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United States Environmental Protection Agency

Windows of Susceptibility

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New Frontiers in Dynamic Toxicology
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The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. EPAor FDA

Office of Research and Development
Center for Computational Toxicology and Exposure

EPA at 50
Progress for a Stronger Future

United States Environmental Protection Agency

Conflict of Interest Statement

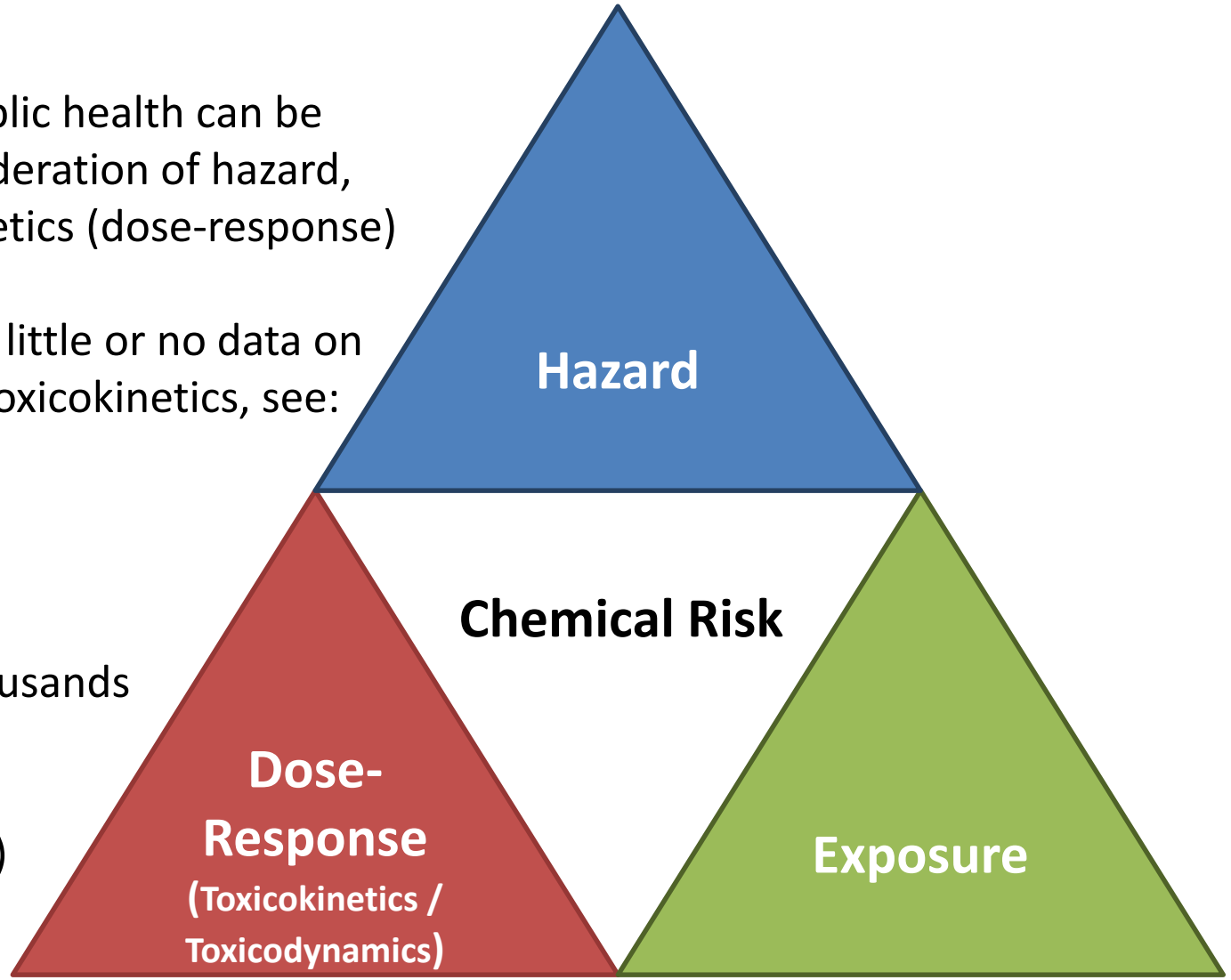
The authors declare no conflict of interest

Hazard, Exposure, and Toxicokinetics

- Chemical risk to the public health can be assessed through consideration of hazard, exposure and toxicokinetics (dose-response)

- Most of chemicals have little or no data on hazard, exposure, and toxicokinetics, see: *Judson et al. (2009), Egeghy et al. (2012), Wetmore et al. (2015)*

- Generating data for thousands of chemicals requires "new approach methodologies" (NAMs)



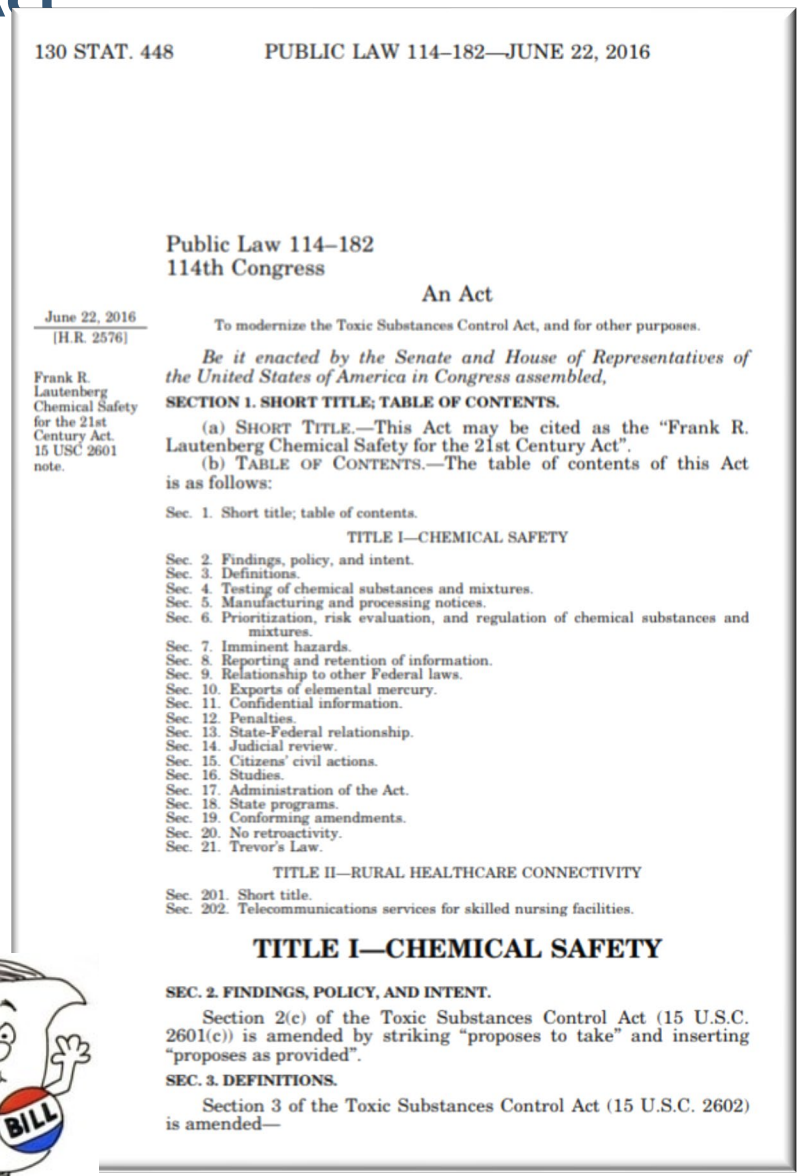
NRC (1983)

The Frank R. Lautenberg Chemical Safety for the 21st Century Act

- Passed by the U.S. Congress in 2016 – modernization of the Toxic Substances Control Act (TSCA)

- Defines "potentially exposed or susceptible subpopulation" to be “a group of individuals within the general population identified by the Administrator who, due to **either greater susceptibility or greater exposure**, may be at greater risk than the general population of adverse health effects from exposure to a chemical substance or mixture, such as **infants, children, pregnant women, workers, or the elderly**”

- “High Priority Substances” present an unreasonable risk of injury to health or the environment, including an **unreasonable risk to a potentially exposed or susceptible subpopulation**



130 STAT. 448 PUBLIC LAW 114–182—JUNE 22, 2016

Public Law 114–182
114th Congress

An Act

To modernize the Toxic Substances Control Act, and for other purposes.

June 22, 2016
[H.R. 2576]

Frank R. Lautenberg Chemical Safety for the 21st Century Act.
15 USC 2601 note.

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled,

SECTION 1. SHORT TITLE; TABLE OF CONTENTS.

(a) SHORT TITLE.—This Act may be cited as the “Frank R. Lautenberg Chemical Safety for the 21st Century Act”.

(b) TABLE OF CONTENTS.—The table of contents of this Act is as follows:

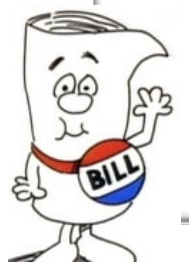
TITLE I—CHEMICAL SAFETY

SEC. 2. FINDINGS, POLICY, AND INTENT.

Section 2(c) of the Toxic Substances Control Act (15 U.S.C. 2601(c)) is amended by striking “proposes to take” and inserting “proposes as provided”.

SEC. 3. DEFINITIONS.

