NTP
National Toxicology Program

Leveraging HTTK, Tox21, and ExpoCast for Prioritizing Potential Human Health Risk

Nisha S. Sipes, Ph.D.

National Toxicology Program (NTP)

National Institute for Environmental Health Sciences (NIEHS)

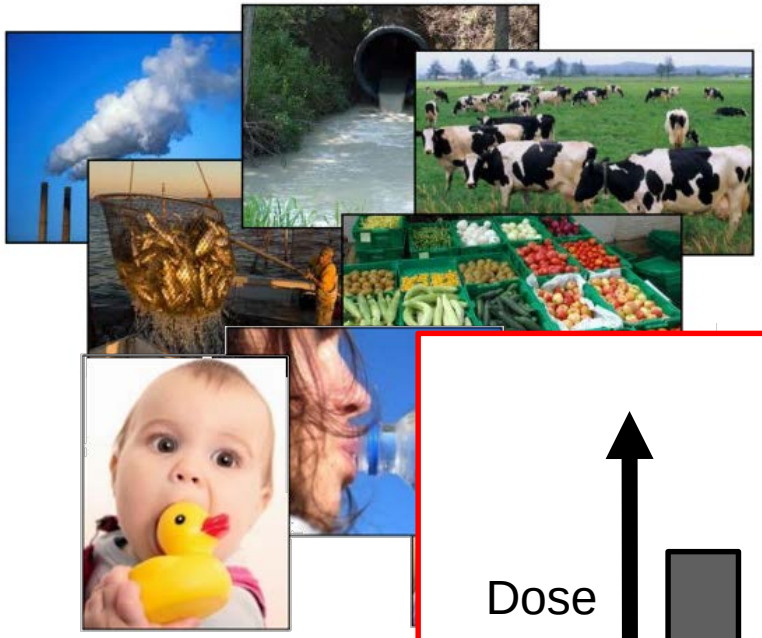
EPA's Computational Toxicology
Communities of Practice
May 25, 2017

The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the NTP, NIEHS, NIH, or the United States government

nisha.sipes@nih.gov

Examining Human Toxicity Risk

Exposure

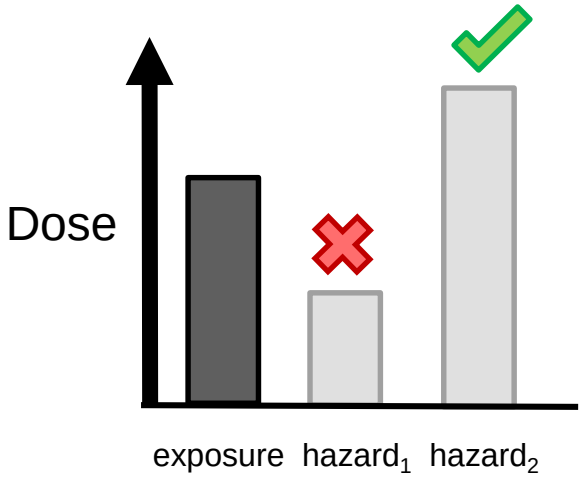


courtesy of J.F. Wamba

vs

compare *External* doses

Hazard

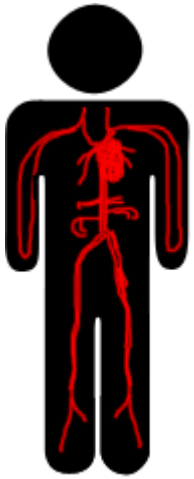


Assume toxicokinetics & biology are similar between humans & animal models

Predicting Human Toxicity Risk

Exposure vs Hazard

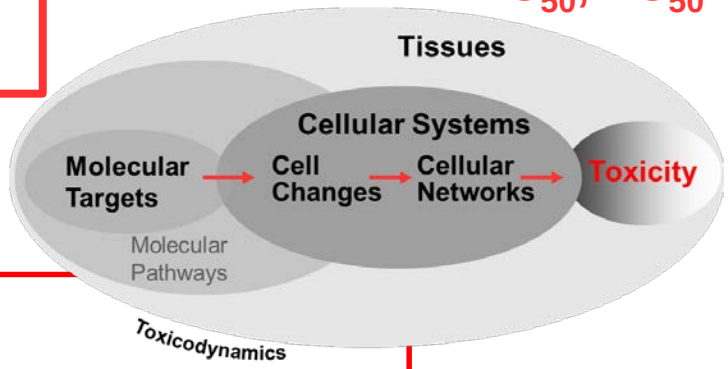
Toxicokinetics



plasma concentration (C_{max})

compare *Internal* doses

IC_{50}, AC_{50}



$$\frac{[I]}{K_i} \sim \frac{[I]}{IC_{50}} \sim \frac{Cmax}{AC_{50}}$$

FDA Guidance for Industry: Drug Interaction Studies (2006)

Estimating likelihood of *in vivo* interaction

$$\frac{[I]}{K_i} \sim \frac{[I]}{IC_{50}} \sim \frac{Cmax}{AC_{50}}$$

As this ratio increases, the likelihood of an interaction increases

'likely'	**Ratio ≥ 1**
'possible'	**1 > Ratio > 0.1**
'remote'	**Ratio ≤ 0.1**

efficacy > 40%

FDA Guidance for Industry: Drug Interaction Studies (2006)

Applying this approach to the Tox21/ToxCast dataset

https://ntp.niehs.nih.gov/go/tox21

$\frac{\text{Cmax}}{AC_{50}}$

10,000 chemicals

- including environmental & pharmaceutical
- *in vitro* HTS assays (>60)
- chemical subset (>1,000) tested in >800 HTS assays (US EPA's ToxCast)

➢ AC_{50} values, efficacy (filtered)
  - o *Hsieh JH et.al.,(2015). J Biomol Screen 20(7):887-97*
  - o *Filer DL et.al.,(2016). Bioinformatics 33(4):618-20*

