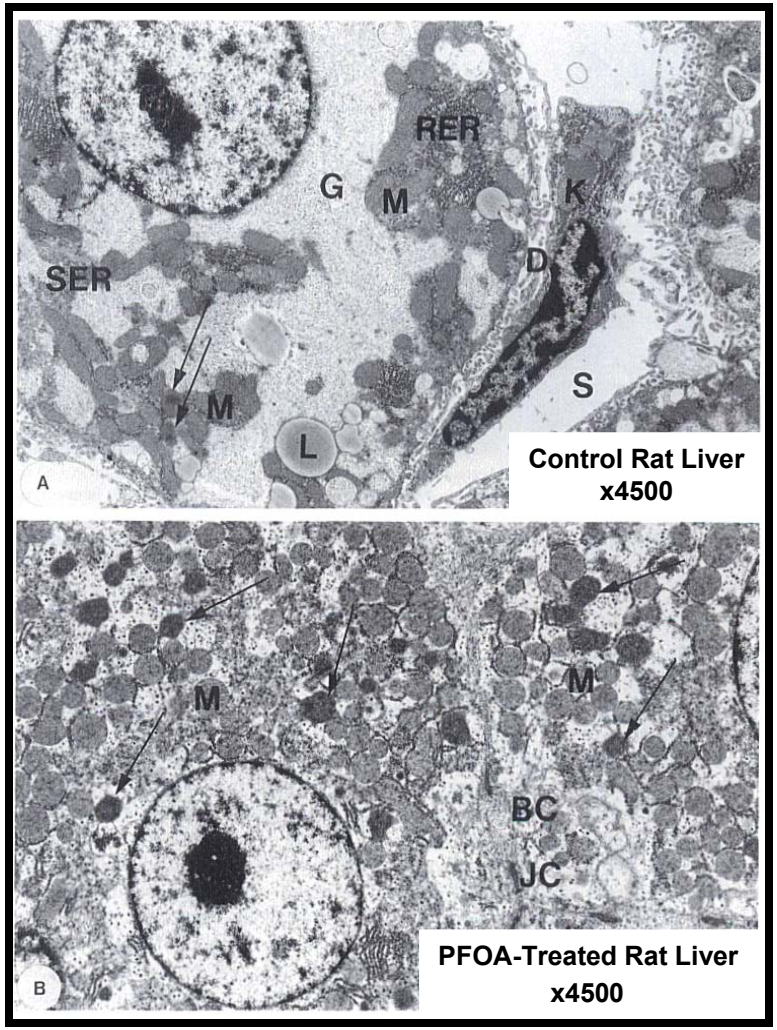
Ligands	Nuclear Receptors	Species
Chemicals Drugs Fatty acids	 Li & Glass, JLR 2004	Humans Rats Mice

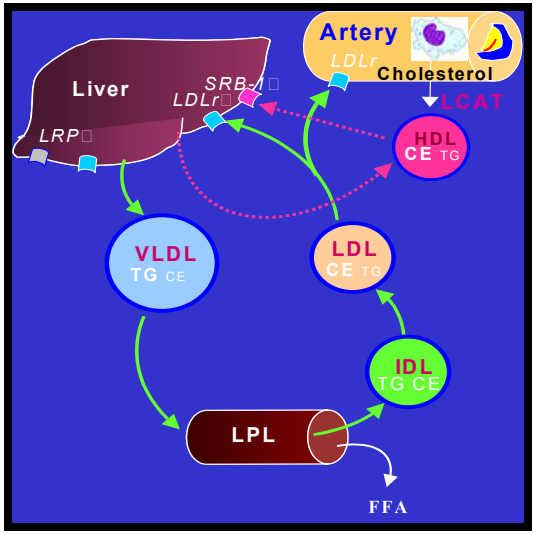
Peter J. Gillies, Ph.D
Study Director
DuPont

John P. Vanden Heuvel, Ph.D.
Principal Investigator
The Pennsylvania State University

Peroxisome Proliferation

Early Pathology to Present Day Molecular Biology





Molecular Biology
- PPAR-α
- PPAR-γ
- PPAR-β/δ
- LXR
- RXR
- Co-activators
- Co-repressors

Exp. Mol. Path. 47: 98-109 (1987)

2005

Today's Discussion Agenda

Evolving View of PPAR Biology

Toxicology

Pharmacology

Nutrition

Understanding PFOA in terms of current biology

- Overview of nuclear receptor biology
- Assessment of published data
- Research goals and study design
- Summary of data
- Insights and perspectives

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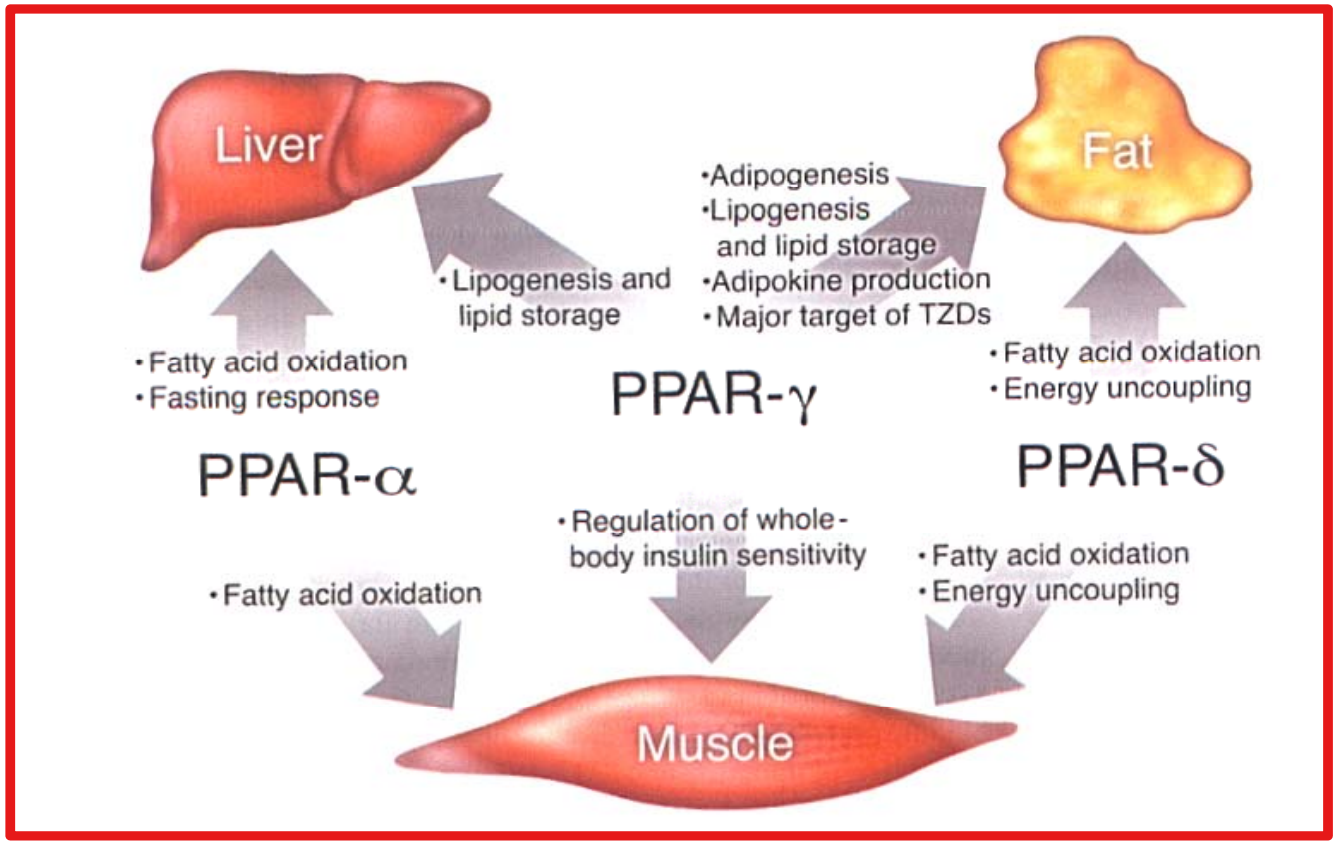
PPARs Are a Family of Nuclear Receptors

- Function as Transcription Factors
- Regulate Multiple Metabolic Processes



Evans etal 2004 Nature Medicine

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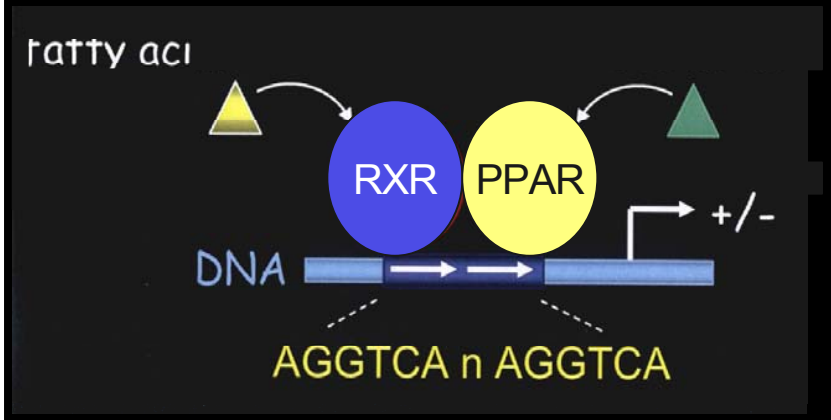
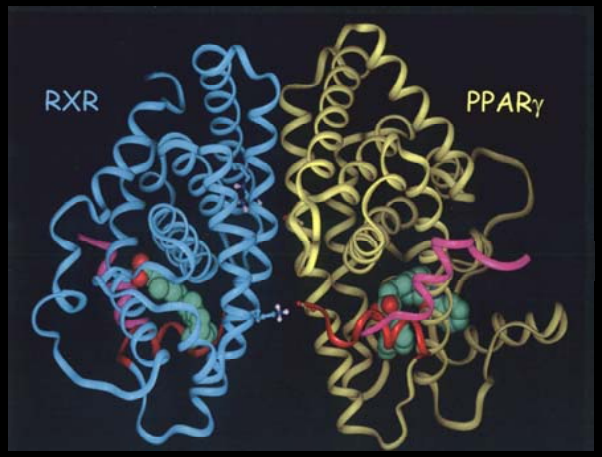
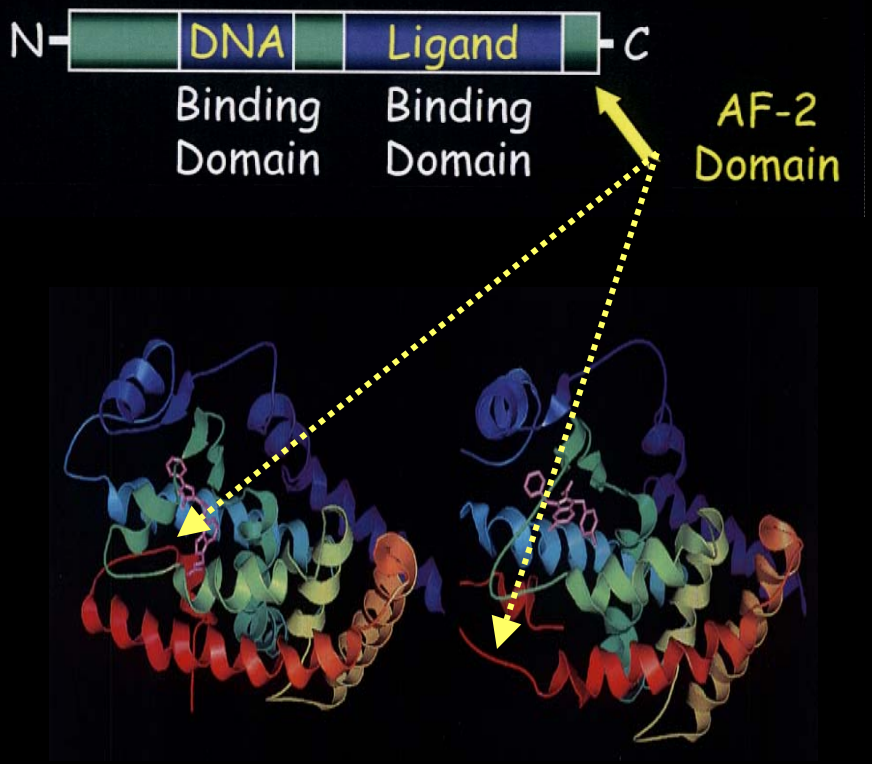
Structural Biology of Nuclear Receptors