Section 3 of the Toxic Substances Control Act (15 U.S.C. 2602) is amended—



June 22, 2016

Research Challenge and Need

- "The normal development of the fetus, infant, and child can be disrupted by relatively low doses of certain chemicals. These developmental stages are '***windows of susceptibility***' when there is increased vulnerability to the effects of toxic chemicals." Birnbaum (2010)

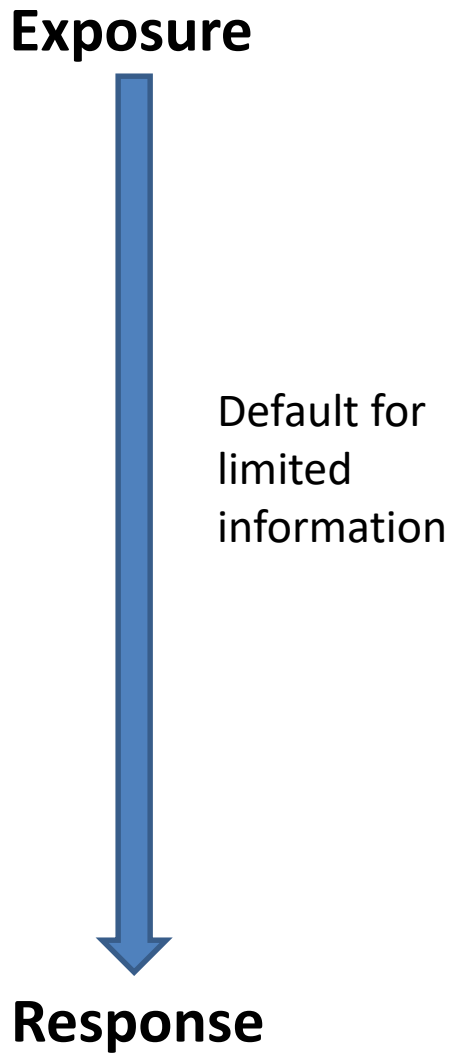
- Too many chemicals to do traditional approaches of developmental toxicity testing

- Need for reliable alternative approaches (that is, NAMs) for

  - **Hazard:** Efficient screening of chemicals for developmental toxicity potential
  - **Toxicokinetics**: Determination of concentration in key tissues as a function of time
  - **Risk** based prioritization for more detailed evaluations

Assessing Chemical Risk

- We wish to link chemical exposure to adverse responses



Barton (2005)

Assessing Chemical Risk

- We wish to link chemical exposure to adverse responses
- Both the **window of susceptibility** (that is, the timing of the toxicodynamics) and the **toxicokinetics** occurring during that window must be addressed
- These analyses involve combining quantitative descriptions of the tissue dosimetry (that is, pharmacokinetics), window of susceptibility, and dose-response behavior within that window
- A major challenge for any modeling, but especially life stage modeling, is how to obtain data sets for model parameterization, calibration, and evaluation

Exposure

Pharmaco- /Toxicokinetics

- Fate of molecules/chemicals in body
- Considers absorption, distribution, metabolism, excretion (ADME)

Pharmaco- /Toxicodynamics

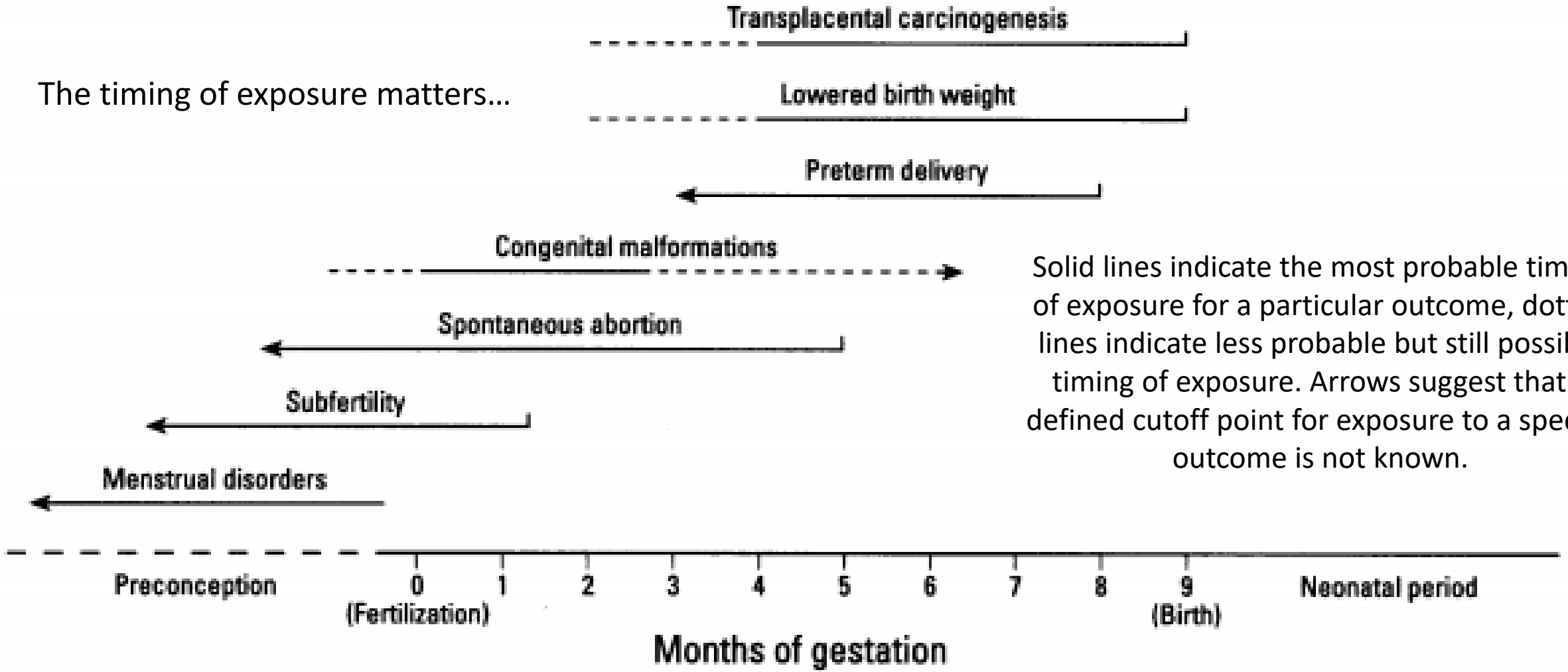
- Effect of molecules/chemicals at biological target *in vivo*
- Perturbation as adverse/therapeutic effect, reversible/ irreversible effects

Response

Barton (2005)

Windows of Susceptibility

The timing of exposure matters…



Solid lines indicate the most probable timing of exposure for a particular outcome, dotted lines indicate less probable but still possible timing of exposure. Arrows suggest that a defined cutoff point for exposure to a specific outcome is not known.

Selevan et al. (1987)

Hazard, Exposure, and Toxicokinetics

- Chemical risk to the public health can be assessed through consideration of hazard, exposure and toxicokinetics (dose-response)
- Most of chemicals have little or no data on hazard, exposure, and toxicokinetics, see: *Judson et al. (2009), Egeghy et al. (2012), Wetmore et al. (2015)*
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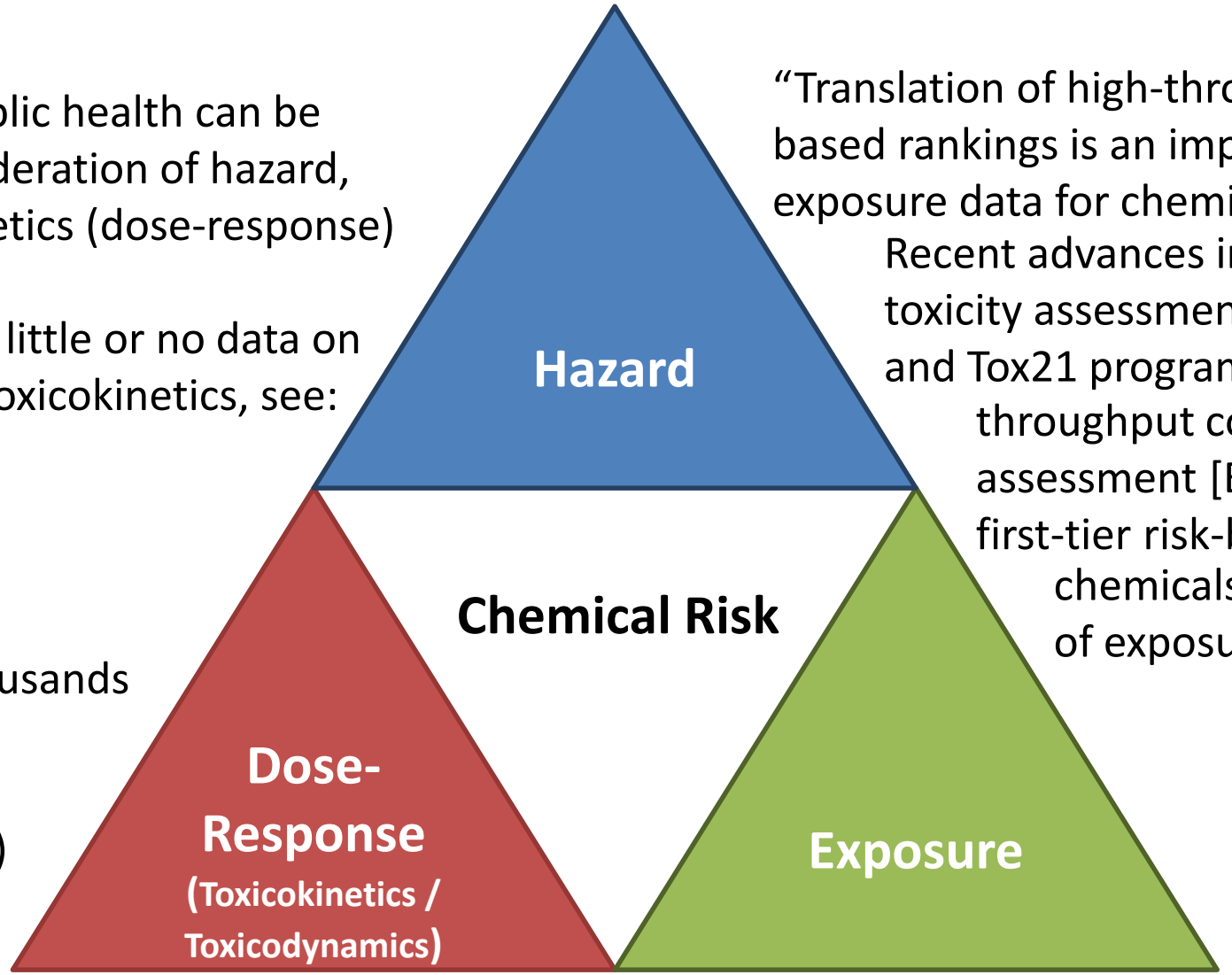


NRC (1983)

"Translation of high-throughput data into risk-based rankings is an important application of exposure data for chemical priority-setting. Recent advances in high-throughput toxicity assessment, notably the ToxCast and Tox21 programs… and in high-throughput computational exposure assessment [ExpoCast] have enabled first-tier risk-based rankings of chemicals on the basis of margins of exposure" - National Academies of Sciences, Engineering, and Medicine (NASEM)