➢ C_{max} values: *in vivo* human (~500)
  - o *DrugMatrix*

1. Evaluate with pharmaceuticals
2. Apply approach to the entire dataset

$\frac{C_{max}}{AC_{50}}$ case studies – targets for pharmaceuticals

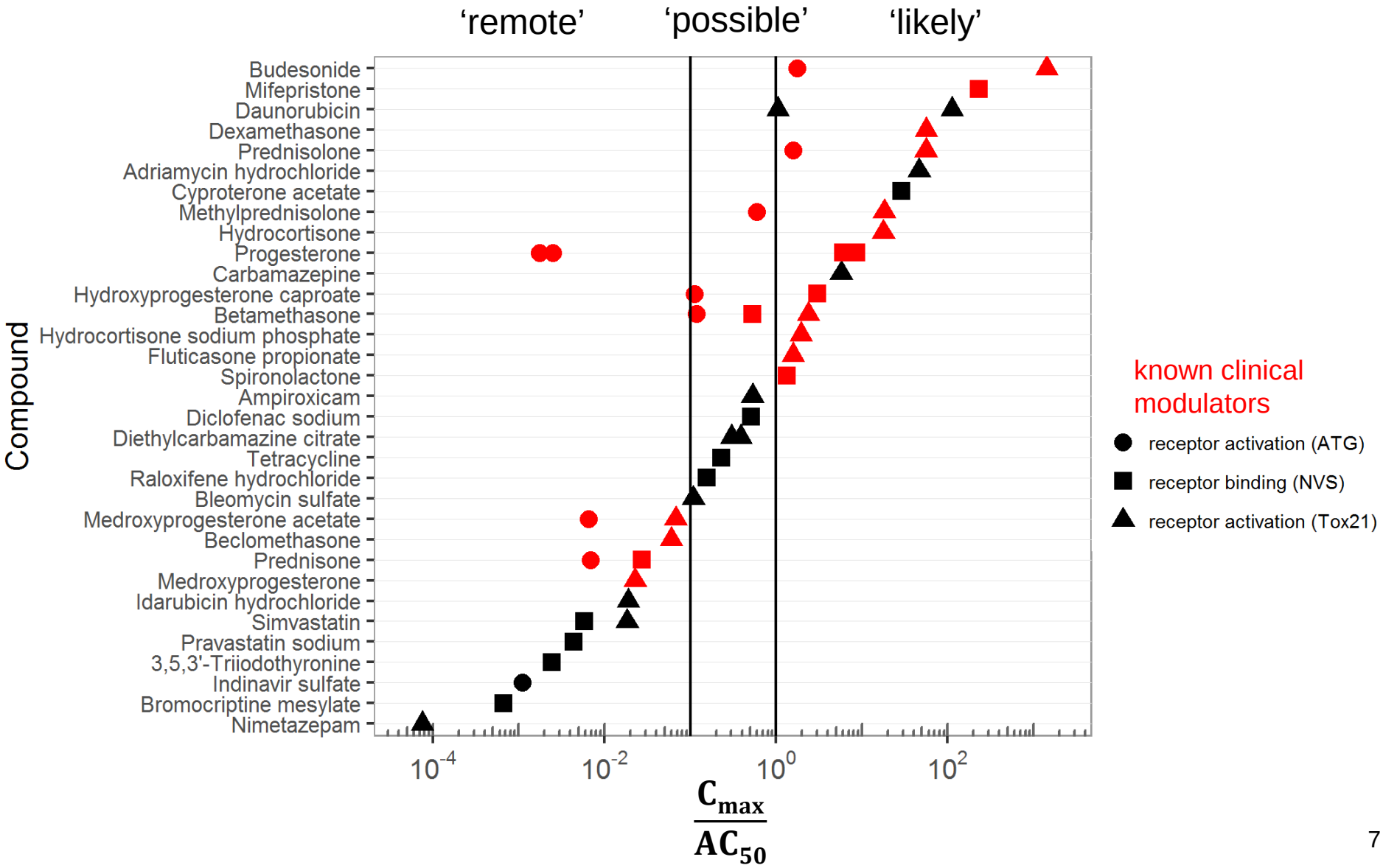
Glucocorticoid Receptor (GR)

- 3 HTS assays
  - receptor activation (HepG2, HeLa), receptor binding
- 30 pharmaceuticals
- Positive controls: dexamethasone and other corticosteroids

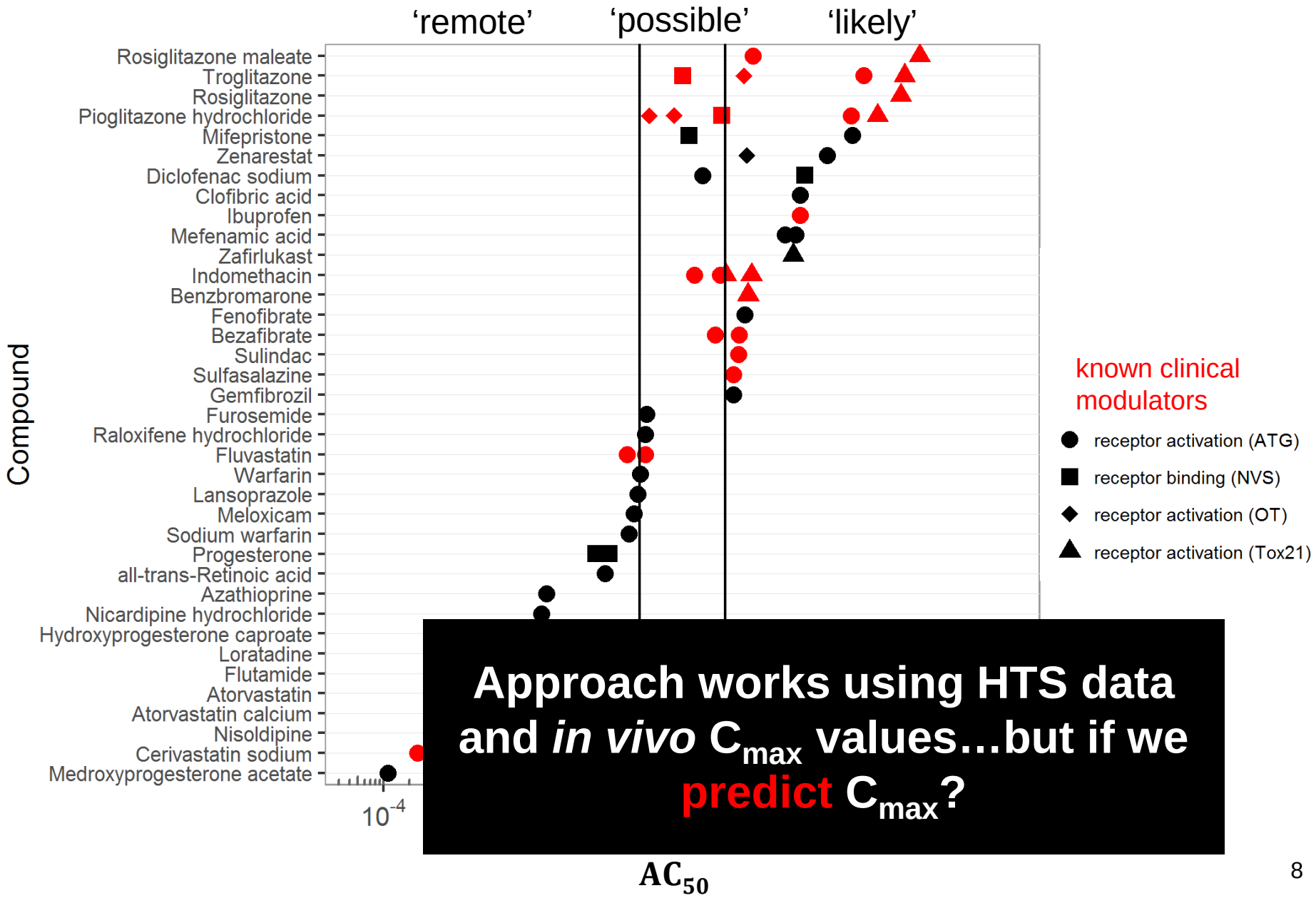
Peroxisome Proliferator-Activated Receptor Gamma (PPARγ)

- 5 HTS assays
  - receptor activation (HepG2, Hek293T), receptor binding
- 45 pharmaceuticals
- Positive controls: pioglitazone, rosiglitazone, troglitazone