Ligand specific conformational change results in the differential recruitment of coactivators / repressors leading to ligand specific gene expression profiles

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Gap Analysis: Transactivation Data

(Publications & Patents)

Ligand	PPAR-α M	PPAR-α R	PPAR-α H	PPAR-β M	PPAR-β R	PPAR-β H	PPAR-γ M	PPAR-γ R	PPAR-γ H	LXR-β M	LXR-β R	LXR-β H	RXR-α M	RXR-α R	RXR-α H	Author Cell line
PFOS (Mixture)	+		+													Shipley, 2004 COS-1 cells
PFOA (Mixture)	+					?			+							Elcombe, 2004 COS-1 cells
	+		+				*		*							Maloney, 1999 COS-1 cells
		+	(+)													Intrasuksri, 1998 CV-1 cells
PFOA (Linear) **PFOA** (Branched)																

M = Mouse, R = Rat, H = Human

+ Activation, (+) Reported + but data not shown, ? Possible, * Inactive at doses tested

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Activation of Nuclear Receptors By Natural and Synthetic Ligands

Principal Investigator
John P. Vanden Heuvel, Ph.D.
Center for Molecular Toxicology and Carcinogenesis
The Pennsylvania State University

Research Goal:
Develop a cross-species (mouse, rat, human) understanding of nuclear receptor activation in response to natural and perfluorinated fatty acids evaluated in a common testing platform

Nuclear Receptors of Interest:
- PPAR and its α-β-γ isoforms [fatty acid and glucose metabolism]
- LXR-β [cholesterol metabolism]
- RXR-α [obligate heterodimer]

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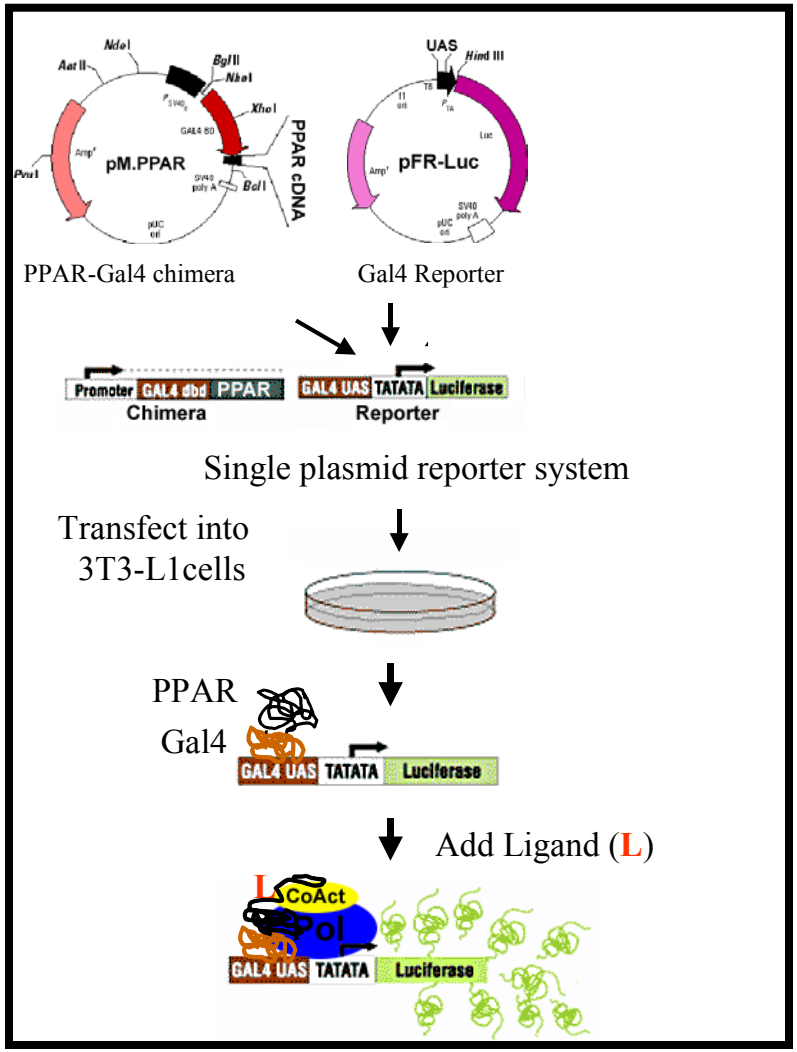
Nuclear Receptor Ligand Binding Assays

(LBD Chimeric Receptor)



3T3-L1 Cell Assay

- Good fatty acid uptake
- Low background noise
- No need for RXR
- Limited metabolic capacity

Assay Protocol

- DMEM + 10% FBS
- DMSO vehicle
- 0-400 µM
- 24 hour incubation

Activity in this assay shows the ability of a chemical to interact with the LBD of the nuclear receptor to produce a conformational change capable of regulating reporter gene transcription; it does not necessarily equate to the chemical's ability to produce a biological or toxicological response *in vivo*.

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Control Ligands

Nuclear Receptor	Positive Control Ligand
PPAR-α	Ciprofibrate (Cipro)
PPAR-β	Tetradecylthioacetate (TTA)
PPAR-γ	Rosiglitazone (Rosi)
LXR-β	T0901317
RXR-α	Methoprene (MP)

Positive controls used to:

- Validate success of transfection process
- Provide a benchmark for comparing activities

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Test Ligands

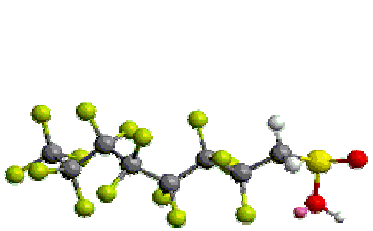
Theoretical Minimized
Energy Configurations

Perfluorinated Fatty Acids

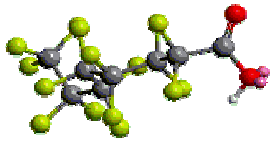
- PFOA-Linear (L)
- PFOA-Linear/Branched (L/B)
- PFOA-Branched (B)
- PFOS

Natural Fatty Acids

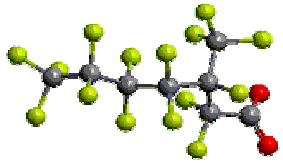
- Octanoate (Oct)
- Conjugated Linoleate (CLA)
- Oleate (OA)
- Linoleate (LA)
- α-Linolenate (ALA)



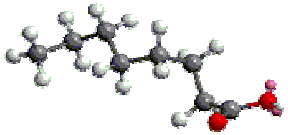
PFOS



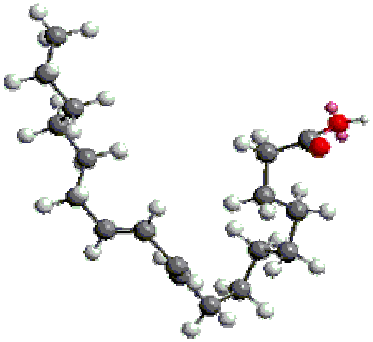
PFOA
(Linear)



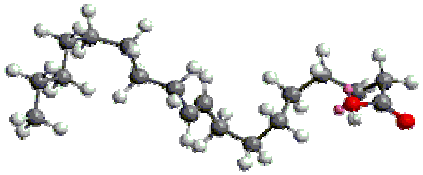
PFOA
(Branched)



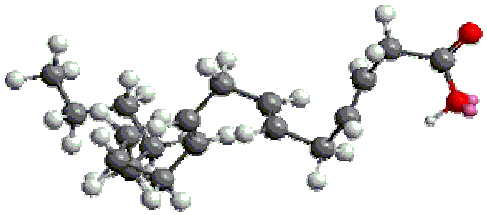
Octanoic Acid
8:0



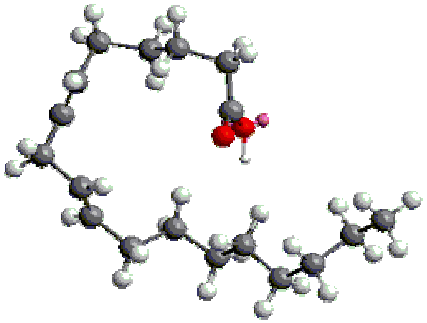
Conjugated Linoleic Acid
18:2 (9,11)



Oleic Acid
18:1(9)

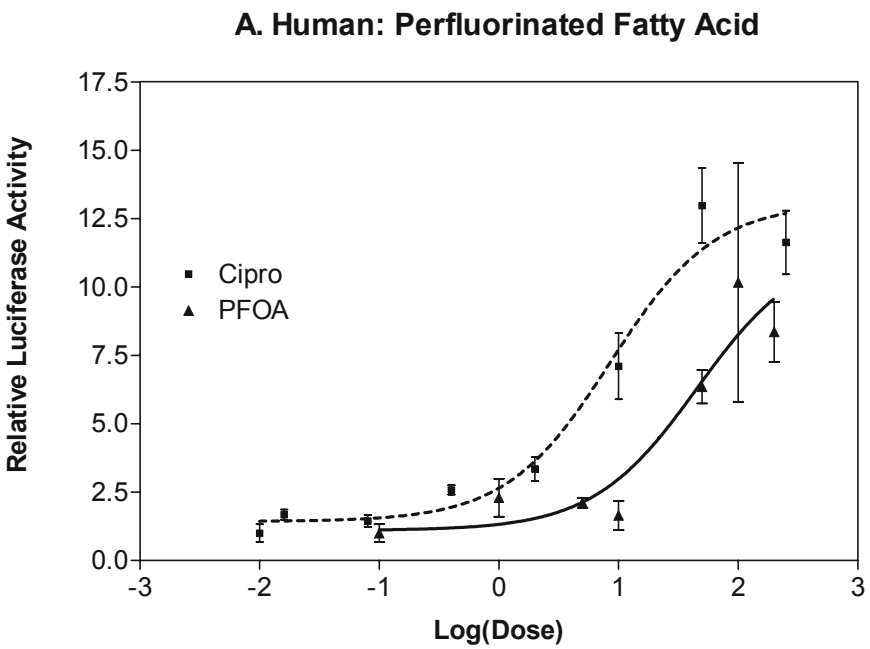


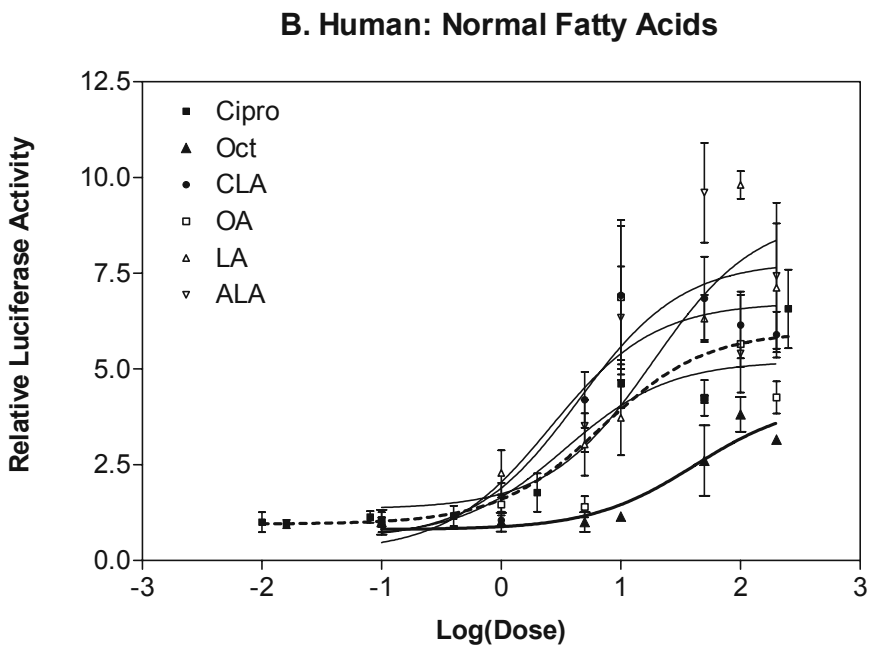
Alpha Linolenic Acid
18:3(9,12, 15)