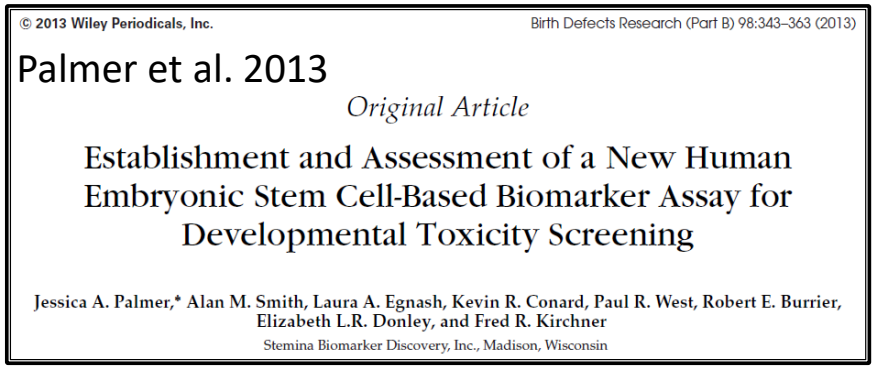
New Approach Methods for Toxicodynamic Windows of Susceptibility

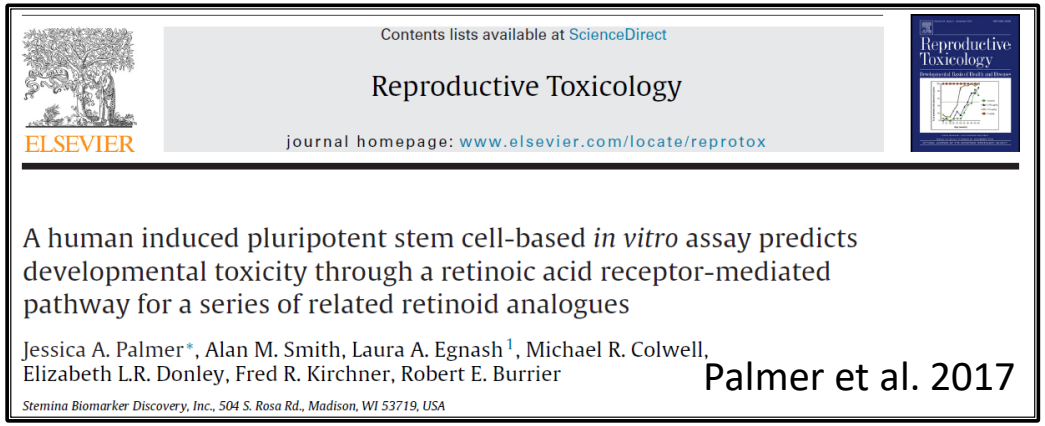
- Palmer et al. (2013) and (2017) reported on *in vitro* biomarker assays for rapid and targeted screening of chemicals for developmental toxicity based on changes in cellular metabolism as early signals

- Specifically, the assay determines the *in vitro* concentration of the test compound that is associated with developmental toxicity potential (dTP)

- Assays have been shown to have good accuracy, sensitivity, specificity, and high concordance to existing *in vivo* models

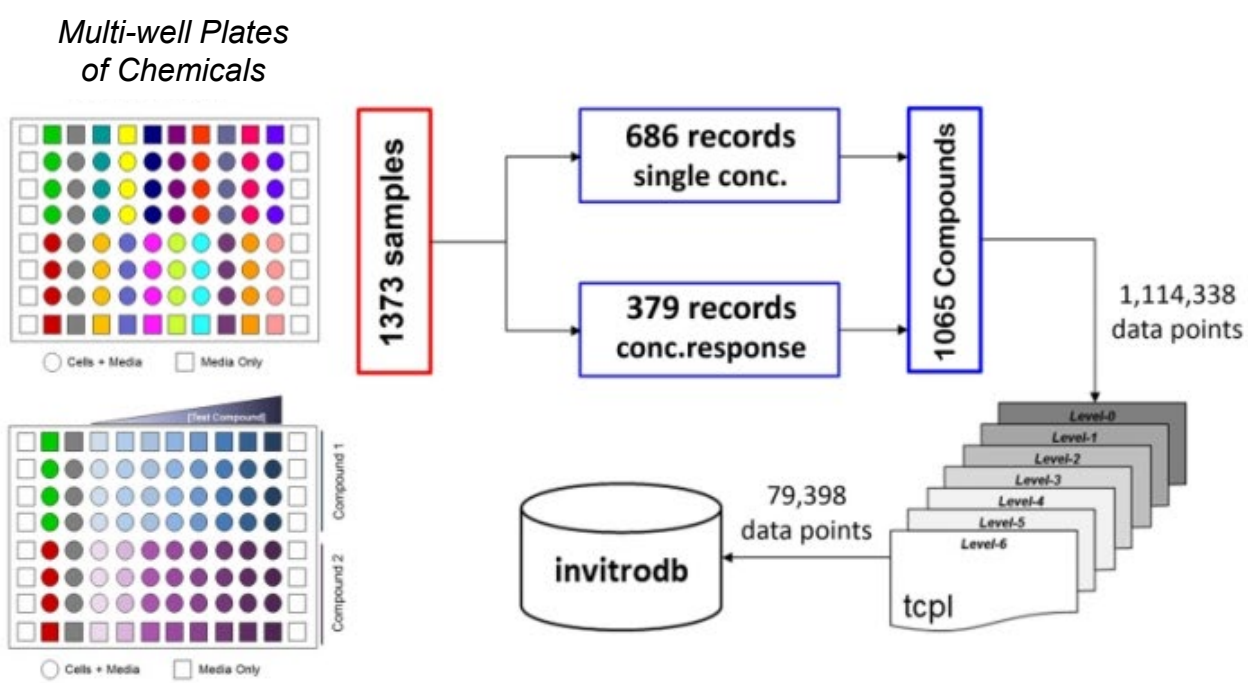
- Zurlinden et al. (2020) describes incorporation of these assays into ToxCast screening program

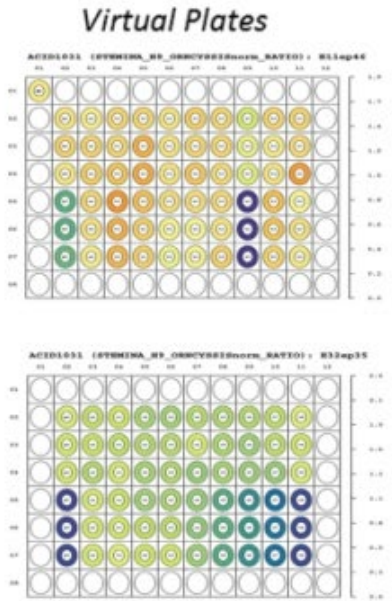


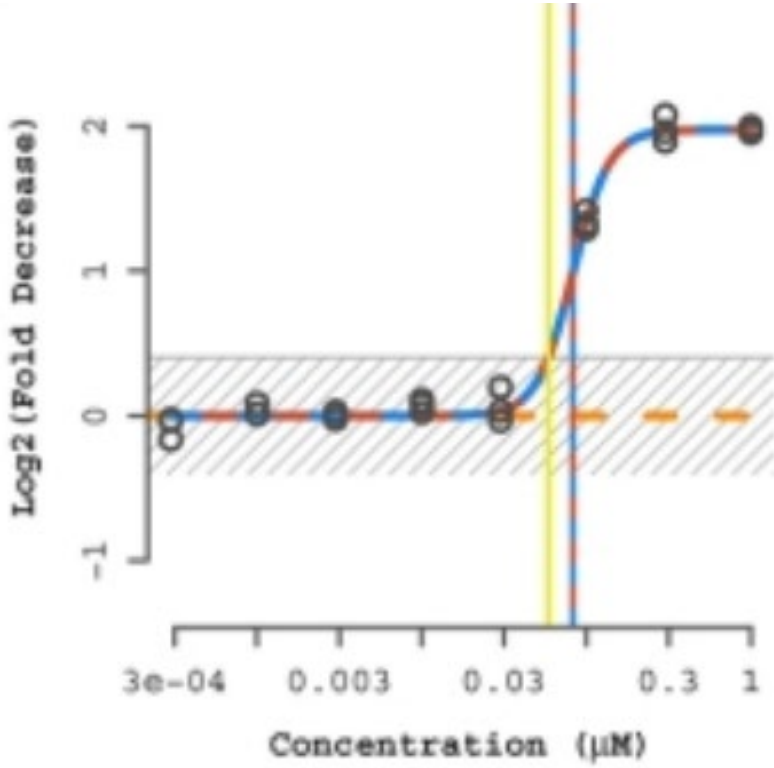


New Approach Methods for Toxicodynamic Windows of Susceptibility

- Zurlinden et al. (2020) describes incorporation of Palmer et al. (2013, 2017) assays into the ToxCast screening program





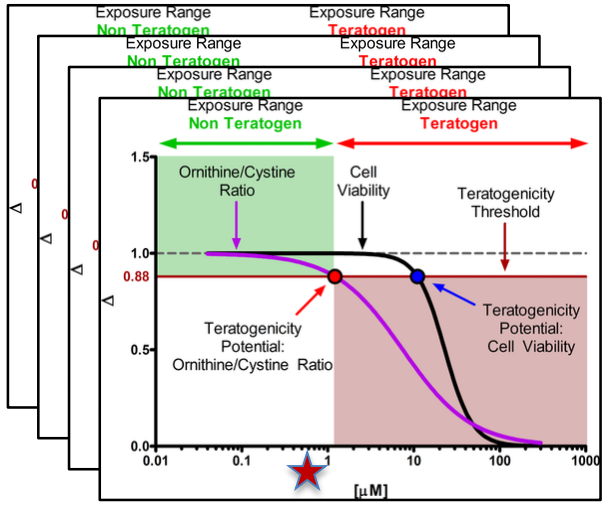


Zurlinden et al. (2020)