Known GR modulators sort to the top



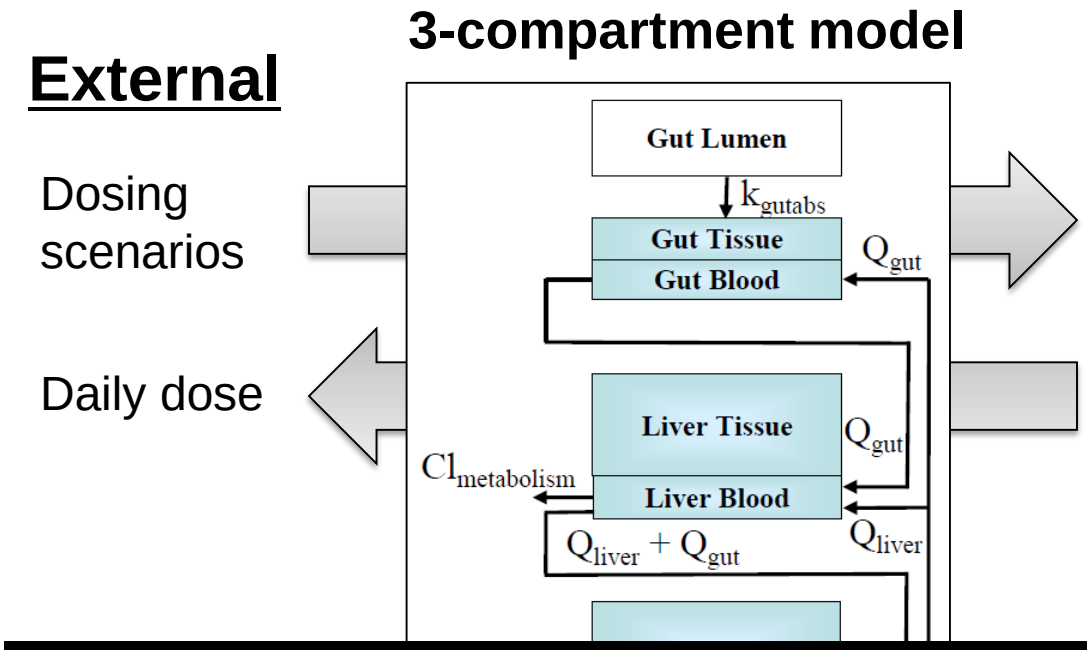
Known PPARγ modulators sort to the top



Approach works using HTS data and *in vivo* C_{max} values…but if we predict C_{max}?

High-throughput Toxicokinetics (HTTK) to estimate C_{max}

Models parameterized using physicochemical properties (QSARs) and *in vitro* parameters (i.e., f_{up} & CL_{int})



only available for ~500 chemicals

Need to **estimate** *in silico*

- *in silico* predictions: Simulations Plus, ADMET Predictor™ 7.2
  - f_{up} & CL_{int}(∑CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4)

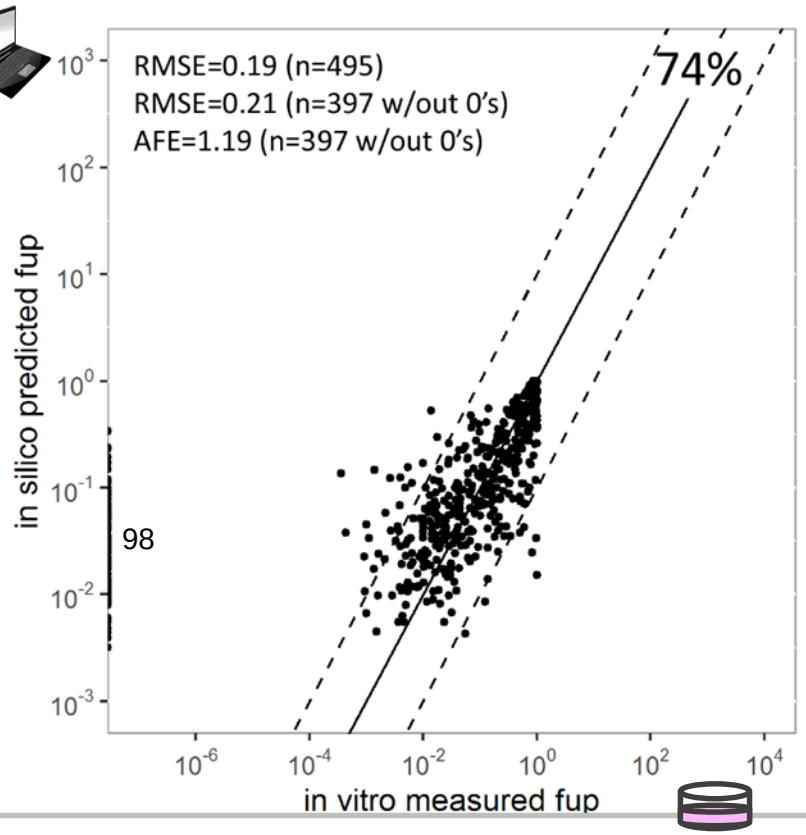
bolism
n liver) or
ltration

Pearce R et. al., Journal of Statistical Software, in press

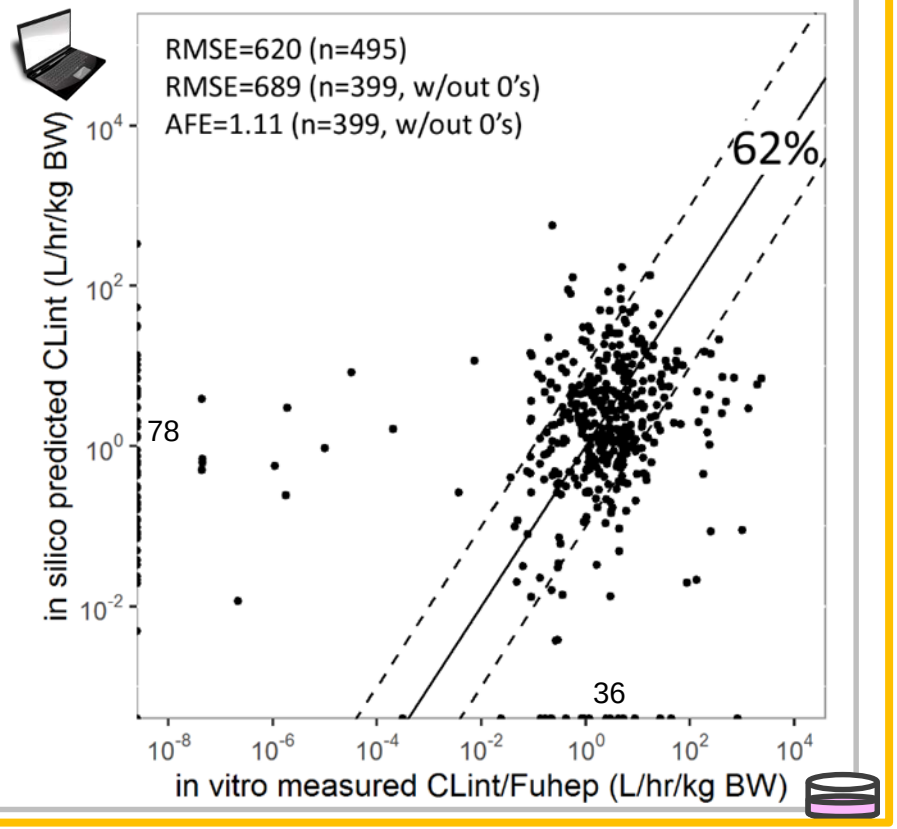
https://cran.r-project.org/web/packages/httk/index.html