Linoleic Acid
18:2(9,12)

Survey of Nuclear Receptor Activity: PPAR-α





Note: The PFOA ligand tested was the linear form of the molecule

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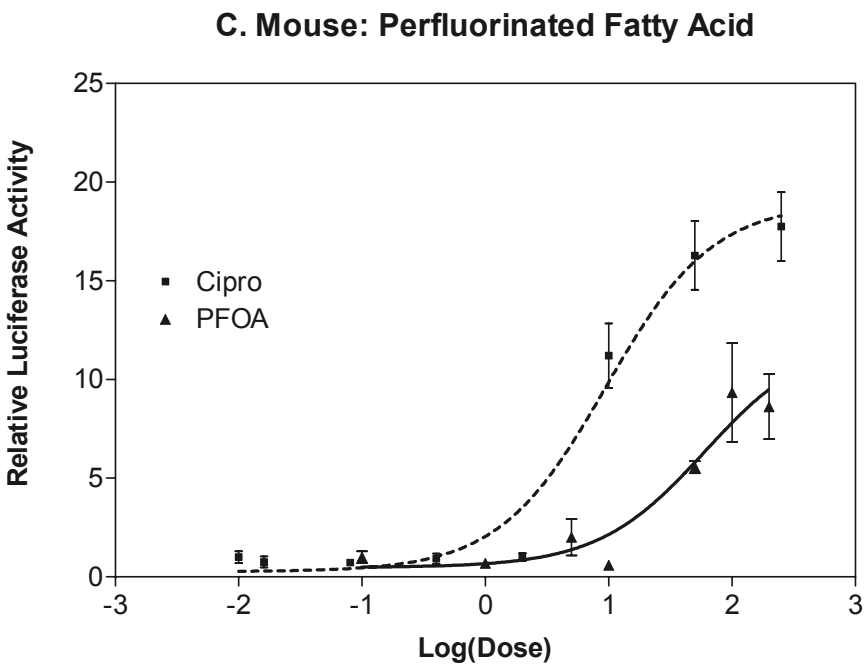
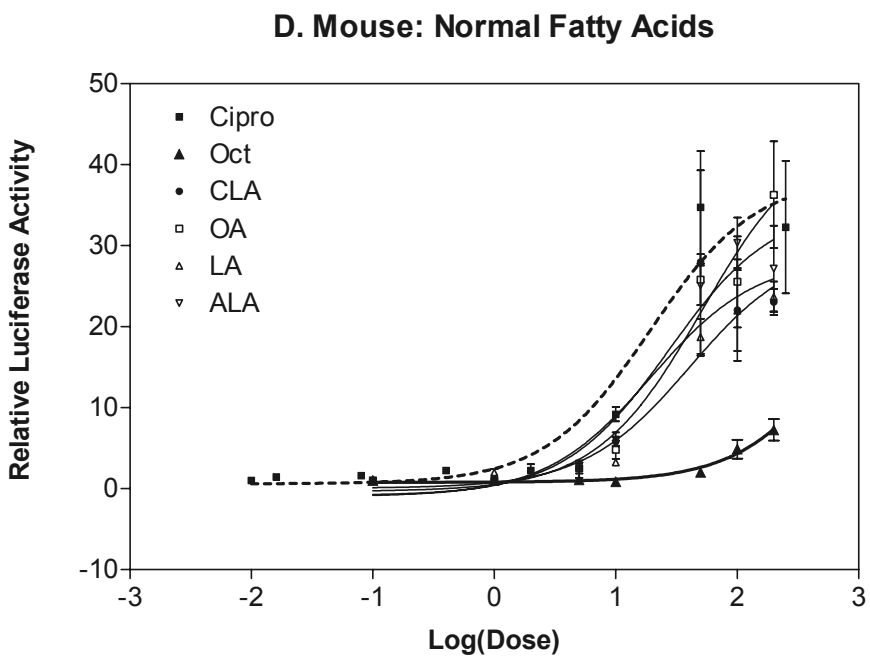
Survey of Nuclear Receptor Activity: PPAR-α





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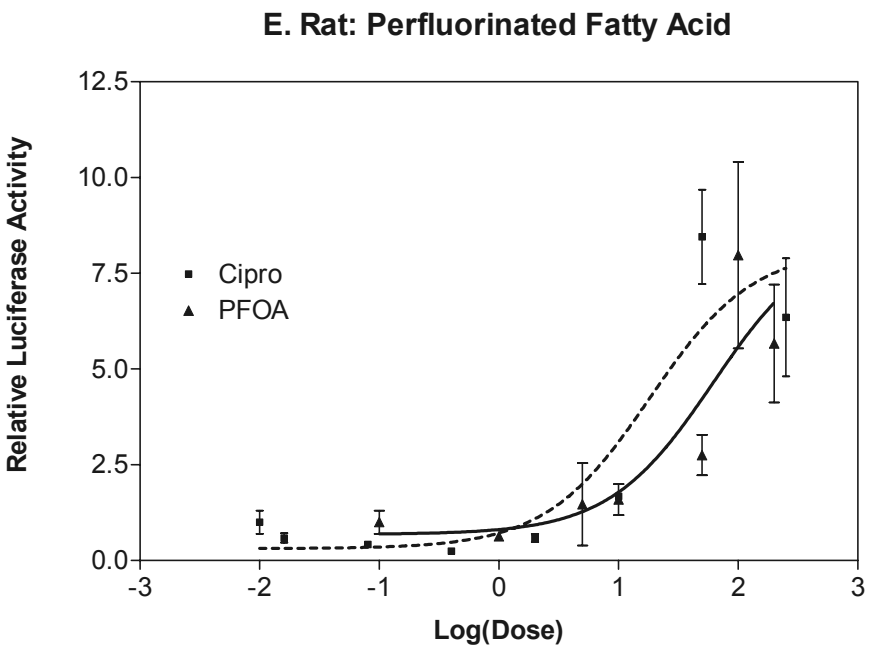
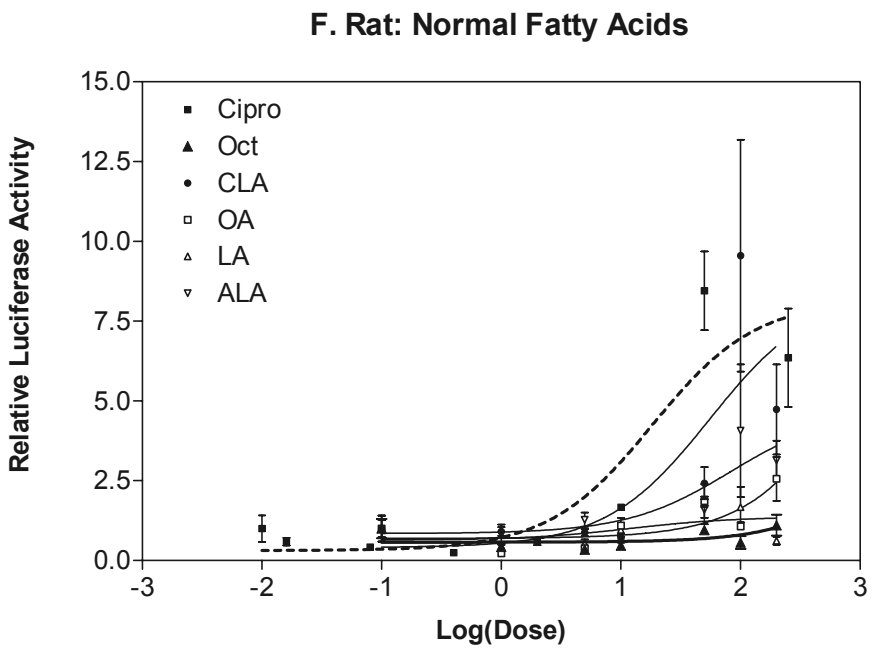
Survey of Nuclear Receptor Activity: PPAR-α





Note: The PFOA ligand tested was the linear form of the molecule

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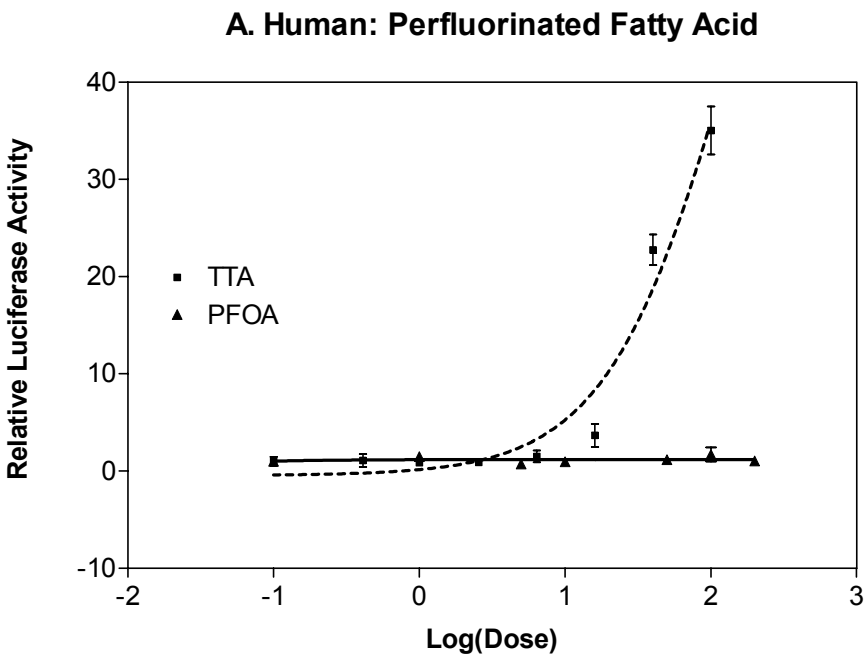
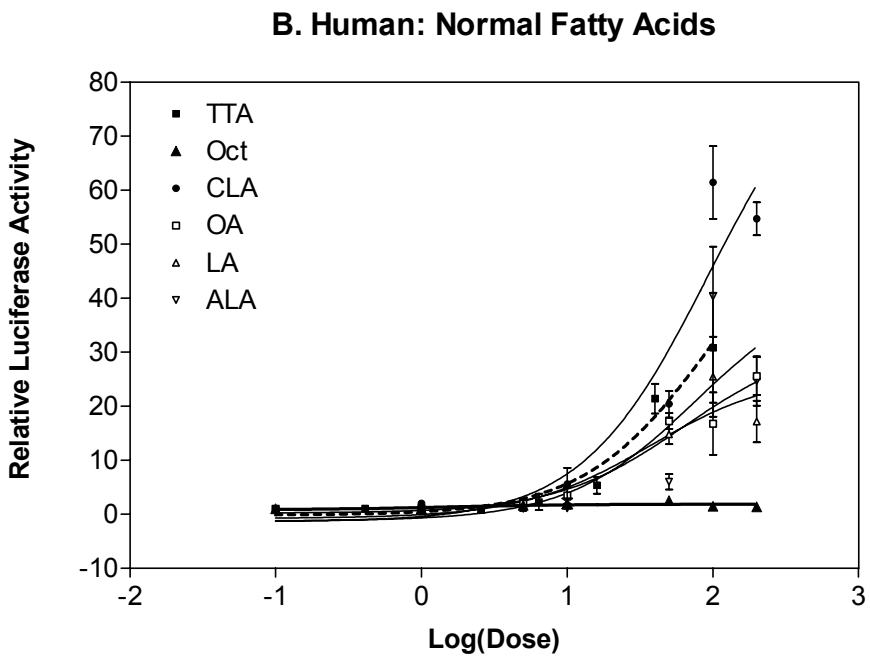
Survey of Nuclear Receptor Activity: PPAR-β