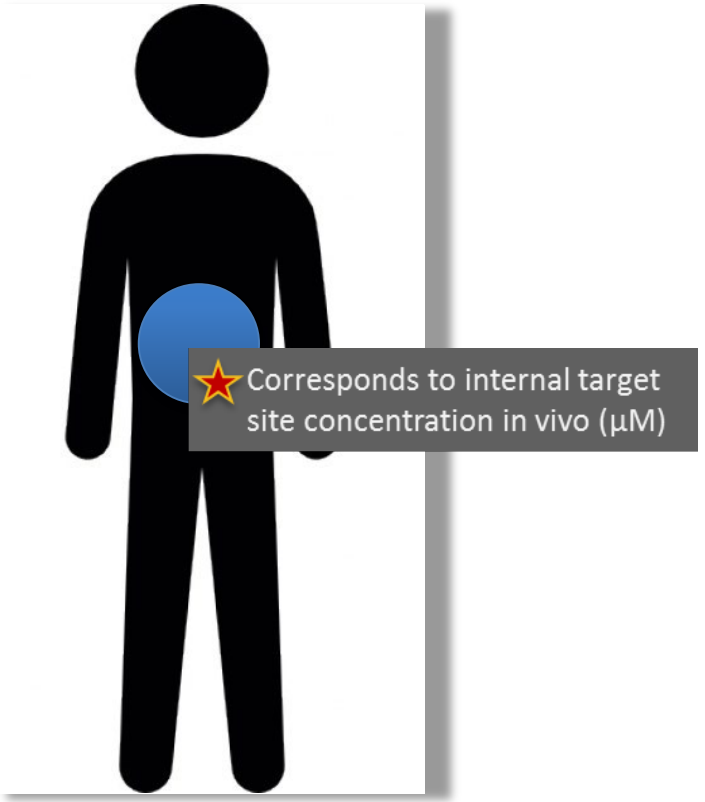
KNOWN: *In vitro* Measured Internal Exposure (µM) associated with Developmental Toxicity

Test Compound #1 ..... Test Compound #*n*

In vitro assay
[e.g., Palmer et al. 2013, 2017]



In vitro concentration [µM]
Identified for developmental toxicity potential ★

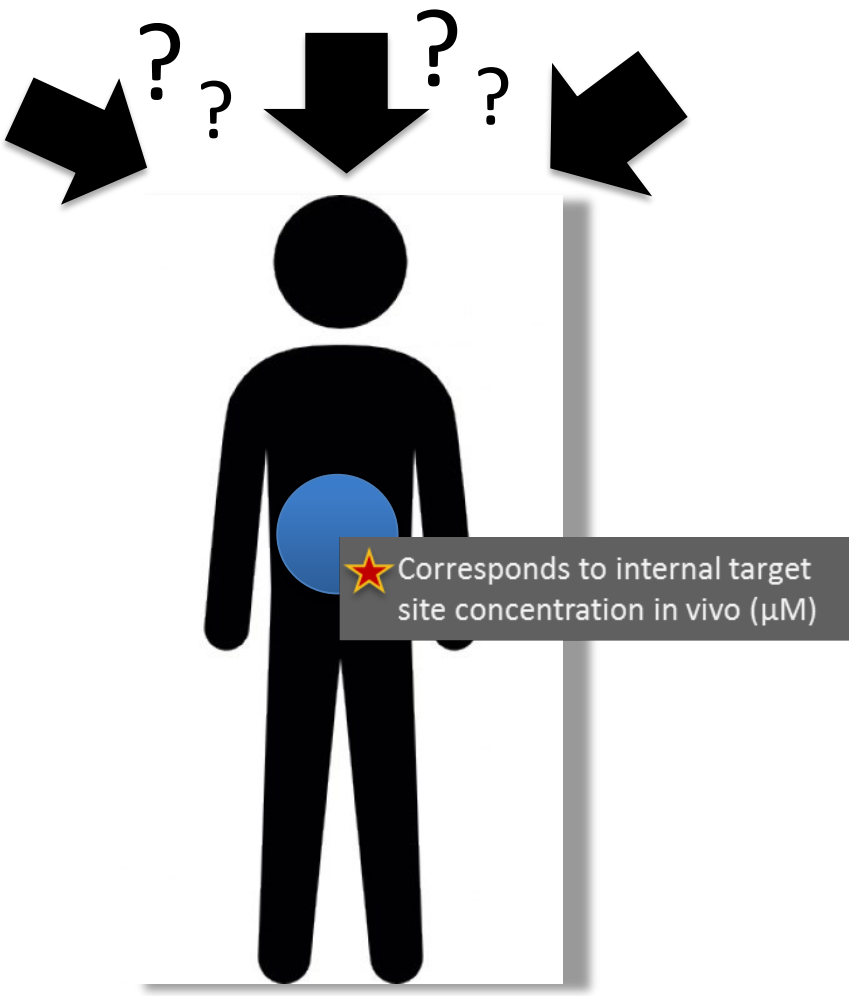


UNKNOWN: *In vivo* Relevant External Exposure (mg/kg) associated with Developmental Toxicity

Specific Research Goal:

What is the level of *in vivo* external exposure (mg/kg) that yields the corresponding internal exposure levels (µM) that are shown to be associated with developmental toxicity *in vitro*?

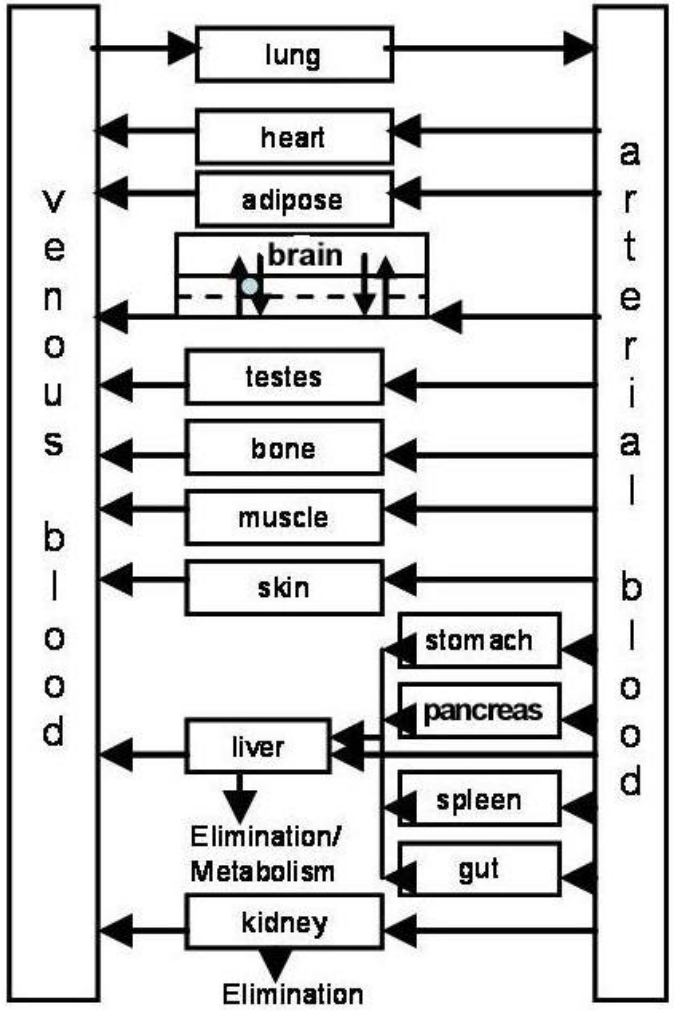
Essence of *In vitro* to *In vivo* Extrapolation (IVIVE)

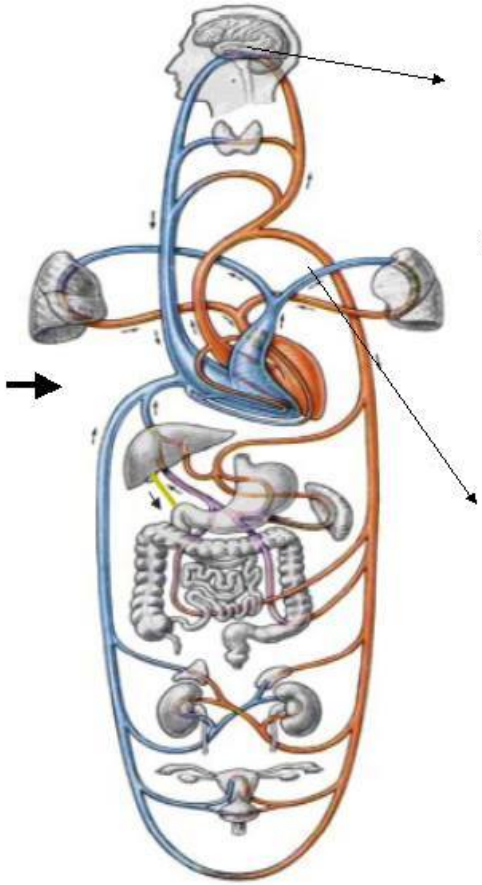


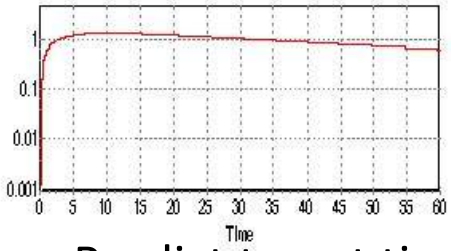
Need a tool that bridges

Internal Exposure ←→ External Exposure:

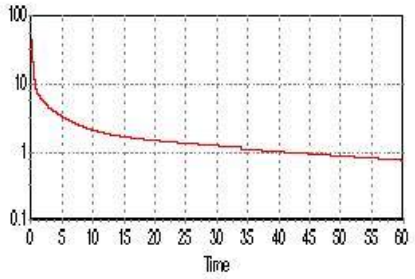
Physiologically Based Pharmacokinetic (PBPK) Modeling







Predict target tissue concentration

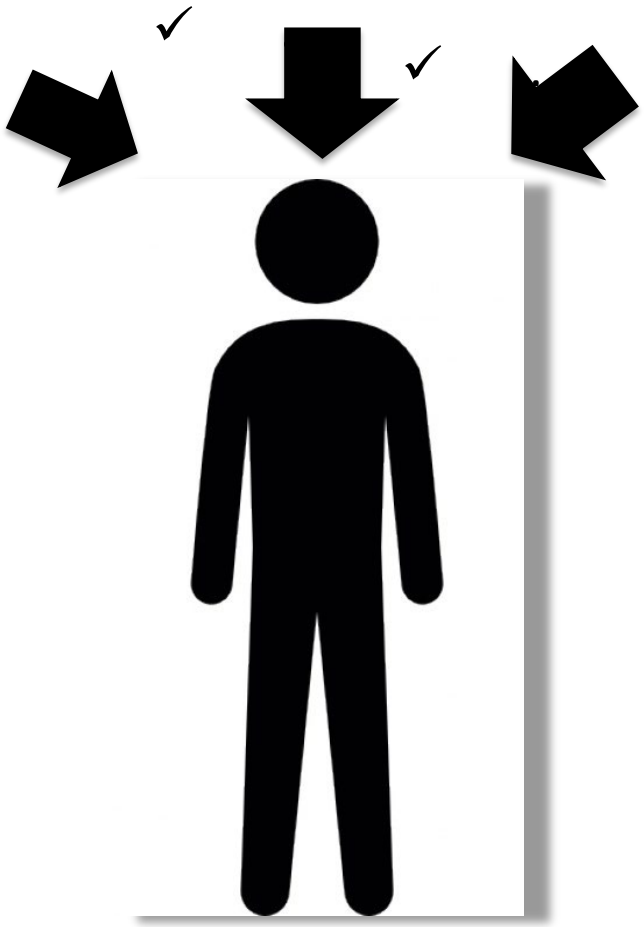


Predict plasma concentration

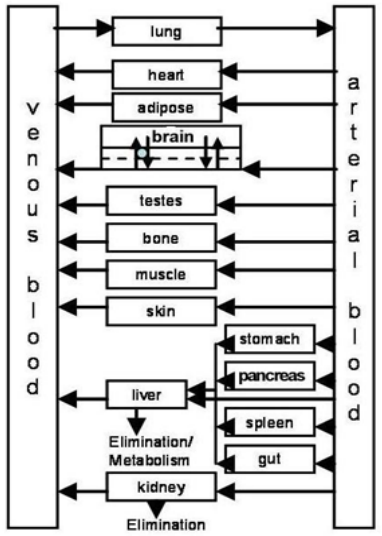
- Mathematical description of what the body does to the drug (Pharmacokinetics)
- Model comprises of physiological parameters and chemical-specific parameters