In silico f_{up} & CL_{int} values are comparable to *in vitro*

f_{up} – plasma protein binding

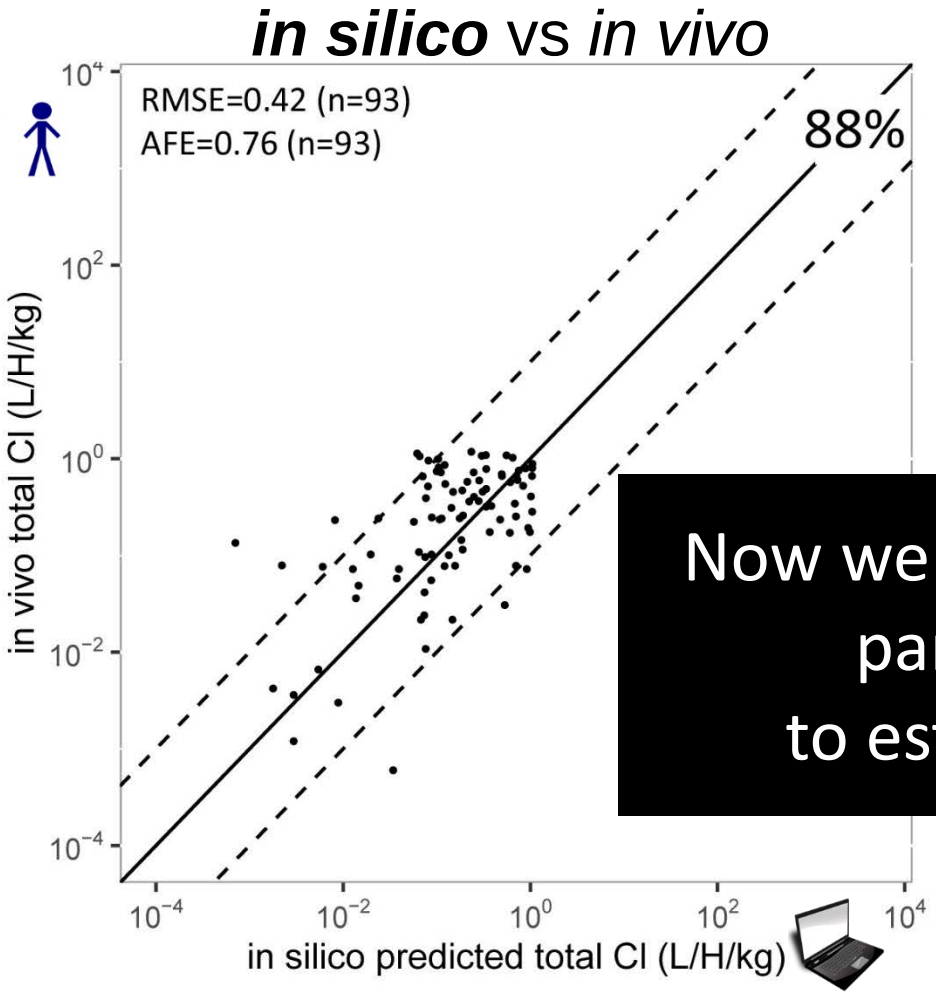


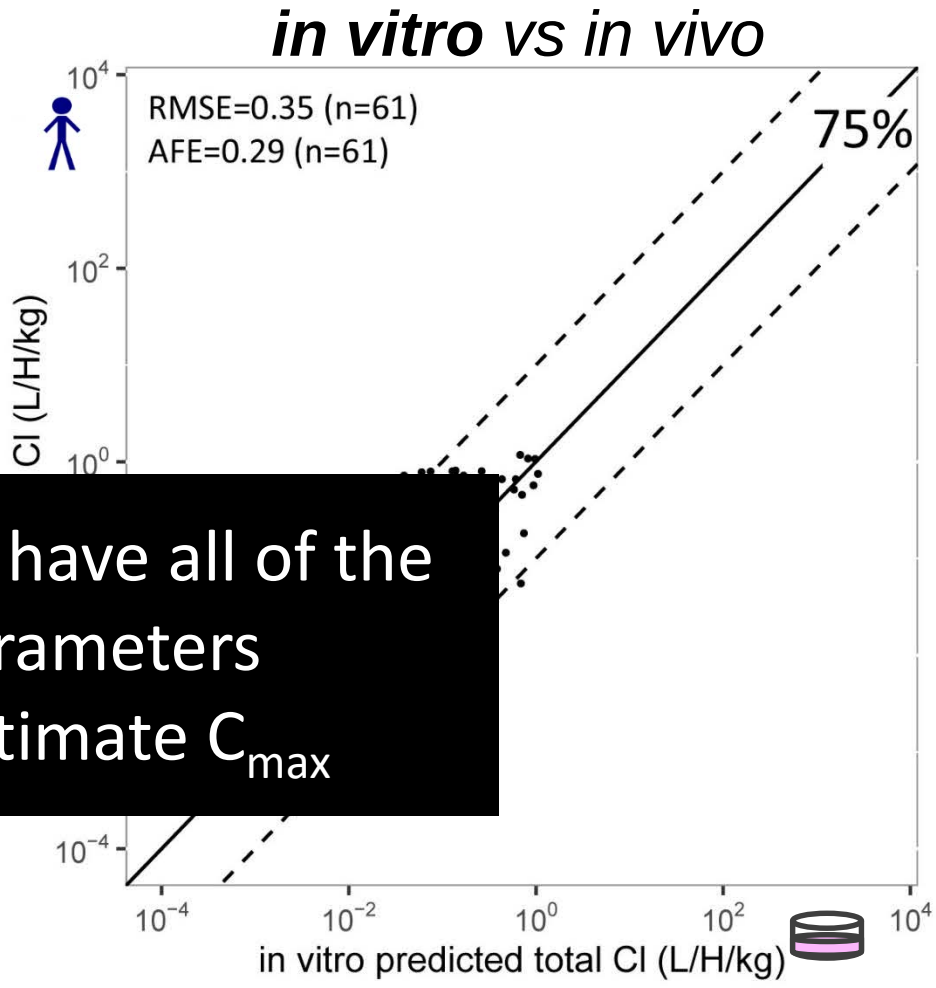
CL_{int} – intrinsic metabolic clearance