Note: The PFOA ligand tested was the linear form of the molecule

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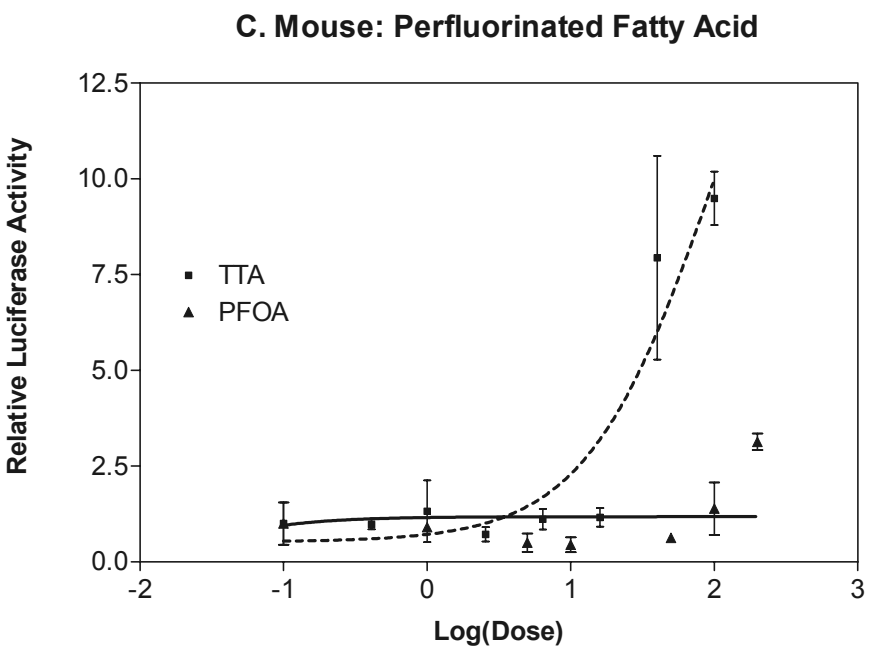
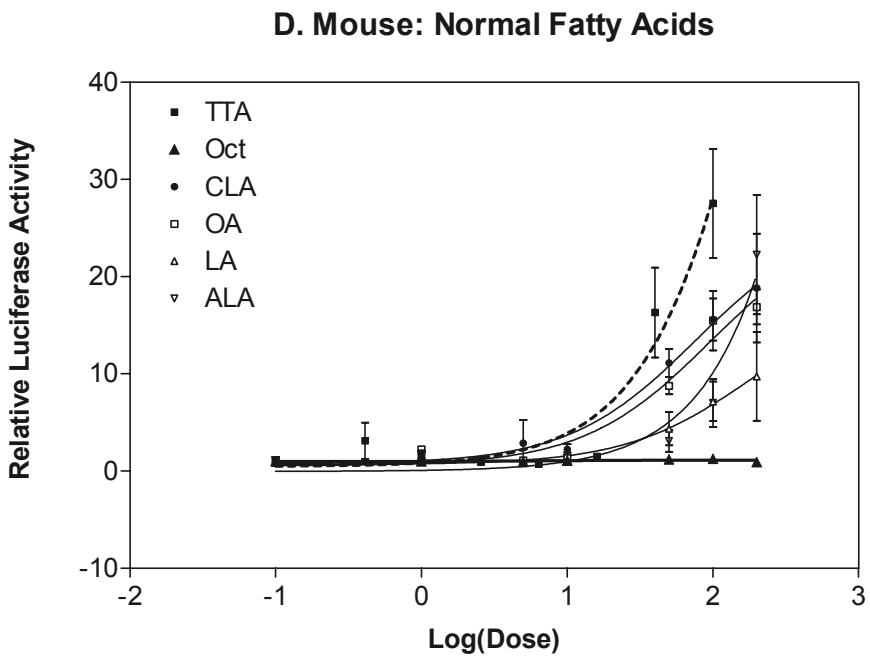
Survey of Nuclear Receptor Activity: PPAR-β





Note: The PFOA ligand tested was the linear form of the molecule

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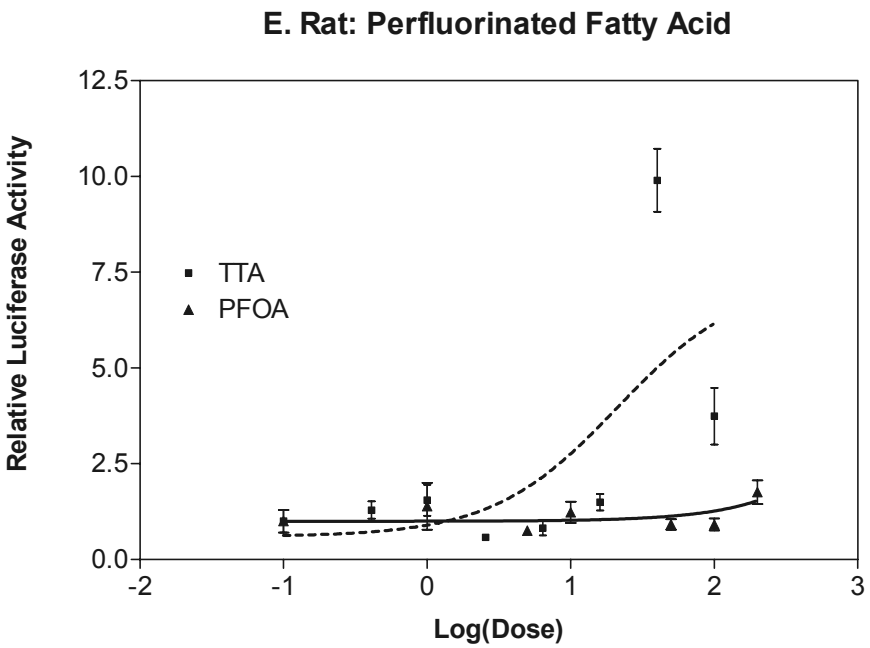
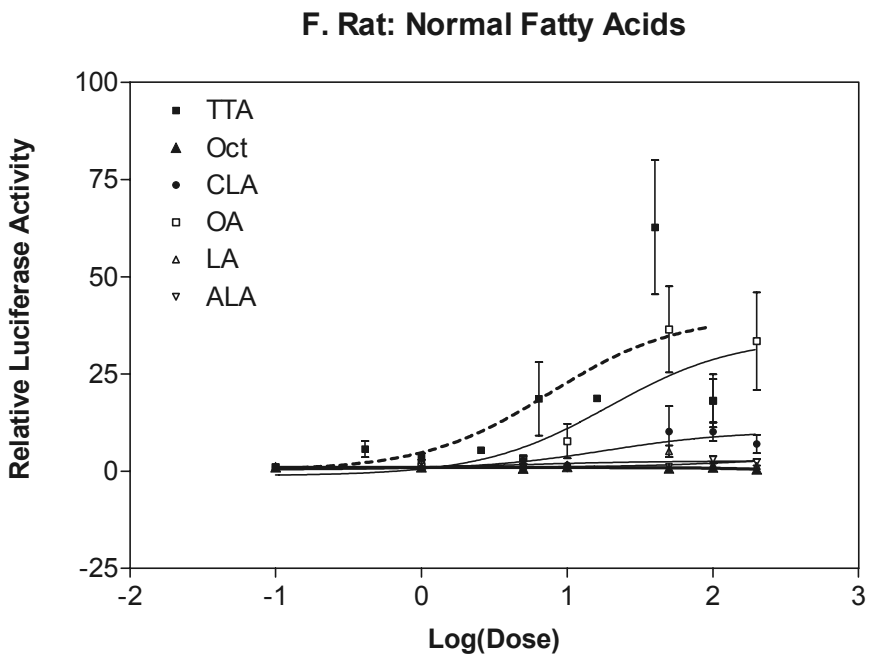
Survey of Nuclear Receptor Activity: PPAR-β





Note: The PFOA ligand tested was the linear form of the molecule

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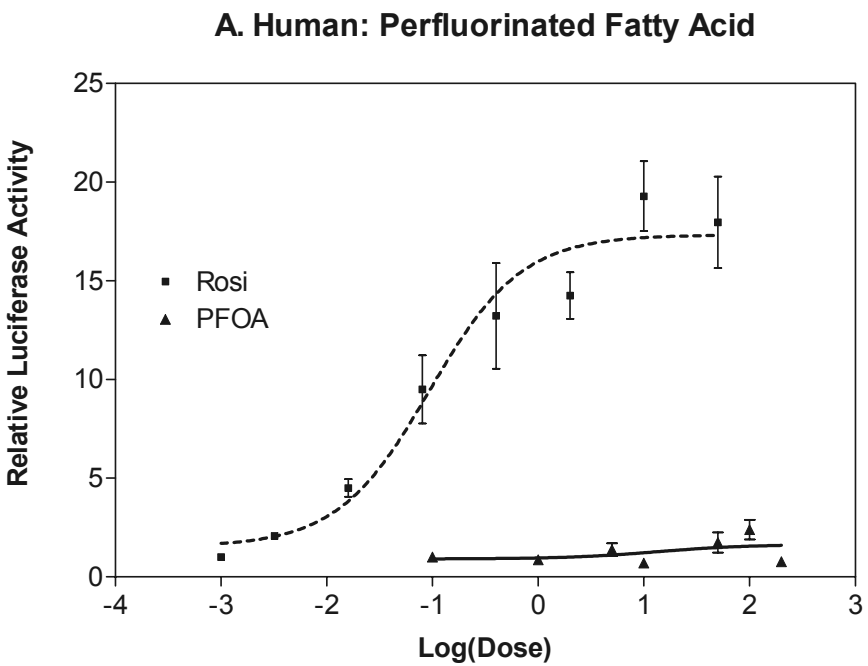
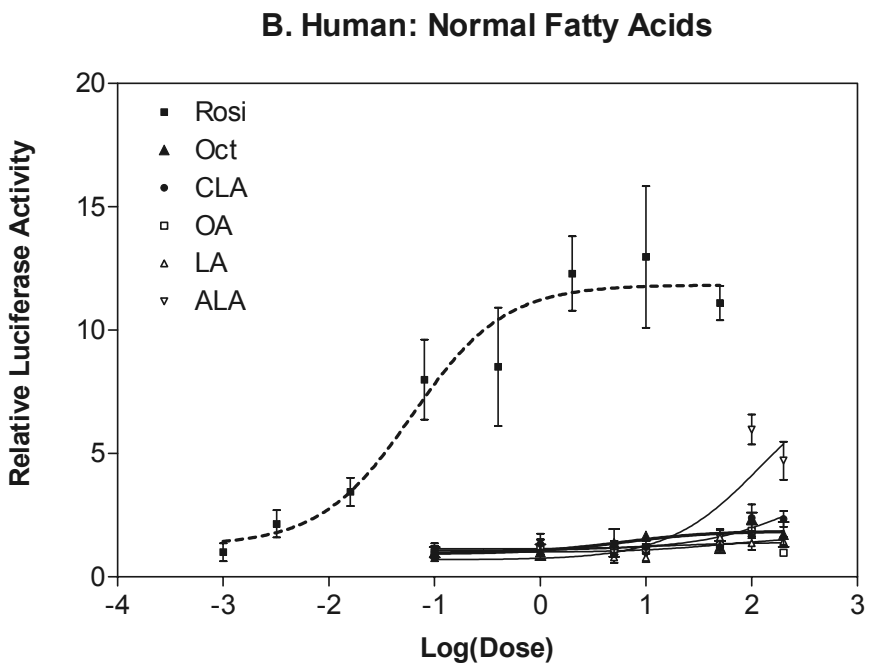
Survey of Nuclear Receptor Activity: PPAR-γ