Utility of Fully Parameterized PBPK: Forward Dosimetry

External Exposure

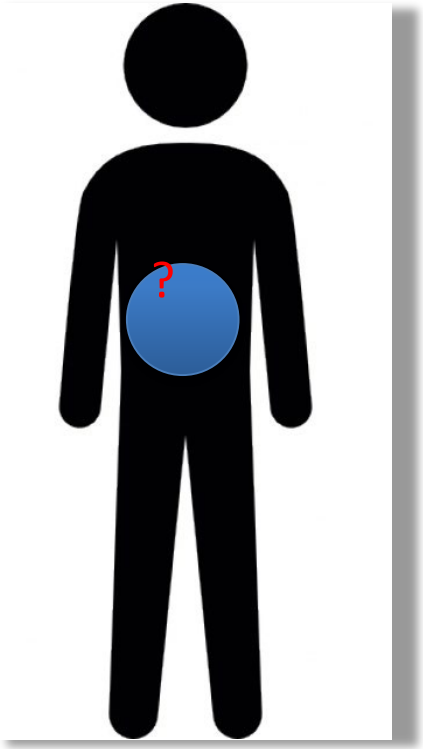


PBPK Model



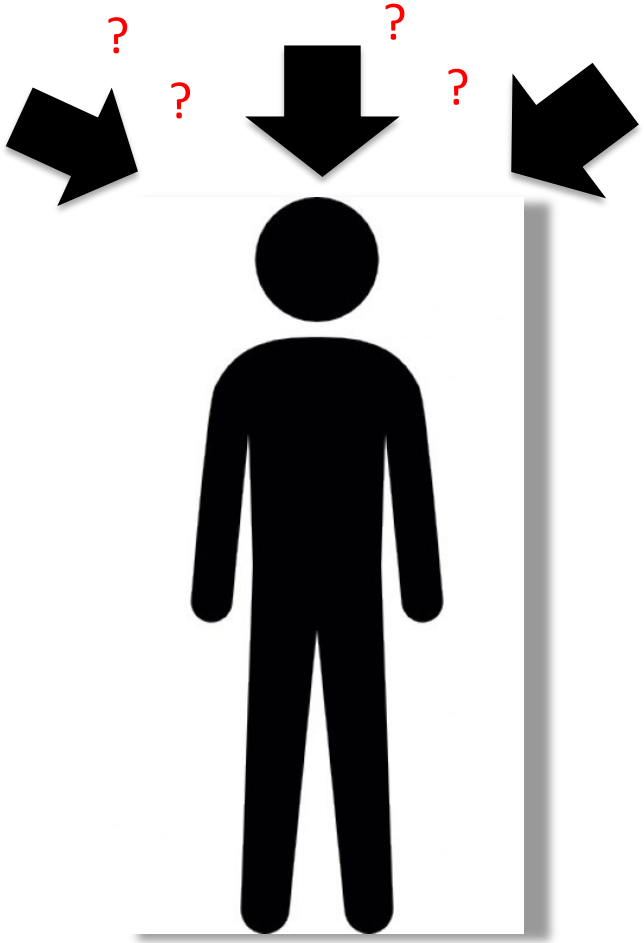
Forward Dosimetry

Internal Exposure

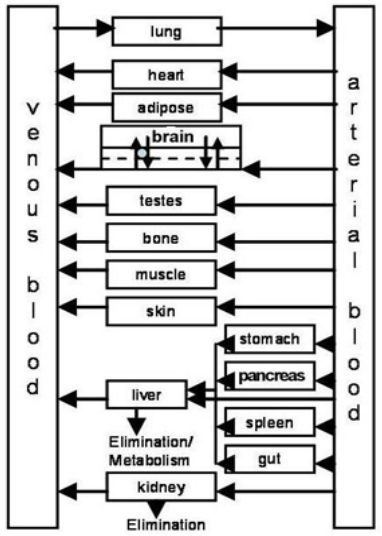


Utility of Fully Parameterized PBPK: Reverse Dosimetry

External Exposure

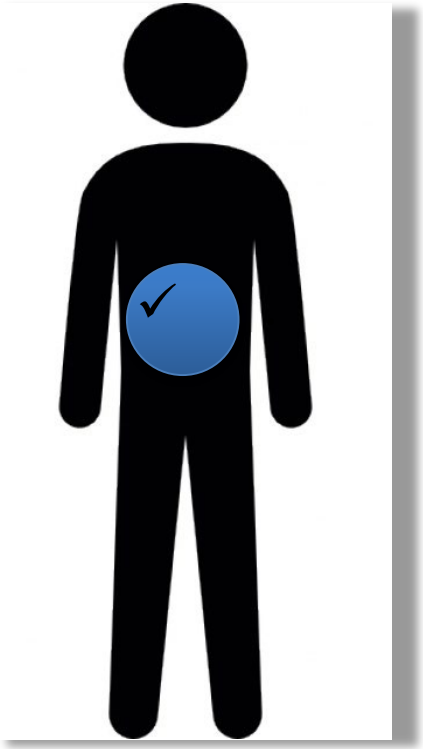


PBPK Model



Reverse Dosimetry

Internal Exposure



~~UN~~KNOWN: External Exposure (mg/kg) associated with Developmental Toxicity

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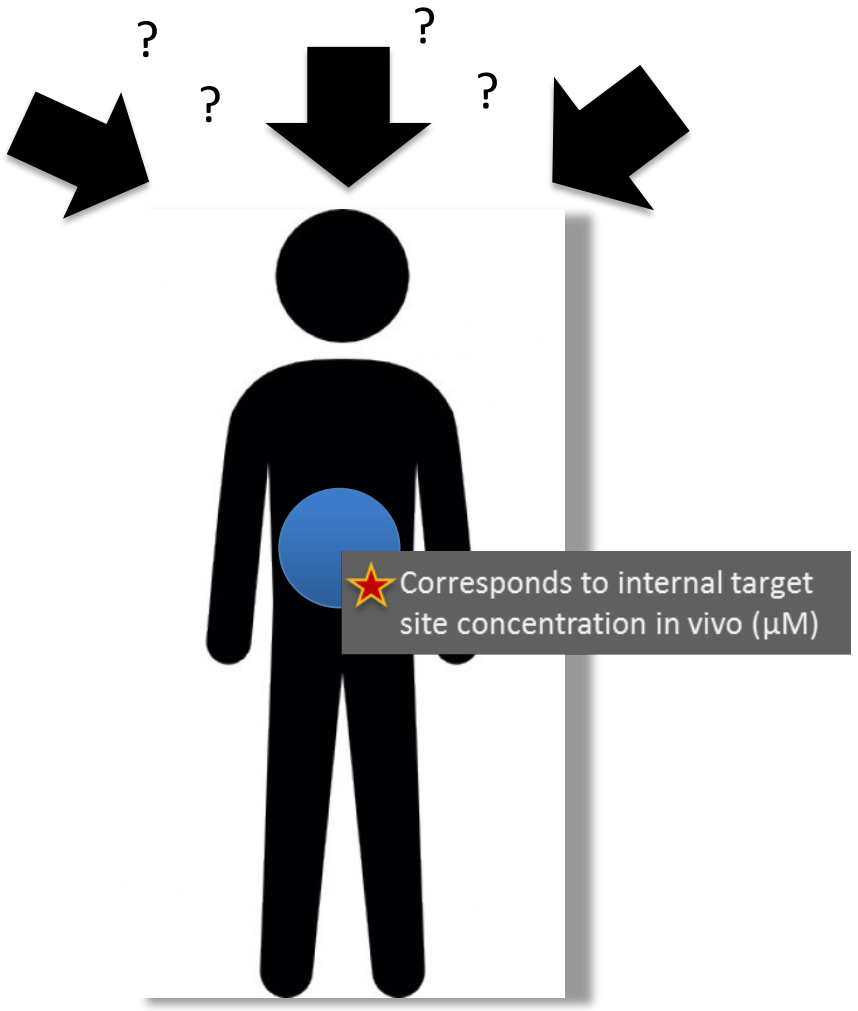


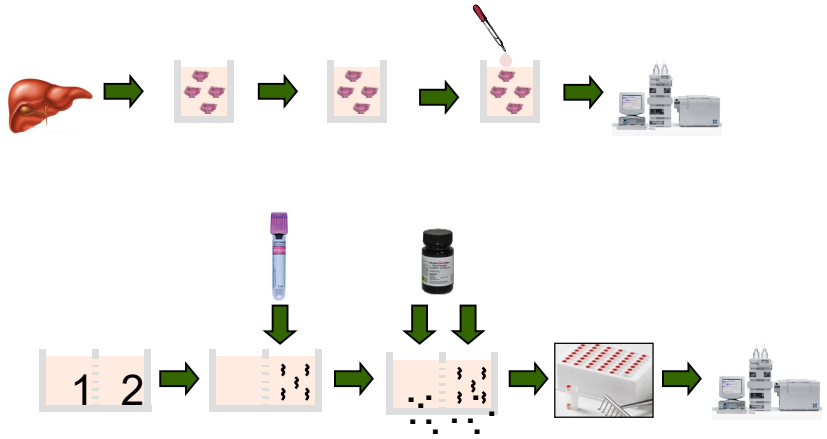
***In vitro* to *In vivo* Extrapolation**
using
PBPK Modeling