Sipes NS et. al., submitted

Total CL values are comparable: *in silico, in vitro, in vivo*



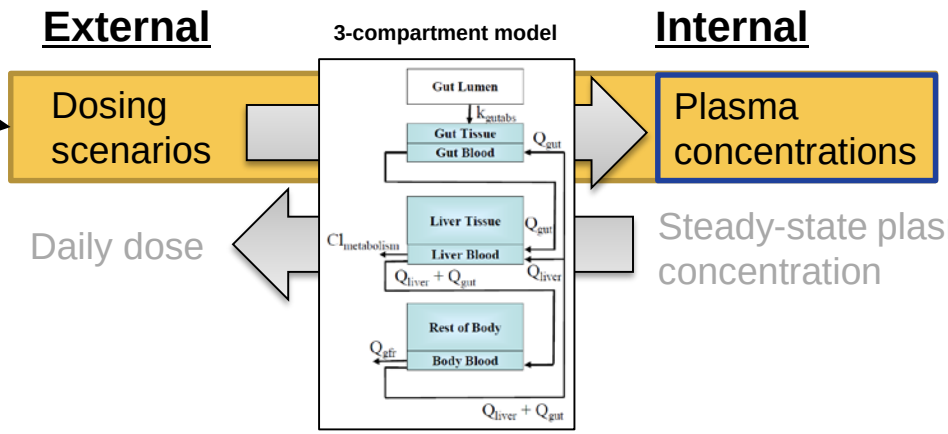


Now we have all of the parameters to estimate C_{max}

Sipes NS et. al., submitted

Comparing C_{max} using therapeutic scenario

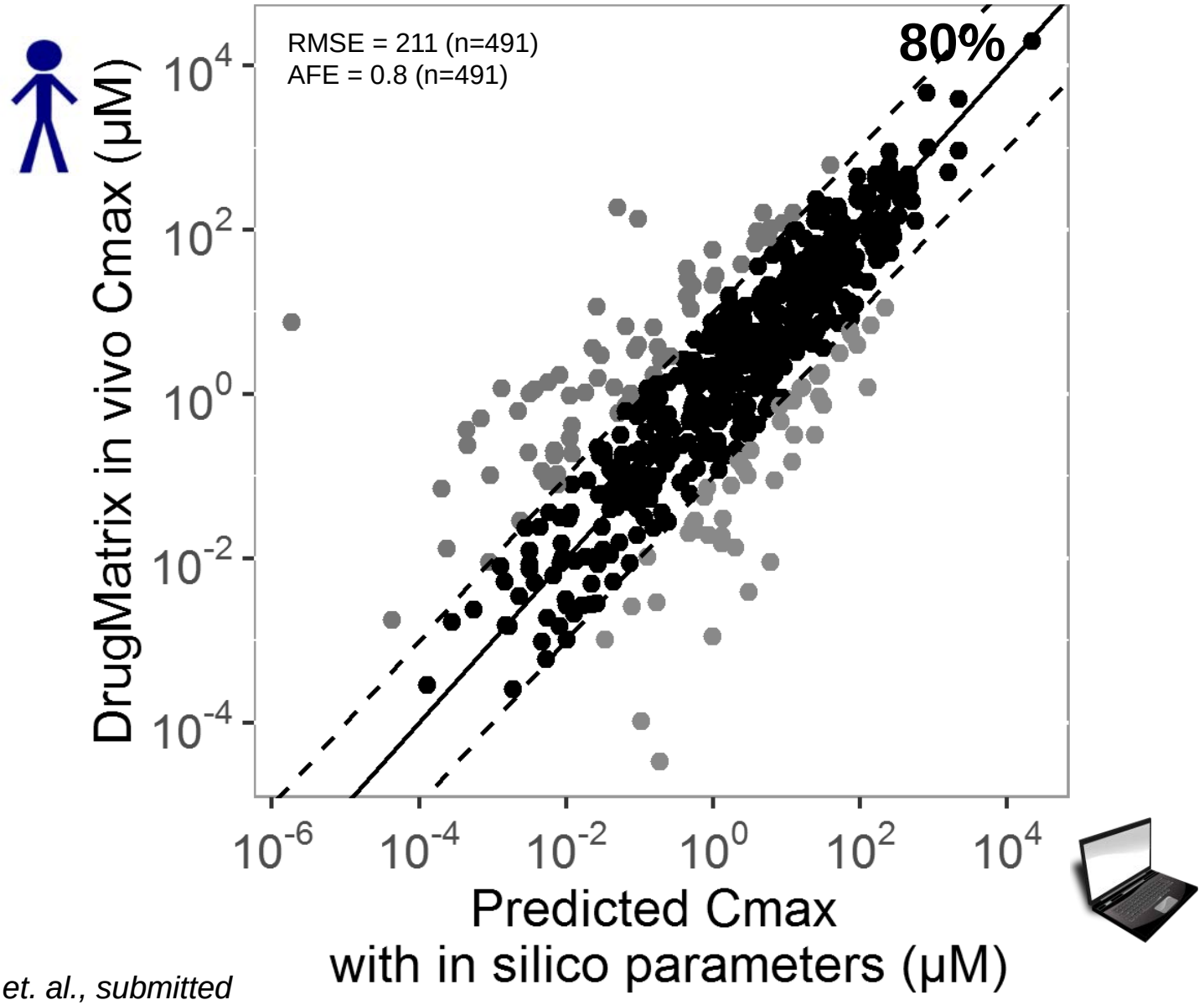
Therapeutic dosing scenarios
corresponding to *in vivo* C_{max}



&

Compare with *in vivo* human C_{max}

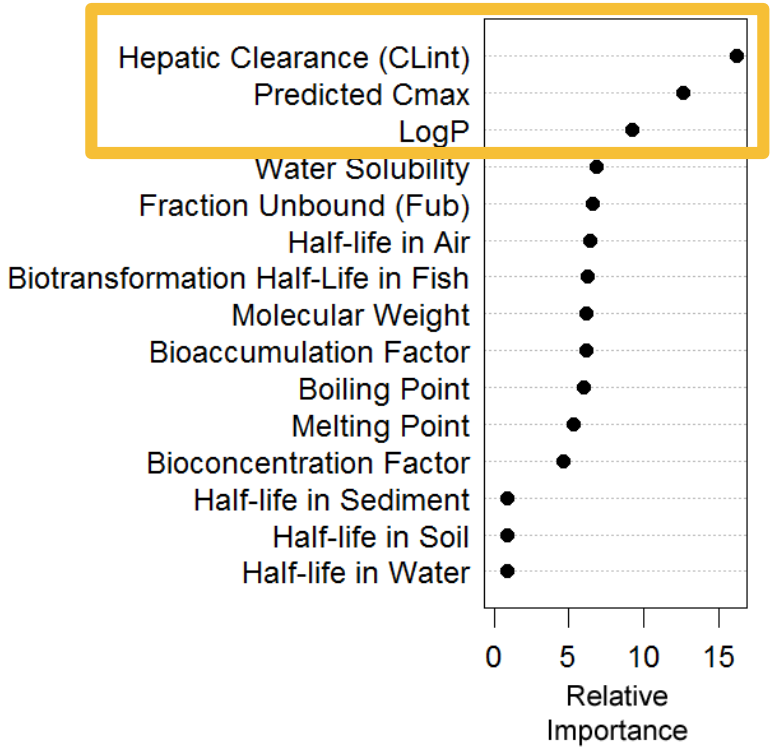
In silico C_{max} predictions are comparable to *in vivo*



Sipes NS et. al., submitted

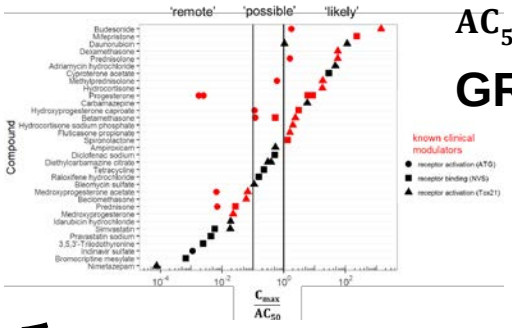
Characterizing confidence in estimating Cmax

importance based on the random forest model



	10 fold under predicted	within 10 fold	10 fold over predicted
Sensitivity	85%	66%	40%
Specificity	82%	68%	87%
Balanced Accuracy	82%	66%	83%

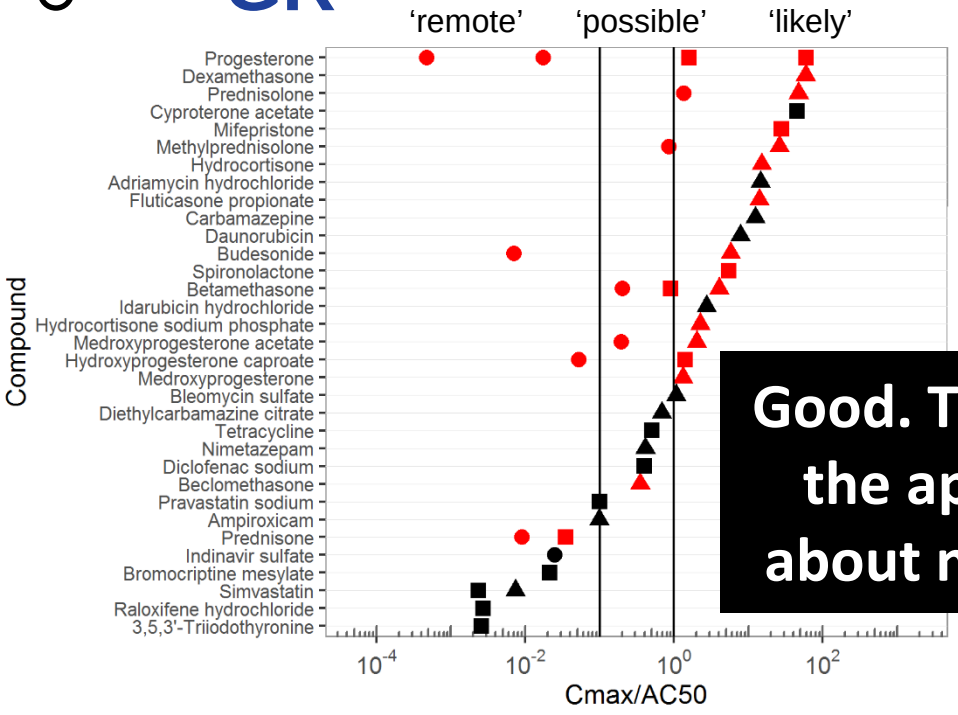
How do $\frac{C_{max}}{AC_{50}}$'s look when using predicted C_{max}?