Note: The PFOA ligand tested was the linear form of the molecule

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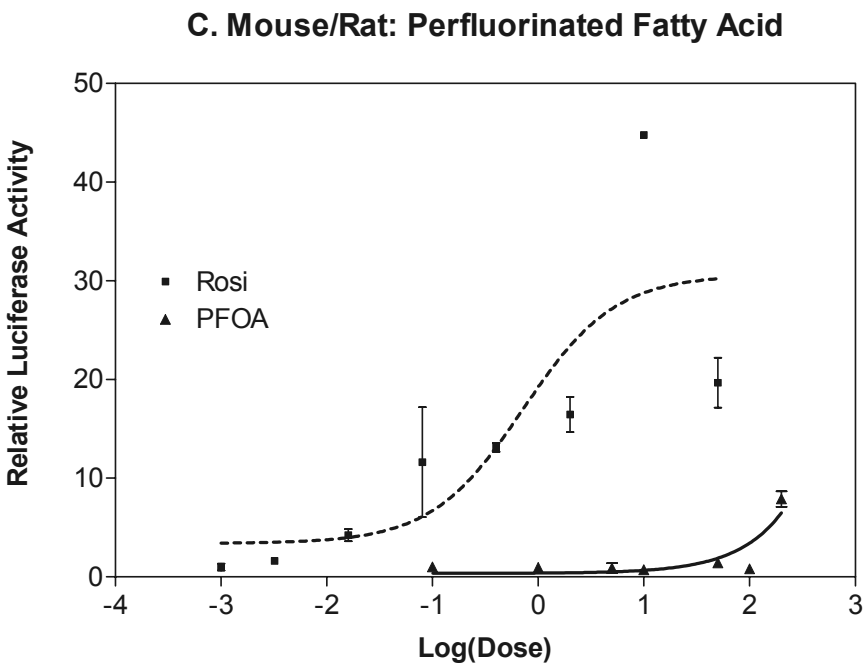
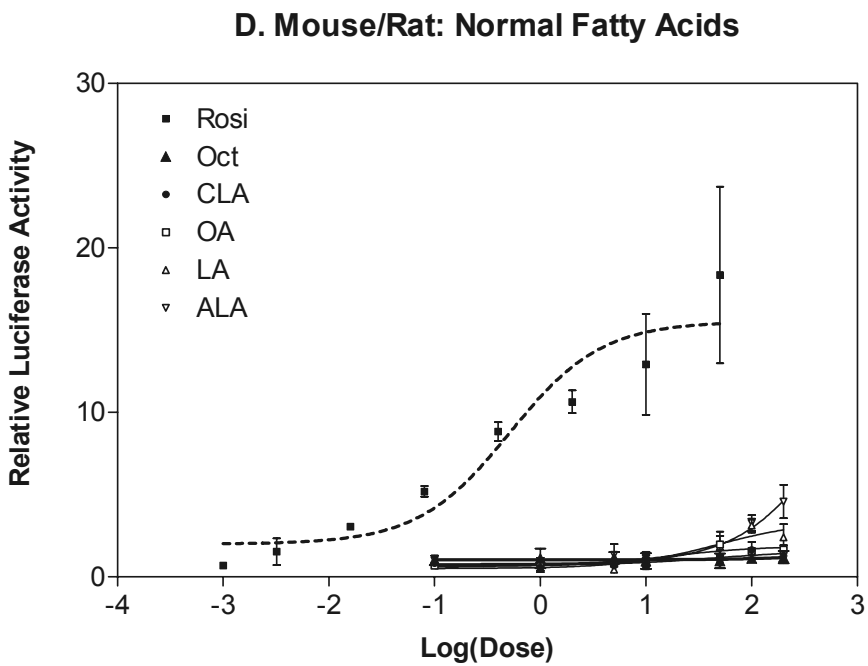
Survey of Nuclear Receptor Activity: PPAR-γ



Note: The PFOA ligand tested was the linear form of the molecule



Note: The LBD for mouse and rat are identical, hence the combined data

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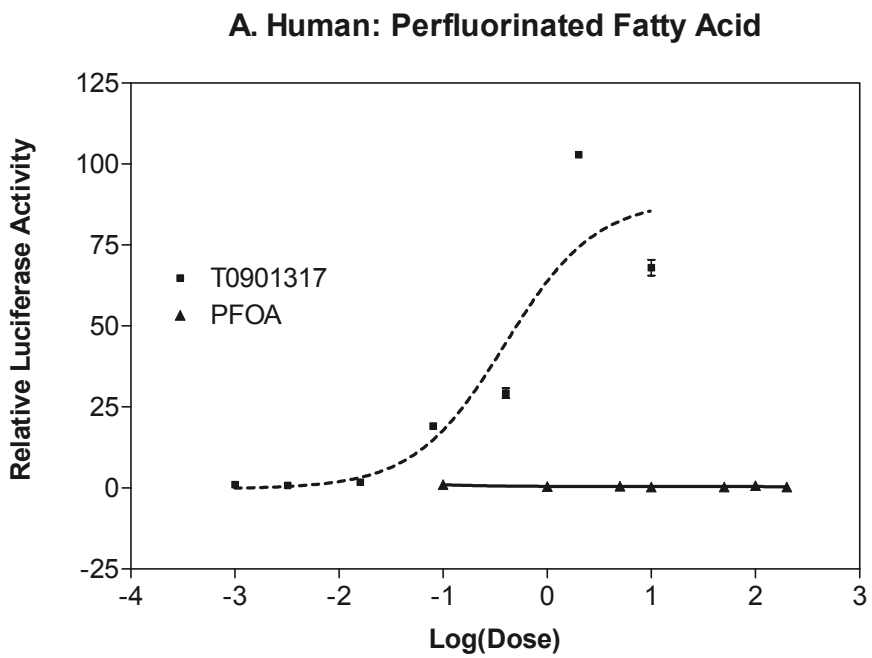
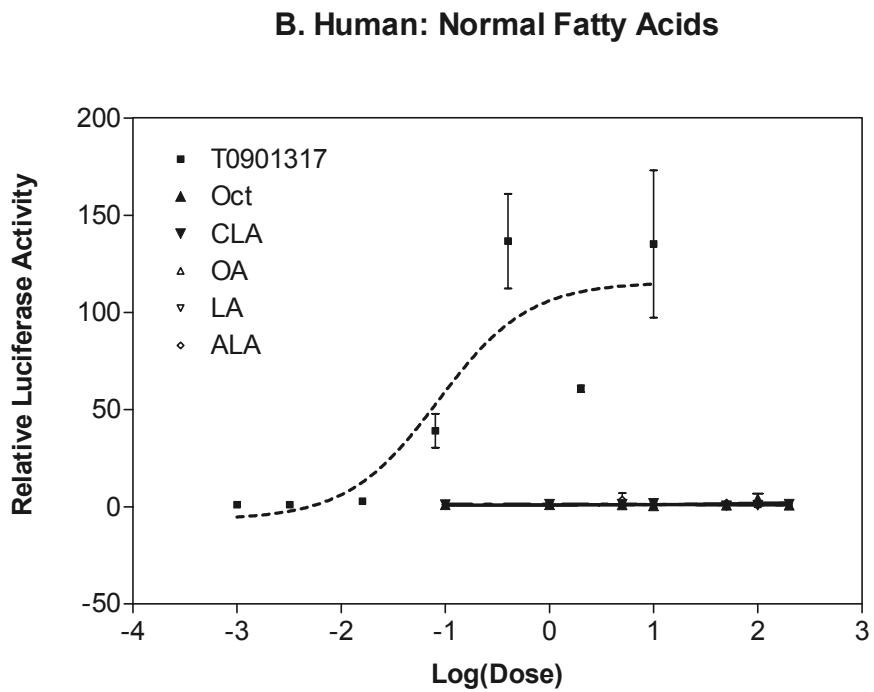
Survey of Nuclear Receptor Activity: LXR-β





Note: The PFOA ligand tested was the linear form of the molecule

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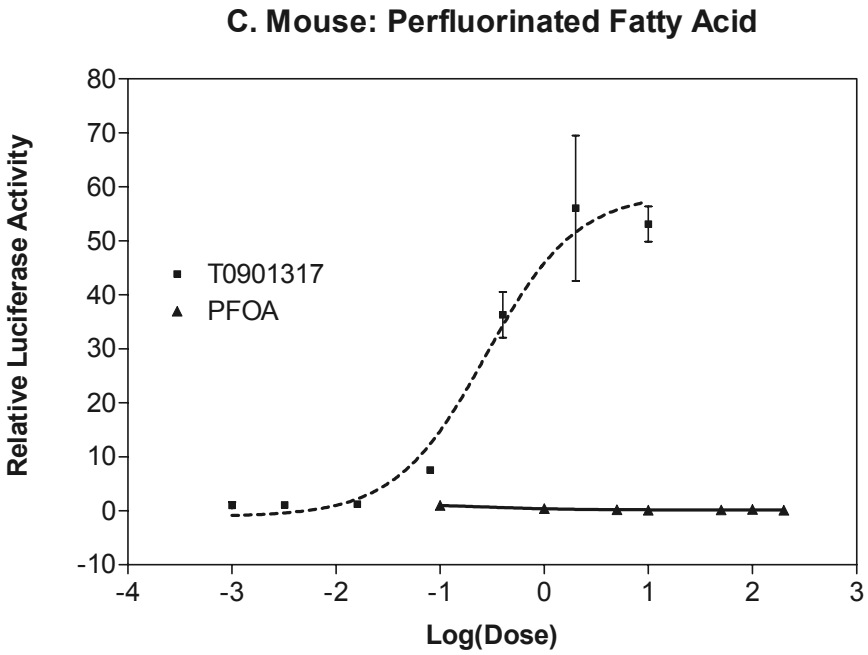
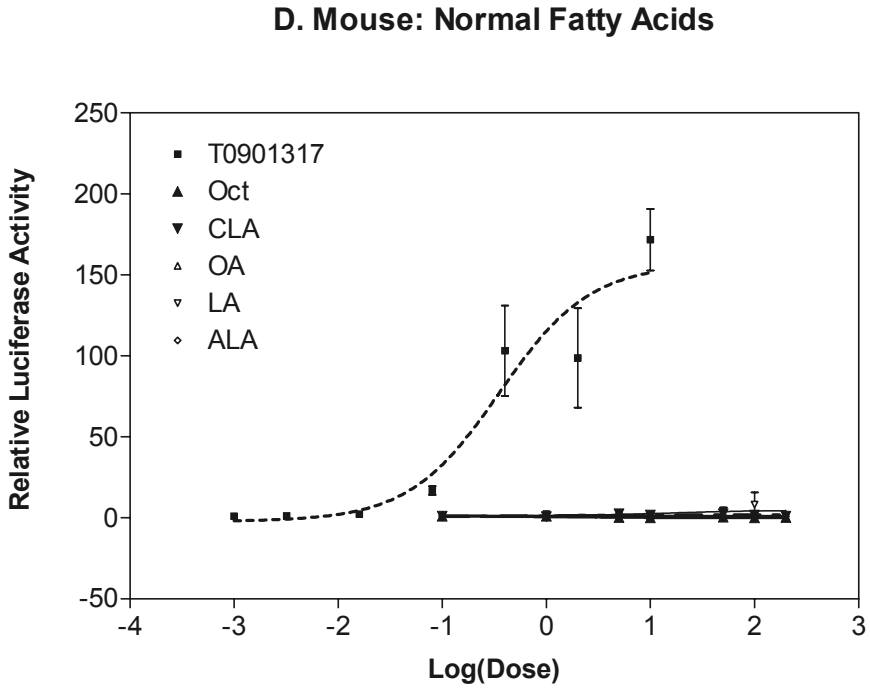
Survey of Nuclear Receptor Activity: LXR-β