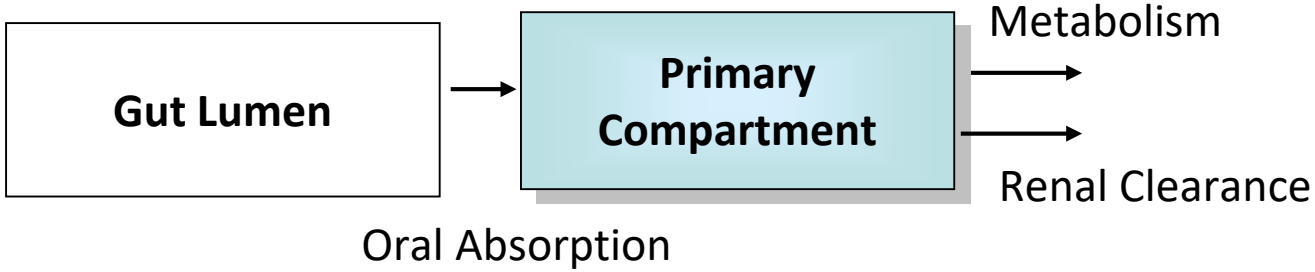
High Throughput Toxicokinetics (HTTK)

Most chemicals lack public toxicokinetic-related data (Wetmore et al., 2012):

In vitro toxicokinetic data + generic toxicokinetic model

= high(er) throughput toxicokinetics

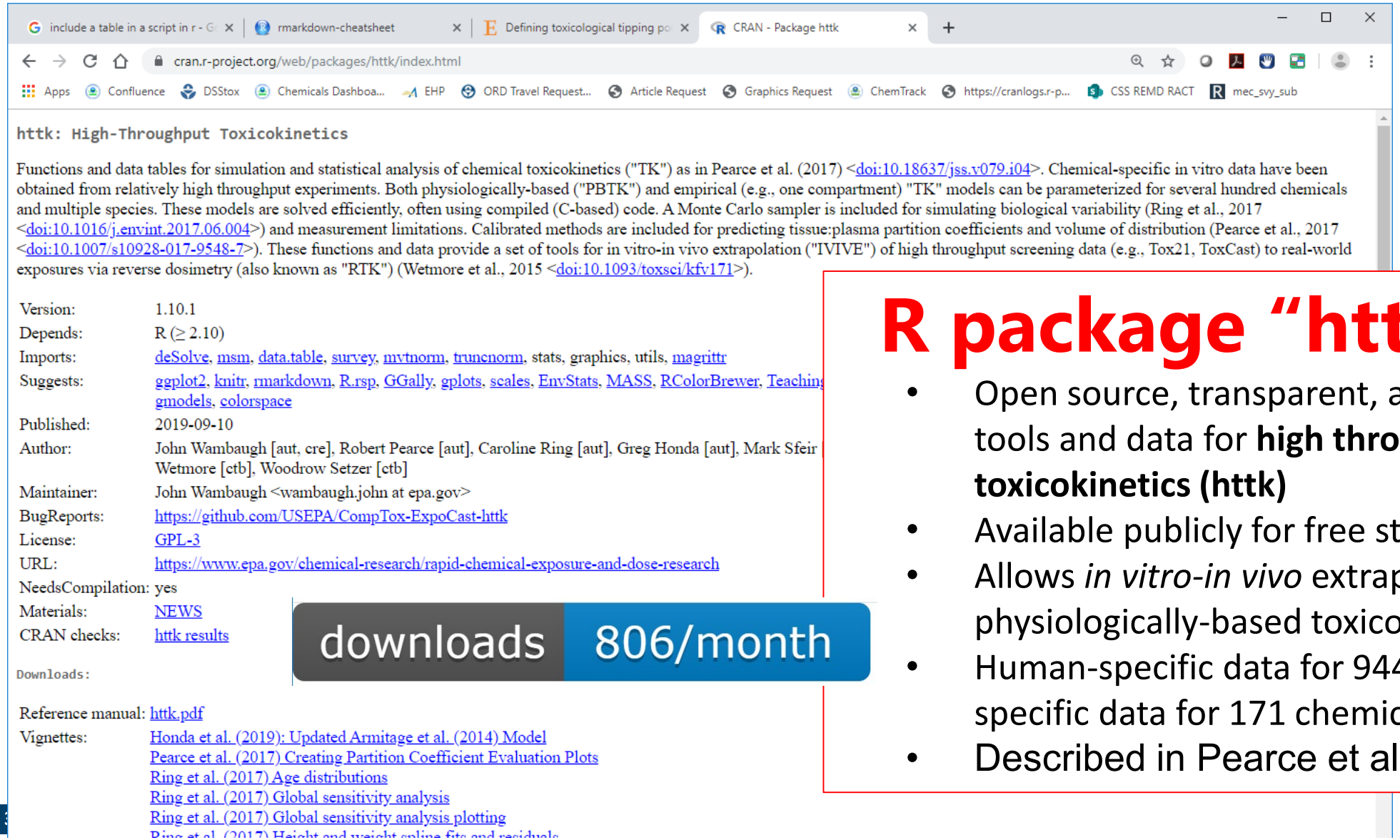




httk

Open Source Tools and Data for HTTK

https://CRAN.R-project.org/package=httk


include a table in a script in r - G | rmarkdown-cheatsheet | Defining toxicological tipping po | CRAN - Package httk

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

Apps | Confluence | DSStox | Chemicals Dashboa... | EHP | ORD Travel Request... | Article Request | Graphics Request | ChemTrack | https://cranlogs.r-p... | CSS REMD RACT | mec_svy_sub

httk: High-Throughput Toxicokinetics

Functions and data tables for simulation and statistical analysis of chemical toxicokinetics ("TK") as in Pearce et al. (2017) <doi:10.18637/jss.v079.i04>. Chemical-specific in vitro data have been obtained from relatively high throughput experiments. Both physiologically-based ("PBTK") and empirical (e.g., one compartment) "TK" models can be parameterized for several hundred chemicals and multiple species. These models are solved efficiently, often using compiled (C-based) code. A Monte Carlo sampler is included for simulating biological variability (Ring et al., 2017 <doi:10.1016/j.envint.2017.06.004>) and measurement limitations. Calibrated methods are included for predicting tissue:plasma partition coefficients and volume of distribution (Pearce et al., 2017 <doi:10.1007/s10928-017-9548-7>). These functions and data provide a set of tools for in vitro-in vivo extrapolation ("IVIVE") of high throughput screening data (e.g., Tox21, ToxCast) to real-world exposures via reverse dosimetry (also known as "RTK") (Wetmore et al., 2015 <doi:10.1093/toxsci/kfv171>).

Version:	1.10.1
Depends:	R (≥ 2.10)
Imports:	deSolve, msm, data.table, survey, mvtnorm, truncnorm, stats, graphics, utils, magrittr
Suggests:	ggplot2, knitr, rmarkdown, R.rsp, GGally, gplots, scales, EnvStats, MASS, RColorBrewer, Teaching gmodels, colorspace
Published:	2019-09-10
Author:	John Wambaugh [aut, cre], Robert Pearce [aut], Caroline Ring [aut], Greg Honda [aut], Mark Sfeir Wetmore [ctb], Woodrow Setzer [ctb]
Maintainer:	John Wambaugh <wambaugh.john at epa.gov>
BugReports:	https://github.com/USEPA/CompTox-ExpoCast-httk
License:	GPL-3
URL:	https://www.epa.gov/chemical-research/rapid-chemical-exposure-and-dose-research
NeedsCompilation:	yes
Materials:	NEWS
CRAN checks:	httk results

Downloads:

downloads 806/month

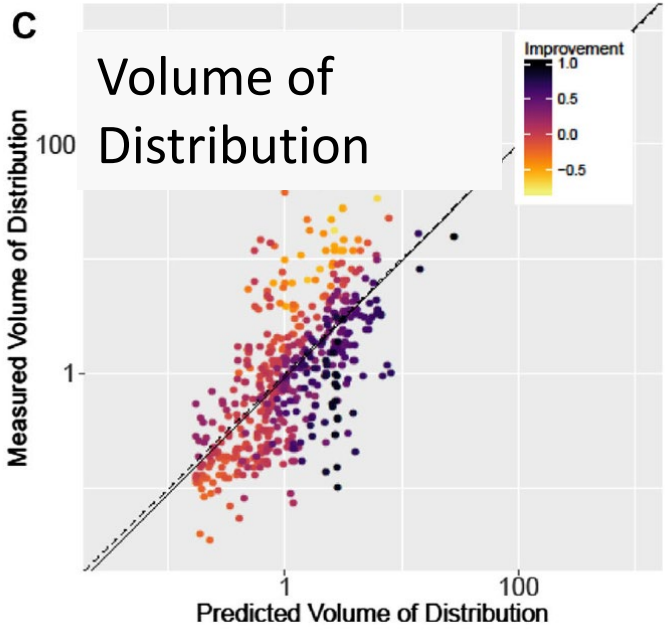
Reference manual: httk.pdf

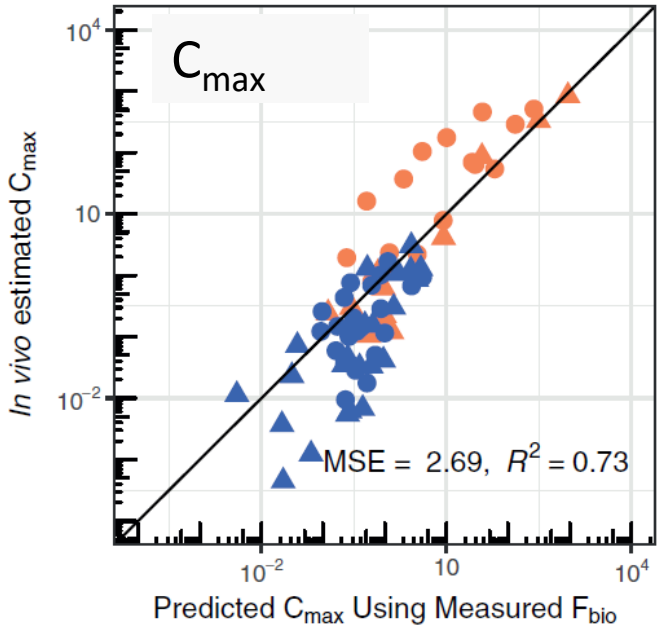
Vignettes:
- Honda et al. (2019): Updated Armitage et al. (2014) Model
- Pearce et al. (2017) Creating Partition Coefficient Evaluation Plots
- Ring et al. (2017) Age distributions
- Ring et al. (2017) Global sensitivity analysis
- Ring et al. (2017) Global sensitivity analysis plotting
- Ring et al. (2017) Height and weight spline fits and residuals