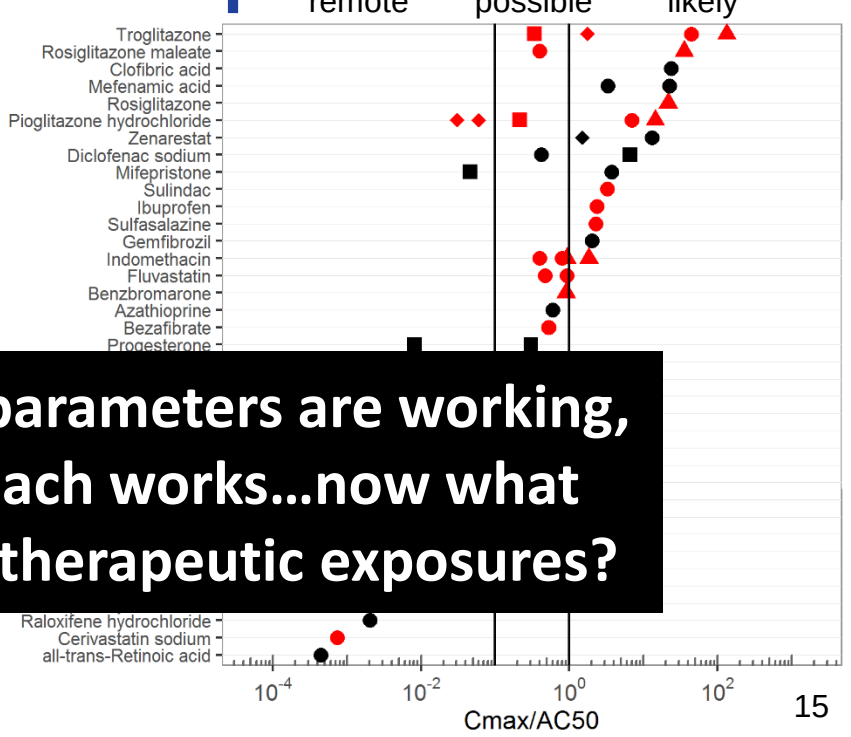
$\frac{C_{max}}{AC_{50}}$ case studies

GR & PPARγ

GR



PPARγ



Good. The parameters are working, the approach works…now what about non-therapeutic exposures?

What is the likelihood of *in vivo* activity based on HTS data at estimated median daily exposures?

'likely' $\frac{Cmax}{AC50} \geq 1.0$ ⟶

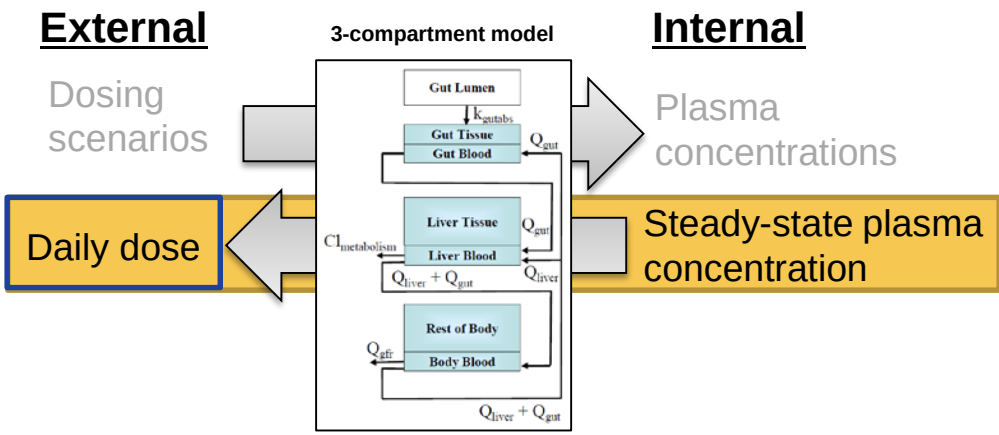
'possible' $1 > \frac{Cmax}{AC50} > 0.1$ ⟶

$AC_{50} >$

$C_{max} \geq AC_{50}$

$C_{max} > 0.1 * AC_{50}$

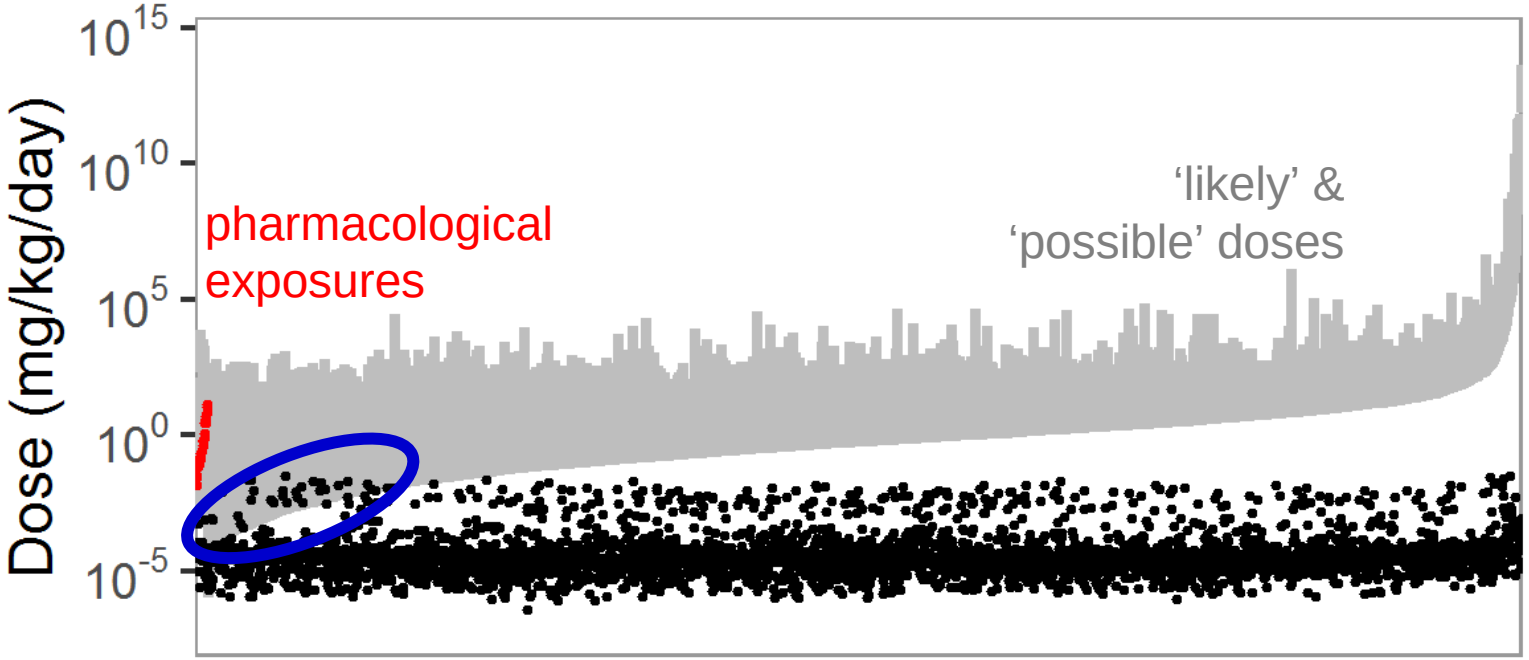
Compare with US EPA's ExpoCast estimated median exposures &