Note: The PFOA ligand tested was the linear form of the molecule

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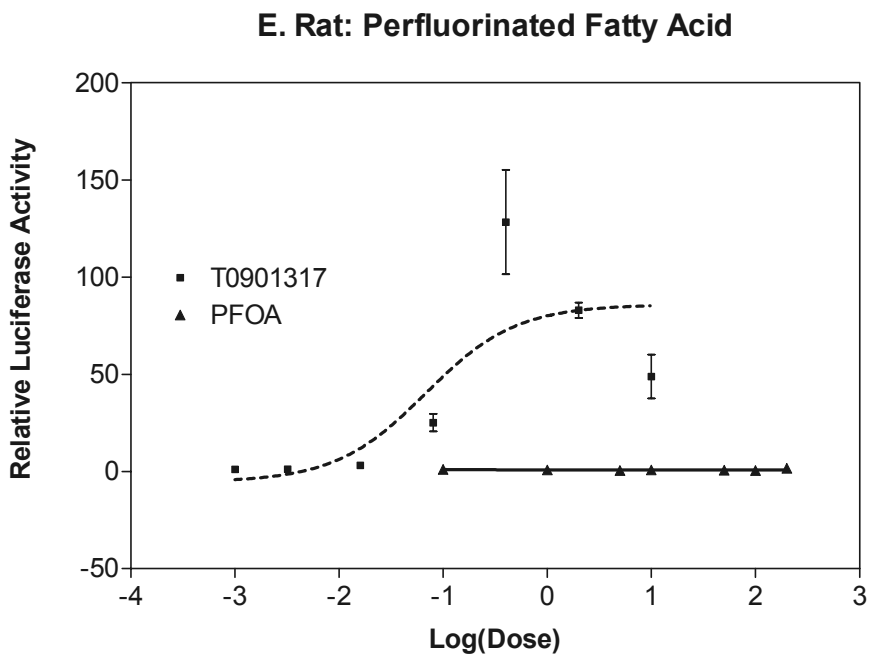
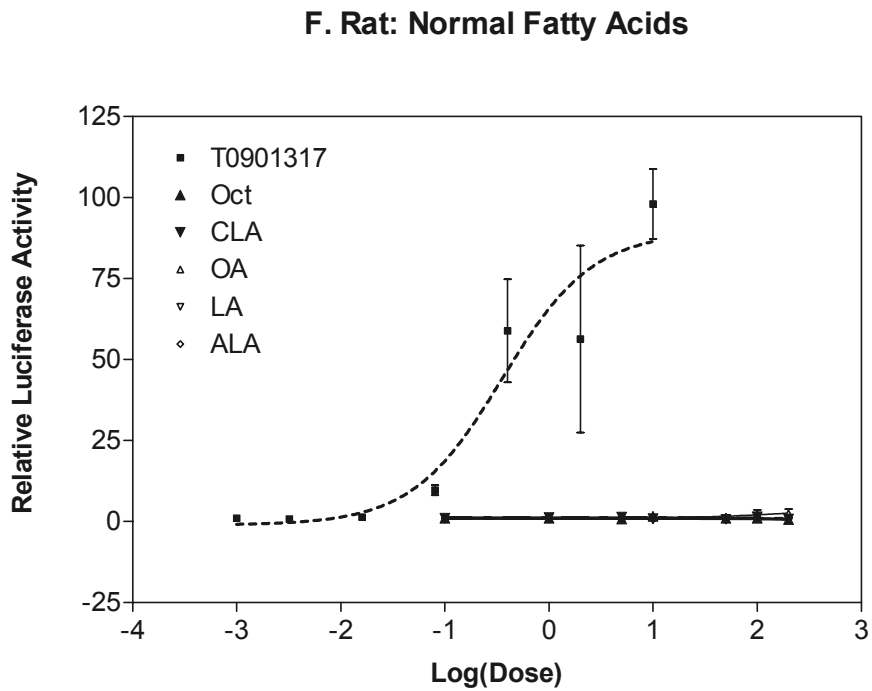
Survey of Nuclear Receptor Activity: LXR-β





Note: The PFOA ligand tested was the linear form of the molecule

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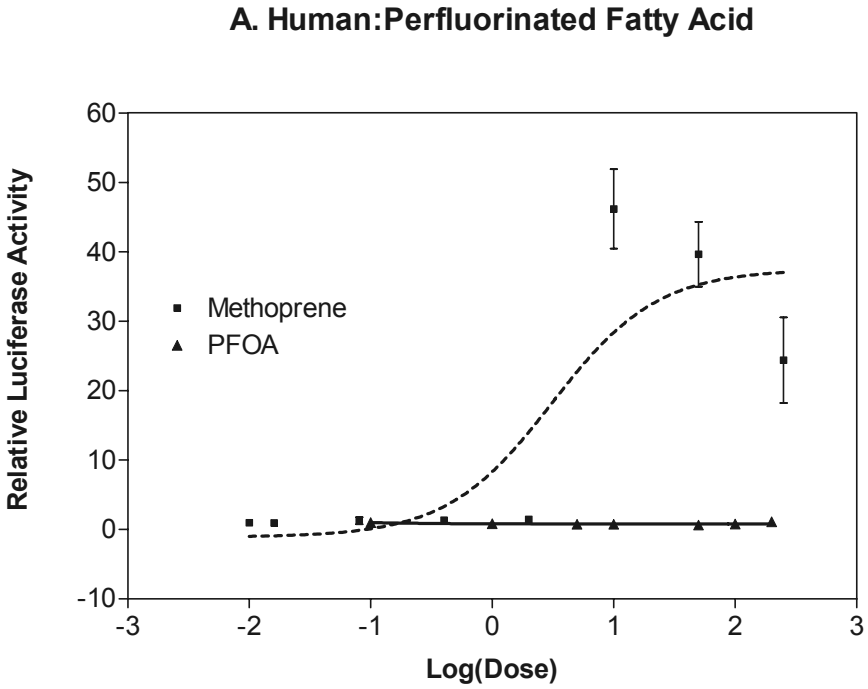
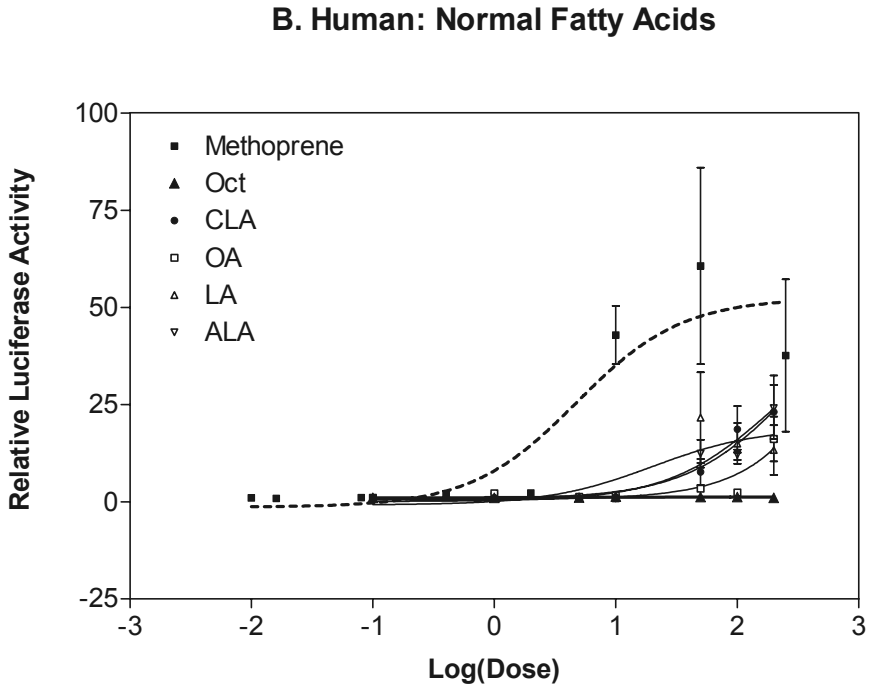
Survey of Nuclear Receptor Activity: RXR-α





Note: The PFOA ligand tested was the linear form of the molecule

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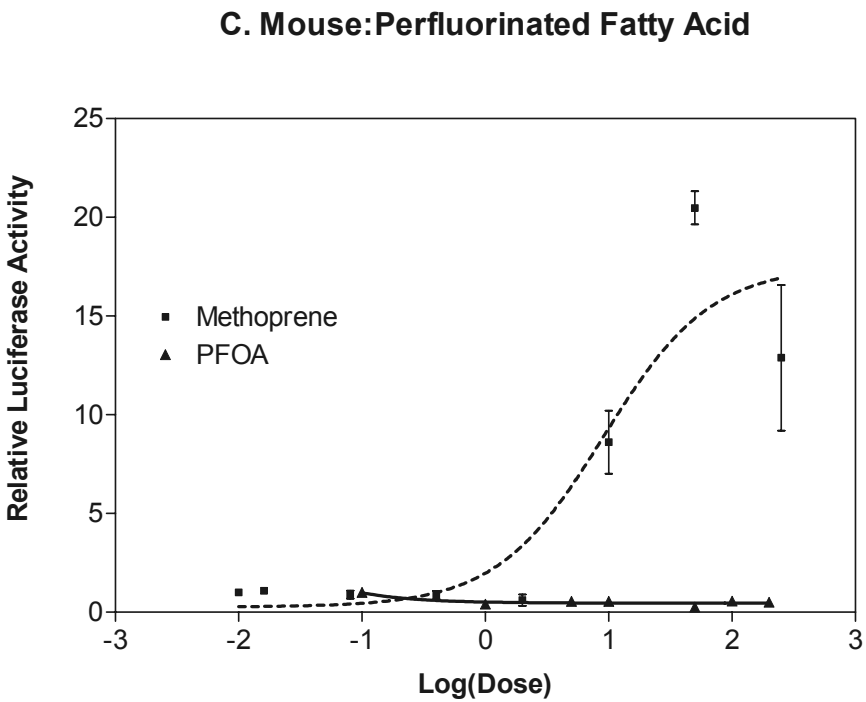
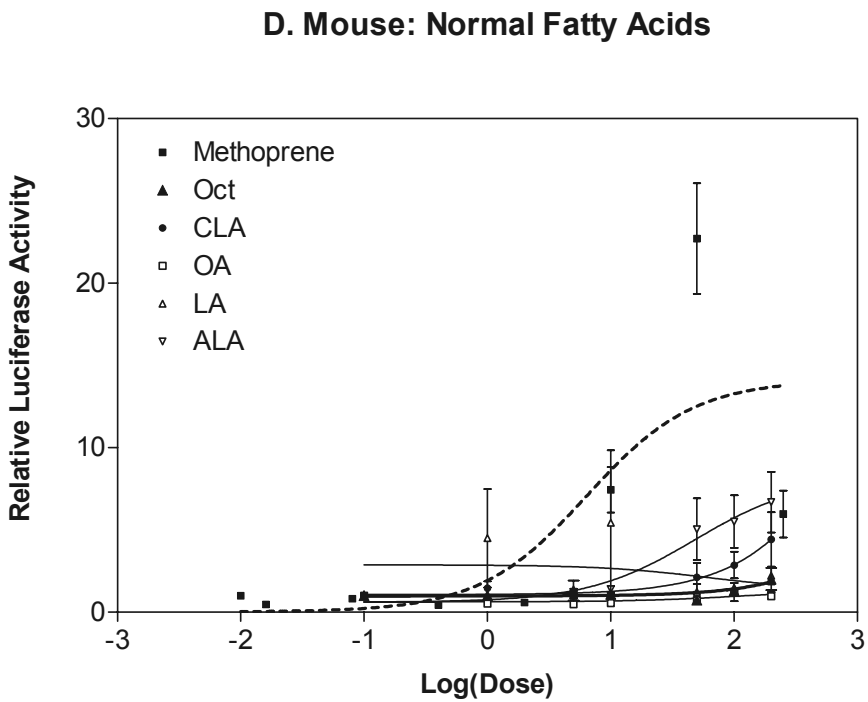
Survey of Nuclear Receptor Activity: RXR-α