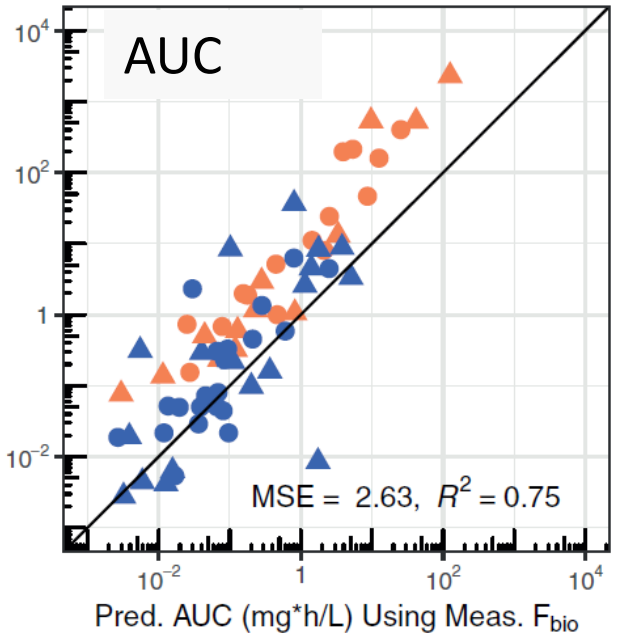
R package "httk"

- Open source, transparent, and peer-reviewed tools and data for **high throughput toxicokinetics (httk)**
- Available publicly for free statistical software R
- Allows *in vitro-in vivo* extrapolation (IVIVE) and physiologically-based toxicokinetics (PBTK)
- Human-specific data for 944 chemicals and rat-specific data for 171 chemicals
- Described in Pearce et al. (2017)

HTTK Model Calibration and Evaluation







HTTK trades reasonable predictions for a range of chemicals for accurate predictions for a specific chemical

More importantly, we can statistically characterize the error in the predictions

Pearce et al. 2017; Wambaugh et al. 2018

‘HTTK’ R-Package Extended to Pregnancy

1-Comp 3-Comp PBTK Maternal/Fetal PBTK

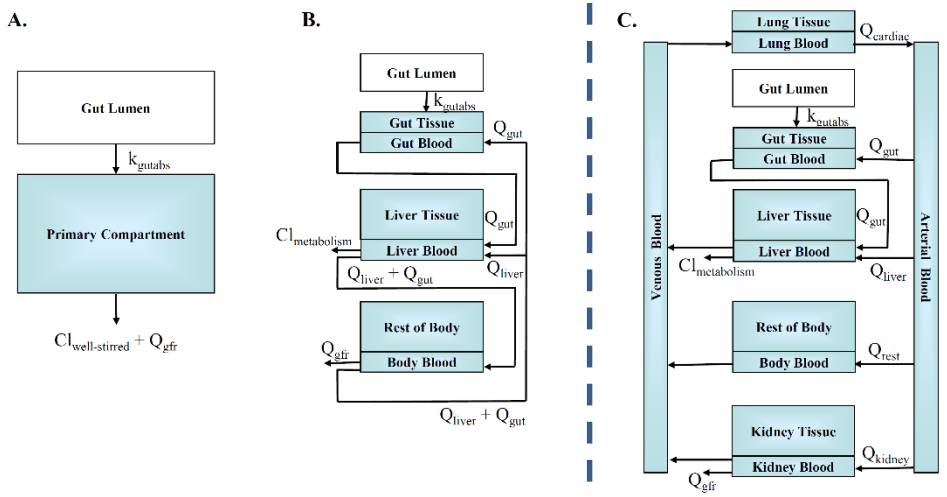


Figure 1: Models (A) 1compartment, (B) 3compartment, and (C) pbtk. In order to preserve mass-balance, Q_{rest} is defined as the difference between $Q_{cardiac}$ and the flow to the liver, kidney, and gut. Variable names are defined in Table 1.

PLOS ONE

RESEARCH ARTICLE

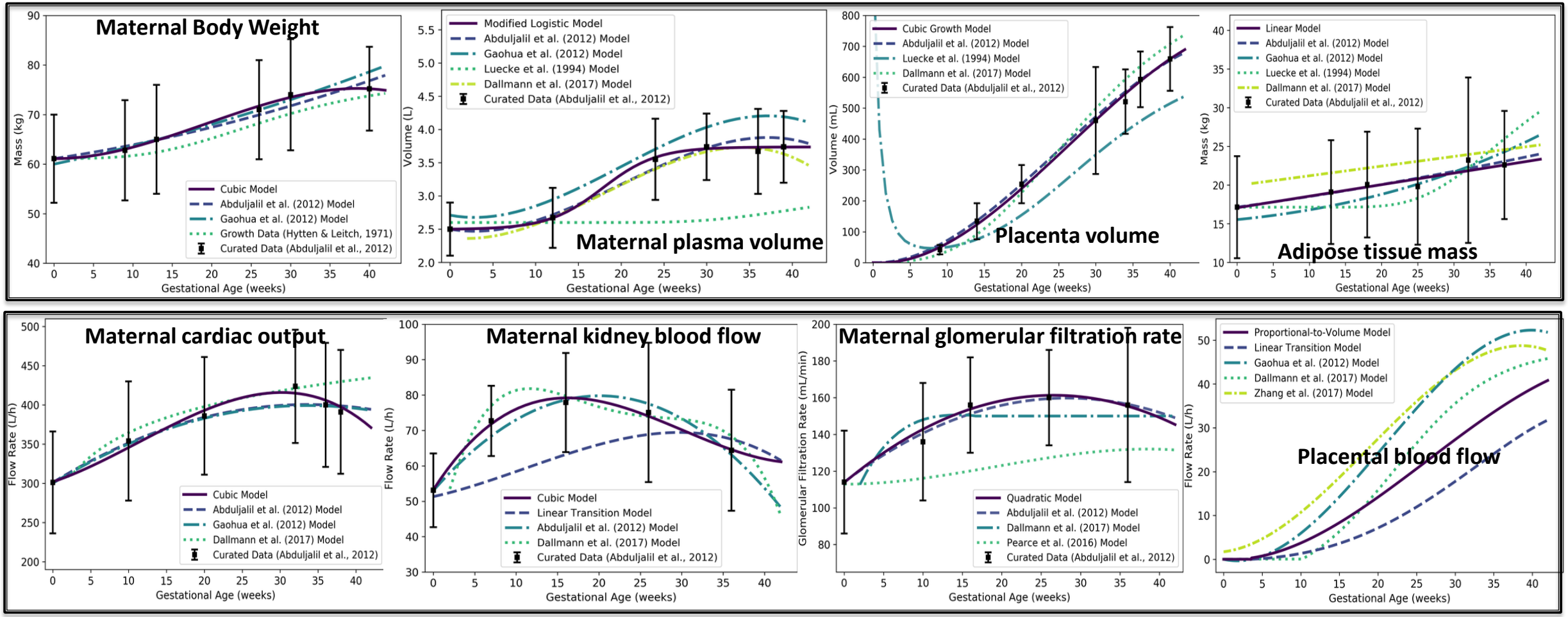
Empirical models for anatomical and physiological changes in a human mother and fetus during pregnancy and gestation

Dustin F. Kapraun[1]*, John F. Wambaugh[2], R. Woodrow Setzer[2], Richard S. Judson[2]

1 National Center for Environmental Assessment, US Environmental Protection Agency, Research Triangle Park, North Carolina, United States of America, 2 National Center for Computational Toxicology, US Environmental Protection Agency, Research Triangle Park, North Carolina, United States of America