J Wambaugh et. al., Environ Sci Technol, 2014, *48*(21), pp 12760–7

& efficacy ≥ 40%

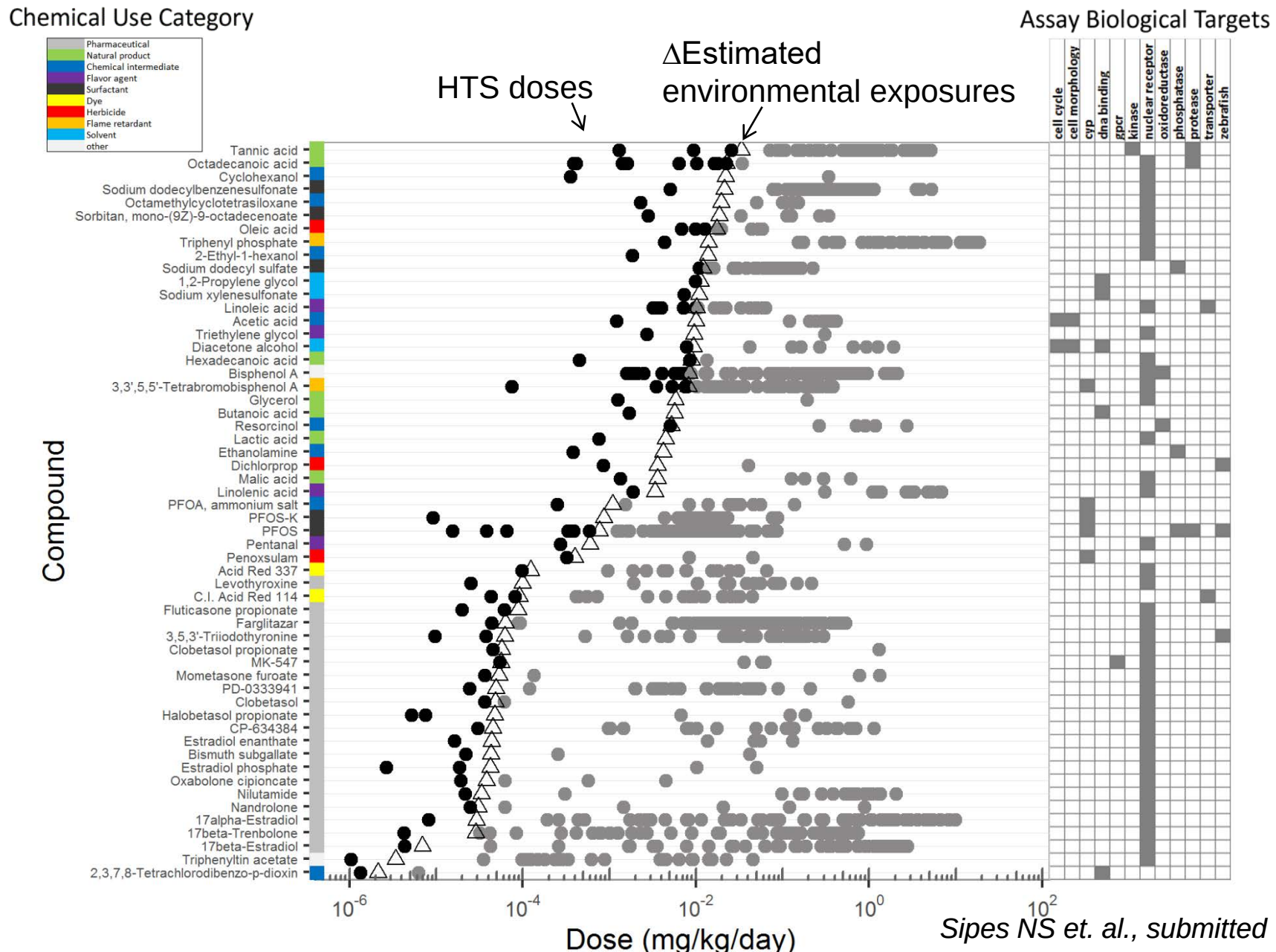
49,789 active compound-assay pairs
3,941 unique compounds, 746 assays



	'likely' & 'possible'
chem-assay pairs	114
unique chemicals	56
unique assays	65

Sipes NS et. al., submitted

‘likely’ & ‘possible’ doses for human *in vivo* interaction compared with estimated daily exposure



Sipes NS et. al., submitted

Next steps toward risk prioritization

Gather more information via literature or experiments

- chemical-biological interactions (*in vitro* & *in vivo)*
- exposures (environmental & occupational)
- parameter estimations

Knowledge gaps

Models will continue to improve with the generation of more publicly available data on thousands compounds

Limited to current biological targets & assay conditions

- Phase III Tox21 includes expanding assays to evaluate toxicogenomics approaches, potentially revealing lower chemical-target potencies

Universal cutoff for C_{max}/AC_{50} – does it exist?

Human variability

Domain of applicability

- QSAR models for f_{up} & CL_{int}
- Likely route of metabolism

Other methods and/or parameters

- AUC - FDA Guidance for Industry: Drug Interaction Studies (2012)
- POD vs AC_{50}

Lessons learned

Intuitive fit-for-purpose framework to prioritize chemicals for a simple risk assessment framework

Novelty

- Uses approach similar to FDA by considering *in vivo* plausibility, estimating likelihood of compound-biological target interaction *in vivo*
- Relies on *in silico* TK parameters
- Applies approach to entire Tox21/ToxCast data, while featuring a conservative plasma concentration estimate

Tox21/ToxCast IVIVE Web Application

National Toxicology Program
U.S. Department of Health and Human Services

Home | Approach | Model | Data | Search by chemical | Browse

High-Throughput IVIVE Daily Dose Equivalent

A goal within the Tox21 collaboration is to research, develop, validate, and translate innovative test methods that will better predict how chemicals may affect humans and the environment. Tox21 and ToxCast efforts have screened ~10,000 chemicals with limited biological information in hundreds of high-throughput screening (HTS) *in vitro* assays. Translating these HTS data into a common language among toxicologists, specifically risk assessors, is an important next step toward making the data useful. This website allows users to estimate doses at which chemical-biological interactions are "likely", "possible", and "possible w/10-fold safety factor" for humans *in vivo*.