Note: The PFOA ligand tested was the linear form of the molecule

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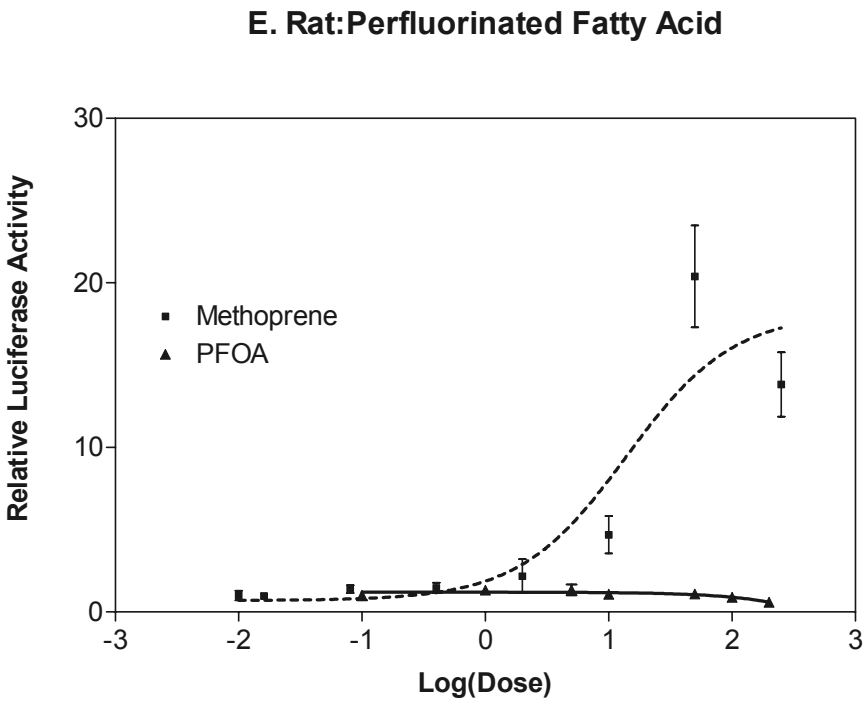
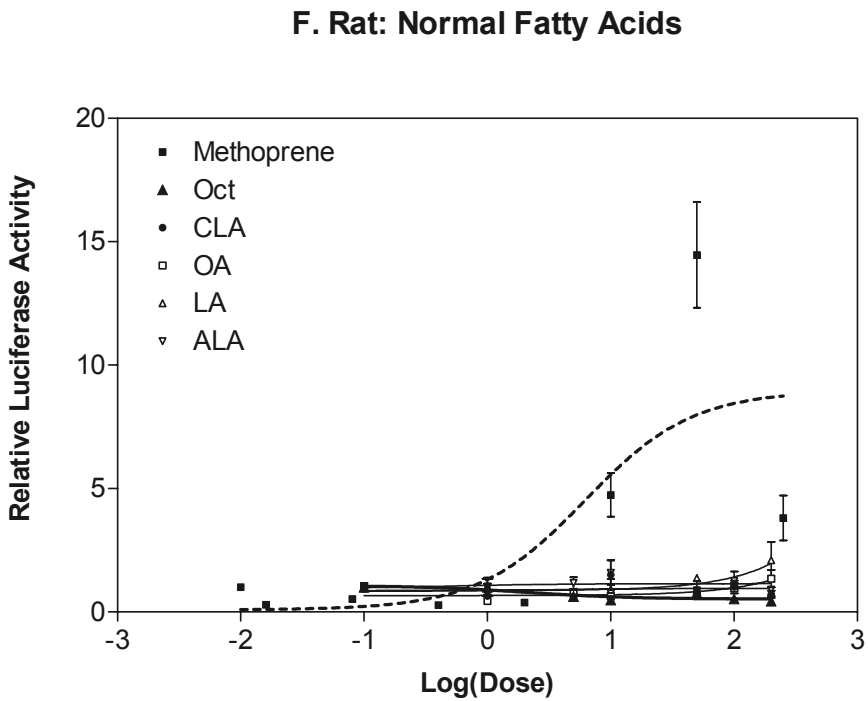
Survey of Nuclear Receptor Activity: RXR-α





Note: The PFOA ligand tested was the linear form of the molecule

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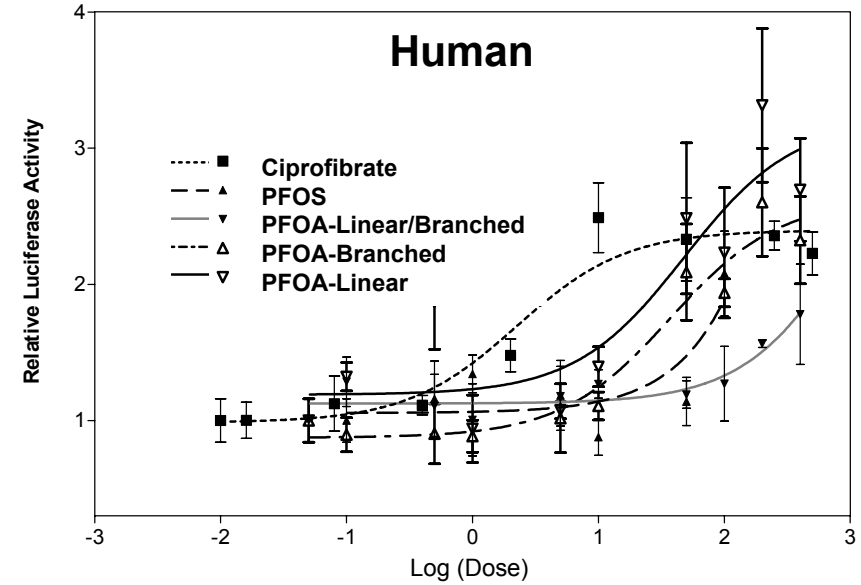
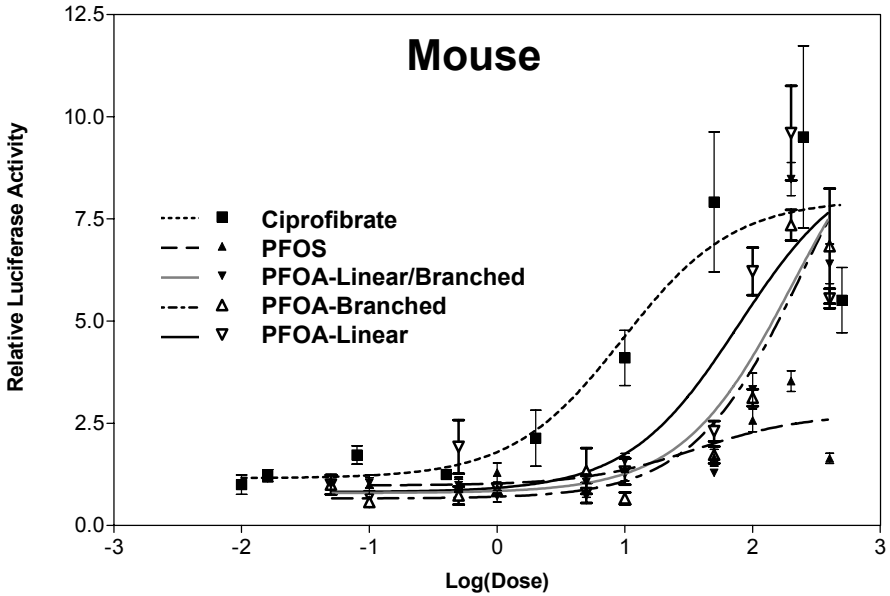
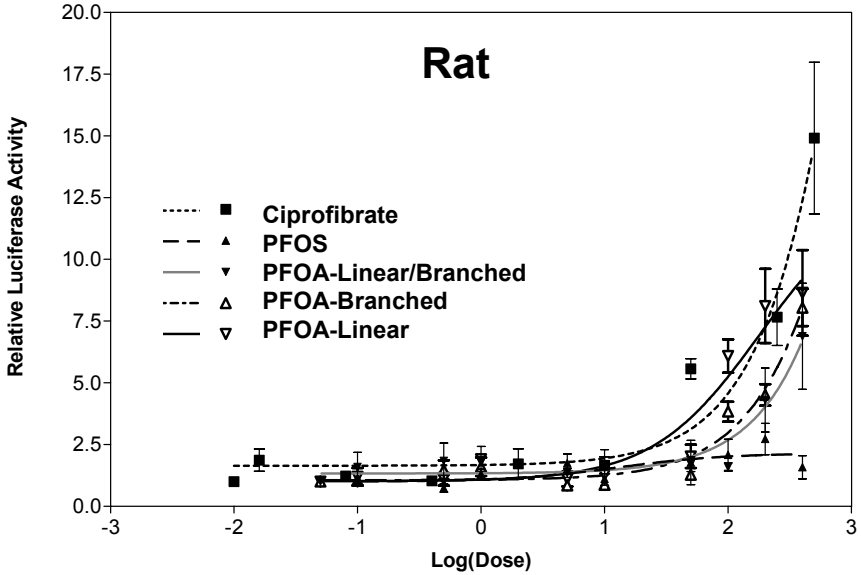
Exploration of PFOS and PFOA Isomers on PPAR-α







Caveats for Testing Mixtures

- Differential uptake of components
- Differential affinity for LBD
- Competitive interactions

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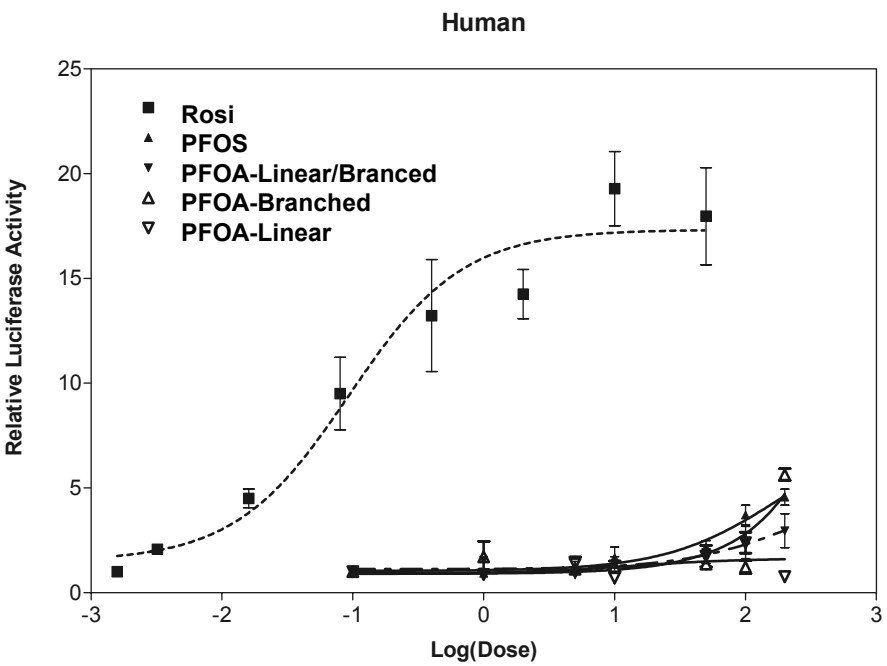
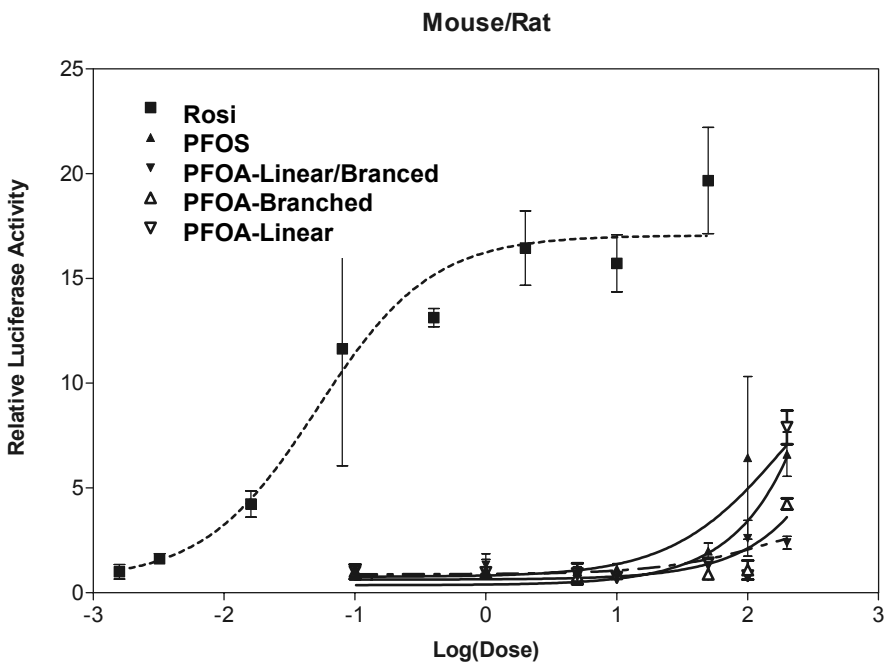
Exploration of PFOS and PFOA Isomers on PPAR-γ





Caveats for Testing Mixtures

- Differential uptake of components
- Differential affinity for LBD
- Competitive interactions