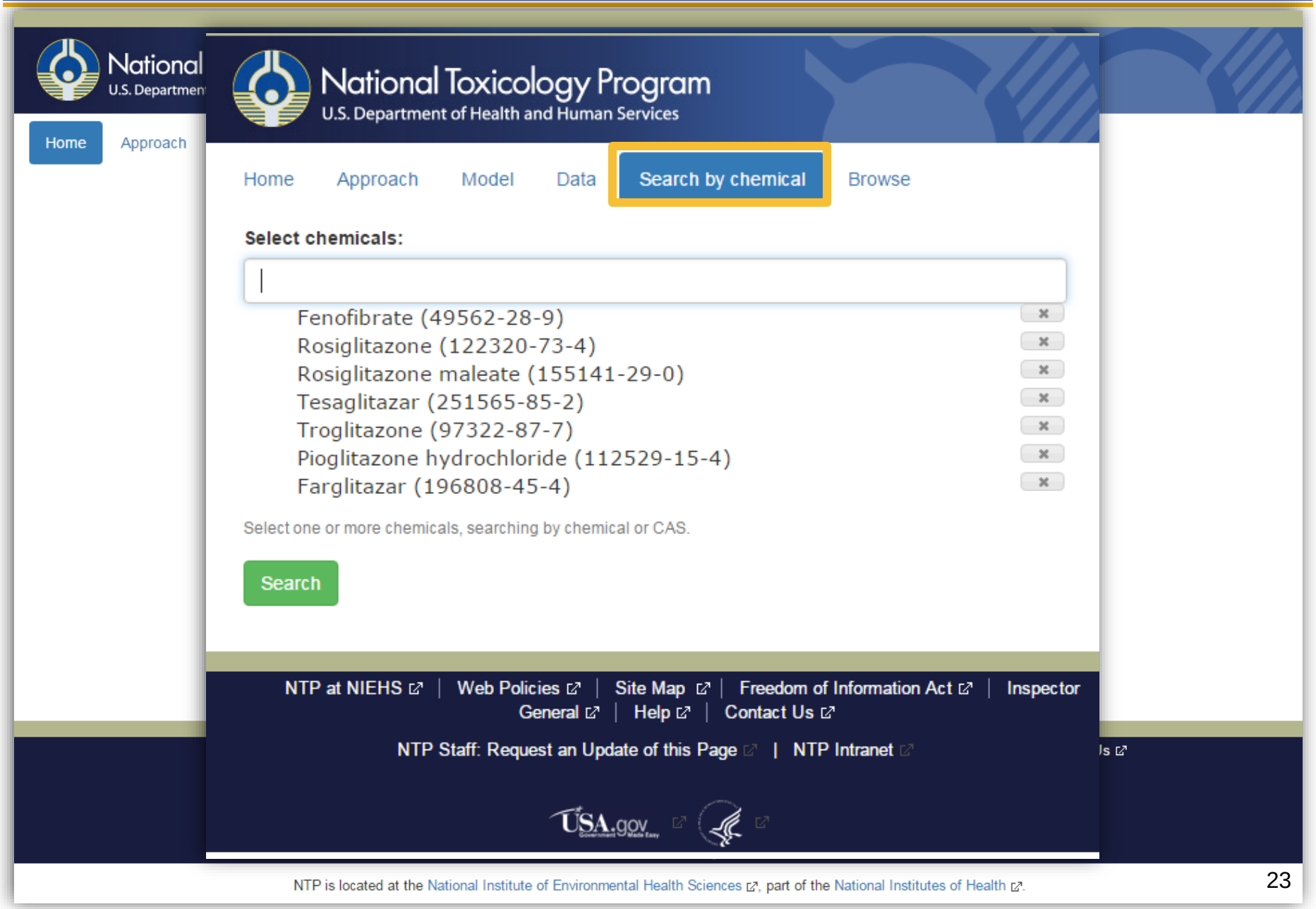
This web-application is a companion to Sipes NS et. al.: [in review]

NTP at NIEHS | Web Policies | Site Map | Freedom of Information Act | Inspector General | Help | Contact Us
NTP Staff: Request an Update of this Page | NTP Intranet

USA.gov

NTP is located at the National Institute of Environmental Health Sciences, part of the National Institutes of Health.

Tox21/ToxCast IVIVE Web Application



Tox21/ToxCast IVIVE Web Application

Nation
U.S. Departn

Home Approach

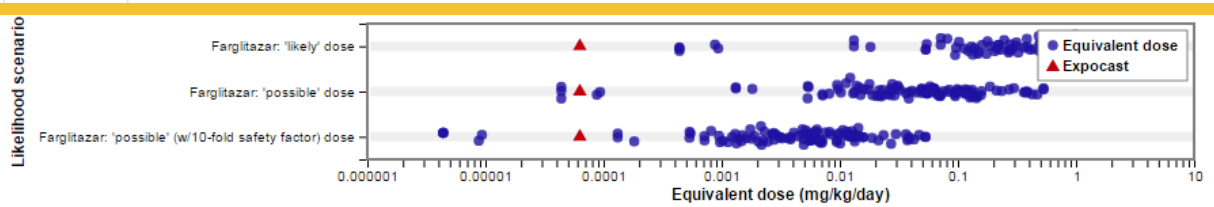
Farglitazar

CAS #: 196808-45-4 (view on EPA comptox dashboard)

Active in **106** Tox21/ToxCast HTS assays (filtered data)

Raw data | Assay summaries

Download data



Assay-specific results:

Chemical	Assay name	Intended target family	AC_{50} (μM)	Expocast dose (mg/kg/day)	'Likely' equivalent dose (mg/kg/day)	'Possible' equivalent dose (mg/kg/day)	'Possible' (w/10-fold safety factor) equivalent dose (mg/kg/day)
Farglitazar	ATG_ERE_CIS_up	nuclear receptor	0.01	6.30E-5	4.39E-4	4.39E-5	4.39E-6
Farglitazar	ATG_PPARg_TRANS_up	nuclear receptor	0.01	6.30E-5	4.39E-4	4.39E-5	4.39E-6
Farglitazar	ATG_PPRE_CIS_up	nuclear receptor	0.01	6.30E-5	4.39E-4	4.39E-5	4.39E-6
Farglitazar	NVS_NR_hPPARg	nuclear receptor	0.02	6.30E-5	8.78E-4	8.78E-5	8.78E-6
Farglitazar	NVS_NR_hPPARa	nuclear receptor	0.021	6.30E-5	9.34E-4	9.34E-5	9.34E-6
Farglitazar	OT_PPARg_PPARgSRC1_0480	nuclear receptor	0.3	6.30E-5	0.013	0.001	1.32E-4
Farglitazar	OT_PPARg_PPARgSRC1_1440	nuclear receptor	0.3	6.30E-5	0.013	0.001	1.32E-4
Farglitazar	ATG_PPARa_TRANS_up	nuclear receptor	0.414	6.30E-5	0.018	0.002	1.82E-4
Farglitazar	NVS_ENZ_hBACE	protease	1.216	6.30E-5	0.053	0.005	5.34E-4

Tox21/ToxCast IVIVE Web Application

Nation
U.S. Depart

Home | Approac

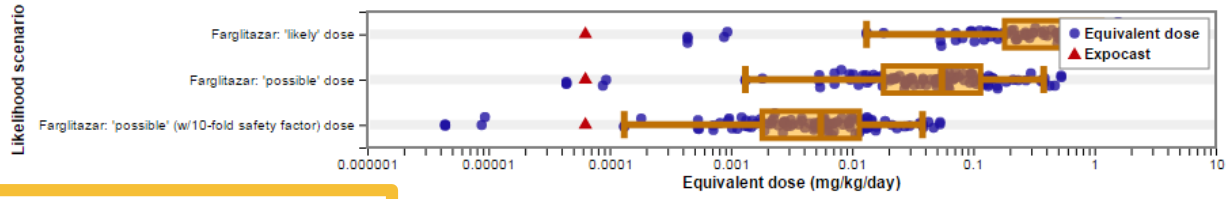
Farglitazar

CAS #: 196808-45-4 (view on EPA comptox dashboard)

Active in **106** Tox21/ToxCast HTS assays (filtered data)

Raw data | Assay summaries

Download data



Summary statistics across assays:

Chemical	Equivalent dose (mg/kg/day)	Number of assays	Percentiles				
			5th	25th	50th	75th	95th
Farglitazar	'Likely'	106	0.013	0.181	0.55	1.149	3.817
Farglitazar	'Possible'	106	0.001	0.018	0.055	0.115	0.382
Farglitazar	'Possible' (w/10-fold safety factor)	106	1.32E-4	0.002	0.006	0.011	0.038

NTP at NIEHS | Web Policies | Site Map | Freedom of Information Act | Inspector General | Help | Contact Us

NTP Staff: Request an Update of this Page | NTP Intranet

USA.gov

NTP is located at the National Institute of Environmental Health Sciences, part of the National Institutes of Health.

USA.gov

NTP is located at the National Institute of Environmental Health Sciences, part of the National Institutes of Health.

Collaborators

National Toxicology Program, NIEHS

Biomolecular Screening Branch

Steve Ferguson
Scott Auerbach
Jui-Hua Hsieh
Dan Svoboda (Sciome)
Michael DeVito (NTPL)
Richard Paules

Program Operations Branch

Andy Shapiro
Joshua Addington

US Environmental Protection Agency

John Wambaugh (NCCT)
Robert Pearce (NCCT/ORISE)
Barbara Wetmore (NERL)

The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the NTP, NIEHS, NIH, or the United States government

Detailed Tox21/ToxCast data processing workflow