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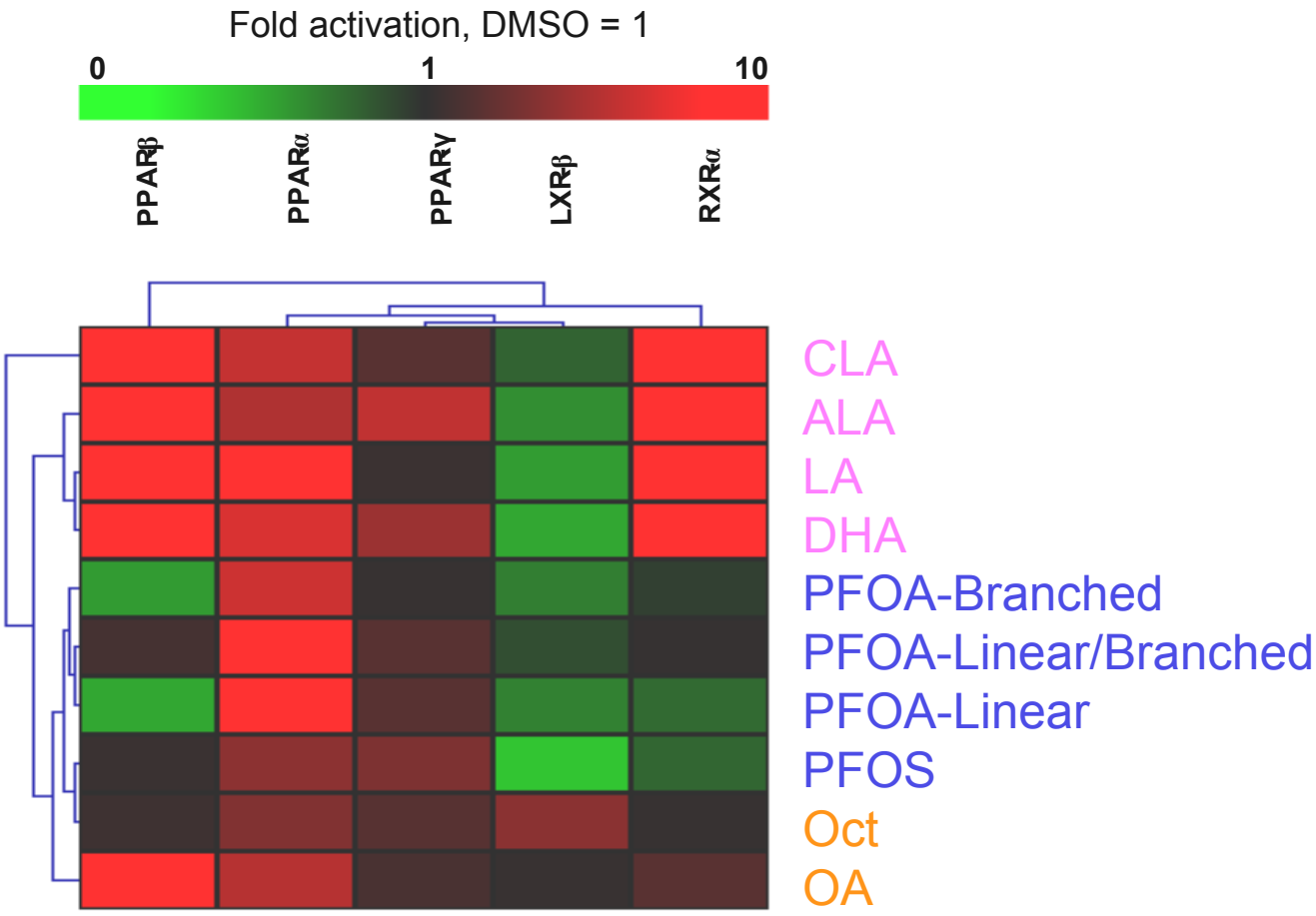
Hierarchical Cluster Analysis

(Fold activation at 100 μM* for human receptors)

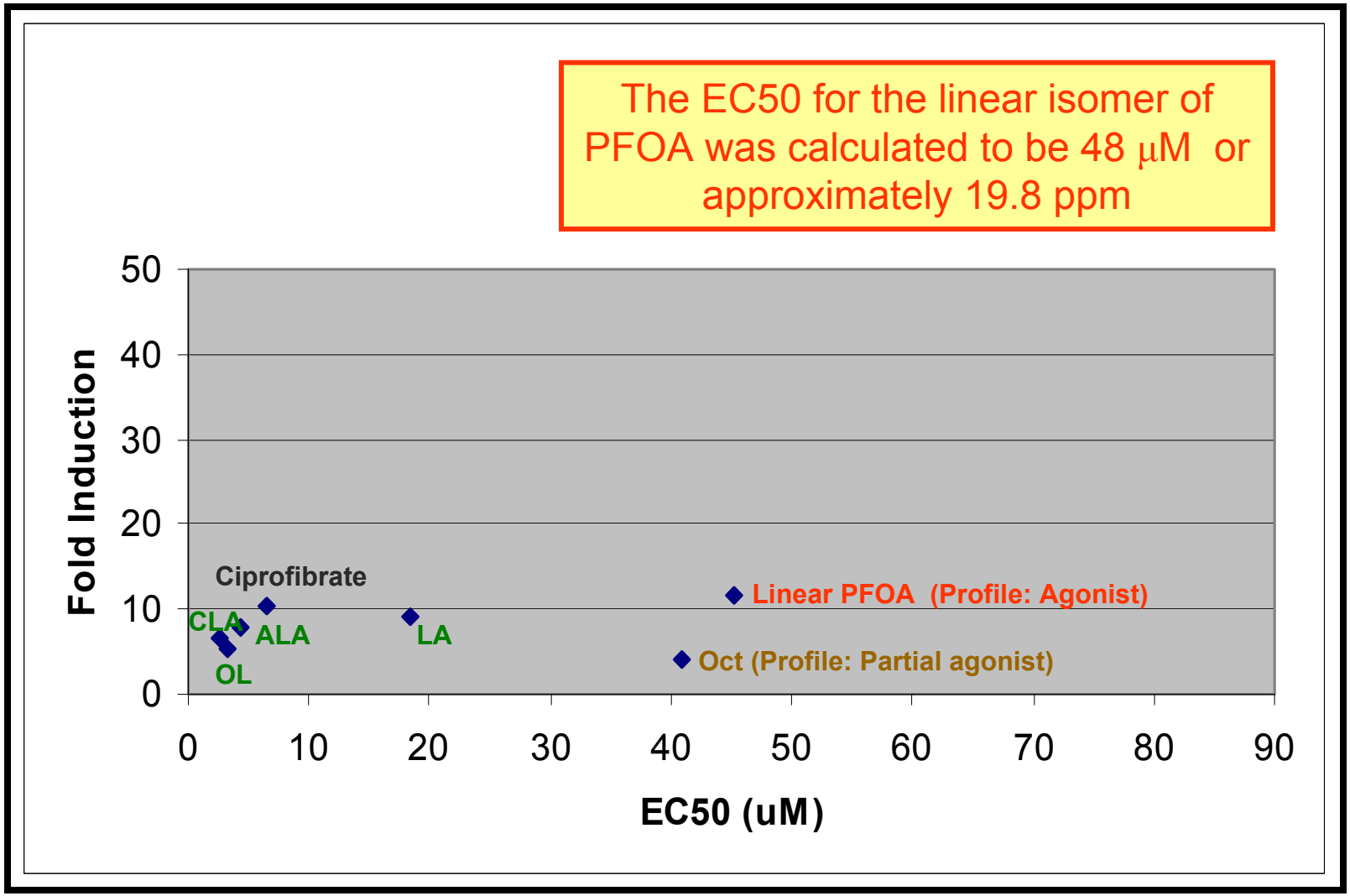


Long chain Polyunsaturated Fatty acids

Saturated and Monounsaturated Fatty acids

* Similar results were obtained at 50 and 200 μM

PFOA vs Dietary Fatty Acids vs Ciprofibrate Human PPAR-α



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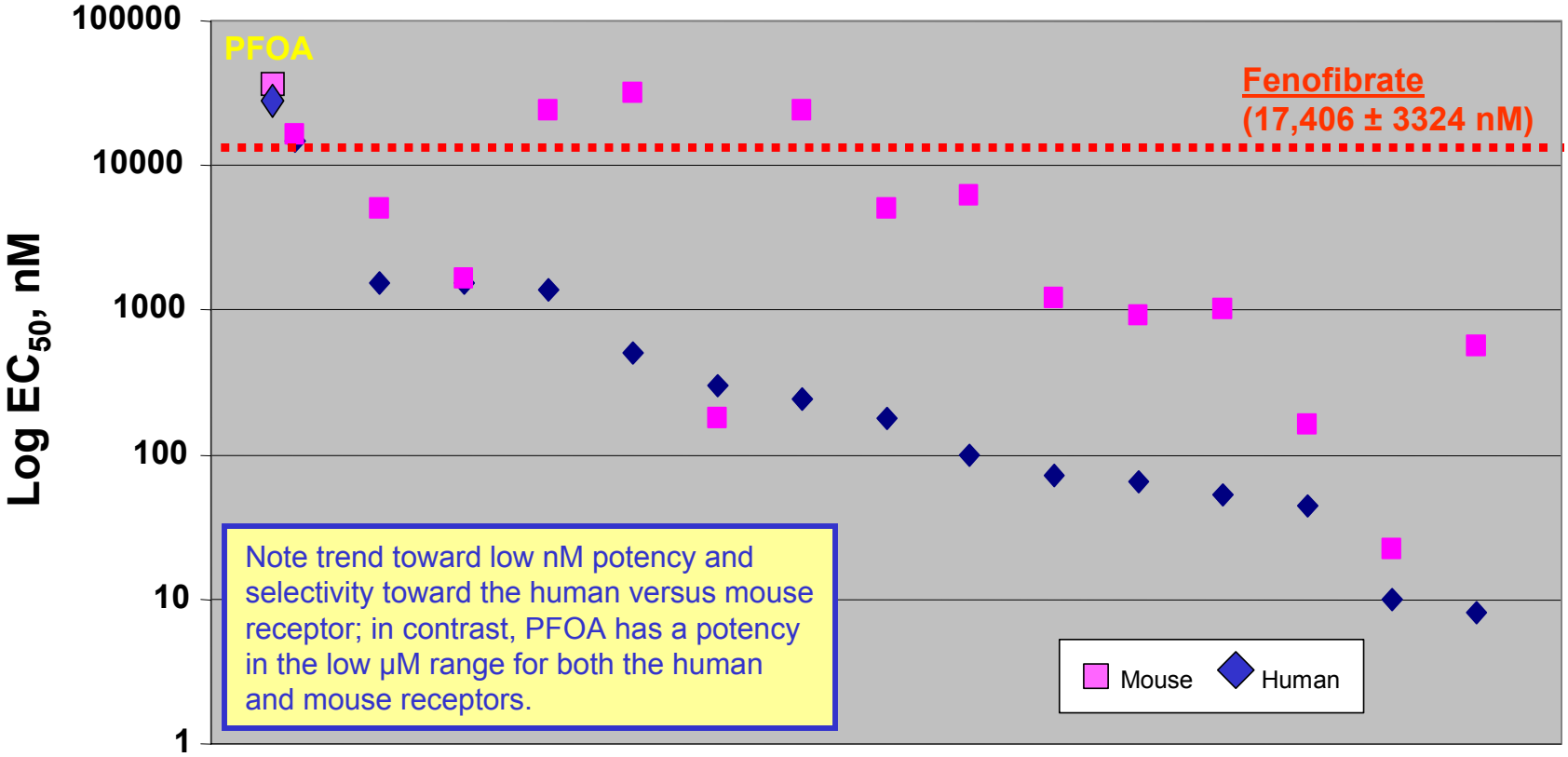
PFOA Differs from Drugs in Development *

* Adapted from Jeri El Hage, Ph.D.
FDA Presentation, 2005

(P , dual, pan agonists)



Candidates in Development
(Transactivation potency data are paired vertically)

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General Summary of the Ability of PFOA to Interact with the Ligand Binding Domain of Nuclear Receptors

- PPAR-α and to a much lesser extent PPAR-γ can be activated by PFOA and its isomers
- Compared to naturally occurring long-chain polyunsaturated fatty acids, the interaction of PFOA with nuclear receptors is more selective, but less potent
- PFOA is considerably less potent than representatives of the fibrate and thiazolinedione drug class currently on the market or in development

Perspectives Regarding Nuclear Receptor Profiling Experiments

1. Nuclear receptor profiling is a powerful new tool in an emerging area of biology

2. Chimeric nuclear receptor assays in cell culture systems are designed to limit biological complexity

3. Extrapolations from molecular biology data to anticipated biological response need to be done cautiously

4. Data generated with mixtures are of limited value due to confounding factors such as differential uptake, variable affinity for the binding domain, and competitive interactions