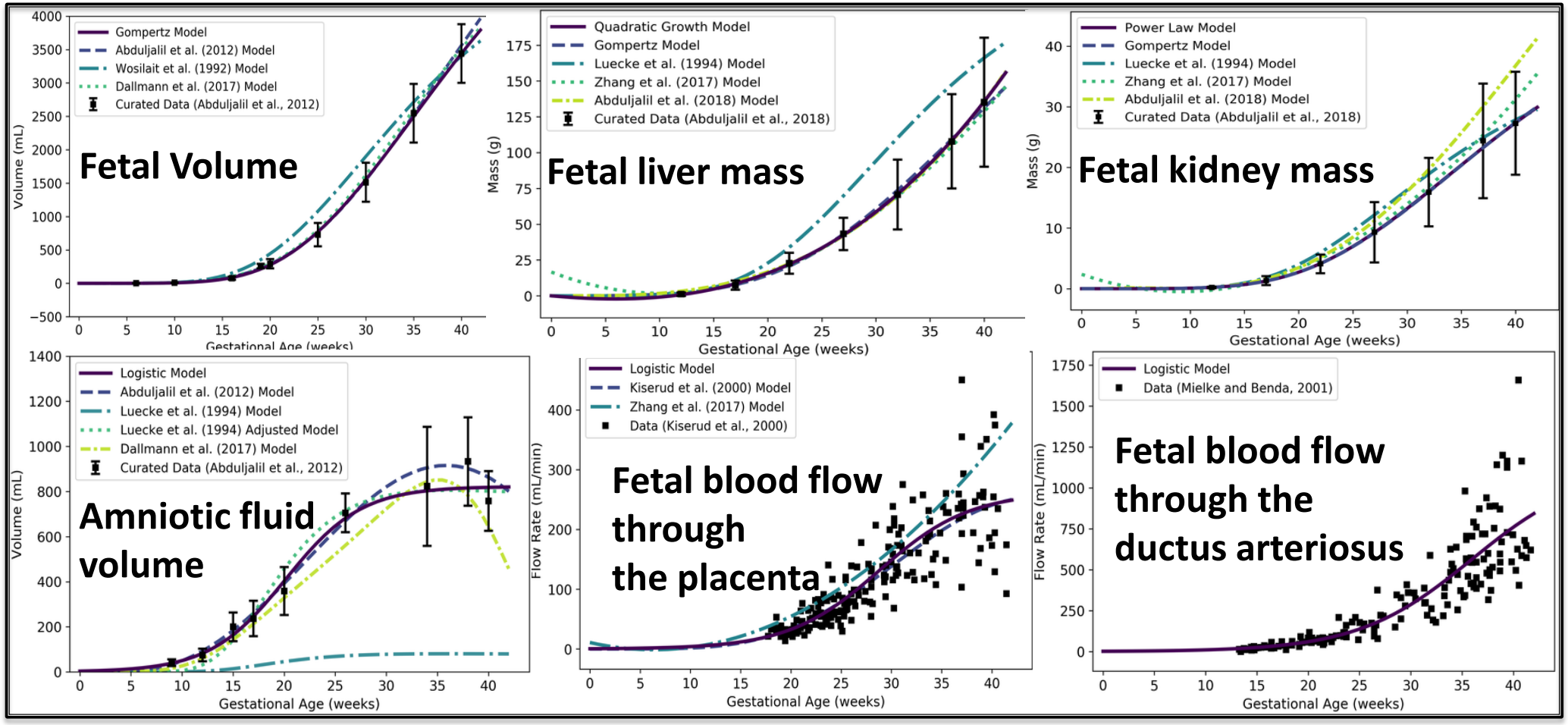
Kapraun et al. 2019

Representative Physiological Parameter Changes in the Mother



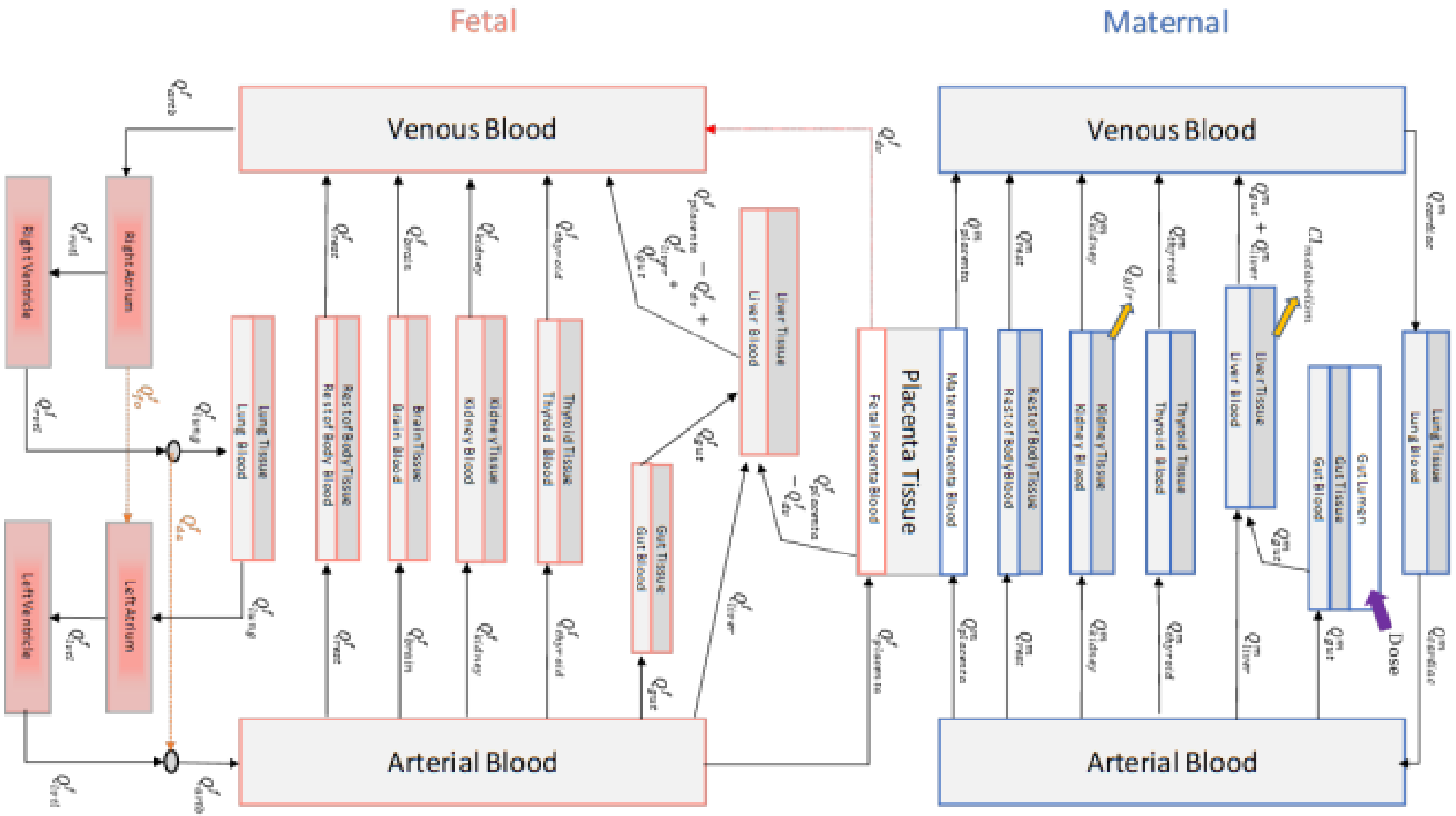
Kapraun et al. (2019)

Representative Physiological Parameter Changes in the Fetus



Kapraun et al. (2019)

Generic Gestational PBTK Model



Come see poster board P569 (Abstract #2939) on Wednesday Morning

"Evaluation of a Rapid, Multi-chemical Human Gestational Physiologically Based Toxicokinetic Model"

Maternal/Fetal HTTK Model: Features

- Description of fetal physiology and the evolving fetal circulatory system in pregnancy PBPK models
- Temporal changes in maternal and fetal physiological parameters (e.g. body weight, blood flow rate, and compartment volumes) informed by the most current human experimental data available
- Designed to simulate ADME in mother and fetus from 13 weeks gestation to term.
- Placental/fetal transfer is described using partition coefficients which might be sufficient for many chemicals
- Accommodates analysis (IVIVE/forward/reverse dosimetry) for >900 chemicals

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Maternal/Fetal HTTK Model: Not Included

- Changes in maternal metabolic enzyme expression levels and activity
- Changes in fetal metabolic enzyme expression levels and activity
- Changes in renal clearance capacities in fetus across gestational age
- Changes in plasma protein binding for both mom and fetus
- Placental metabolism contributions
- Placental barrier descriptions (permeability rate constants or active transporter function to determine extent of fetal exposure might be important for some chemicals)

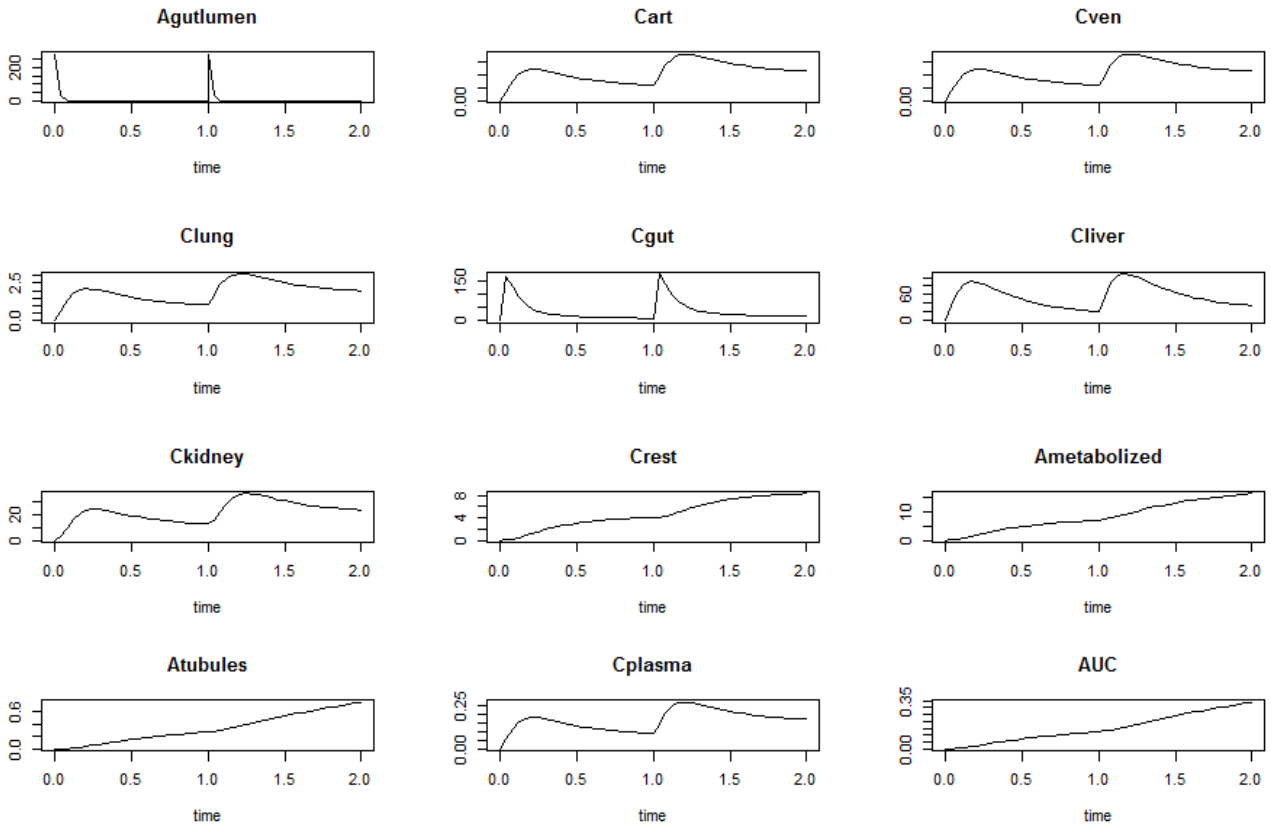
Forward Dosimetry Evaluations
Generic HTTK Model to Predict ATRA Kinetics in Humans

all-*trans*-retinoic acid (ATRA)

Sample Studies	Dose	PK parameters	Observed	Predicted	Predicted/ Observed Ratio
Ozpolat et al. 2003	1.2 mg/kg	Cmax (μM)	1.3 ±1.2	0.1	0.1
		AUC(0,∞) (μM*h)	3.0±2.6	3.12	1
Thudi et al. 2011	0.14 mg/kg	Cmax (uM)	0.1±0.04	0.02	0.2
		AUC(0,∞) (μM*h)	0.3±0.1	0.4	1
Peng et al. 2014	0.3 mg/kg	Cmax (uM)	0.5±0.1	0.04	0.1
		AUC(0,∞) (μM*h)	1.2±0.4	0.8	1

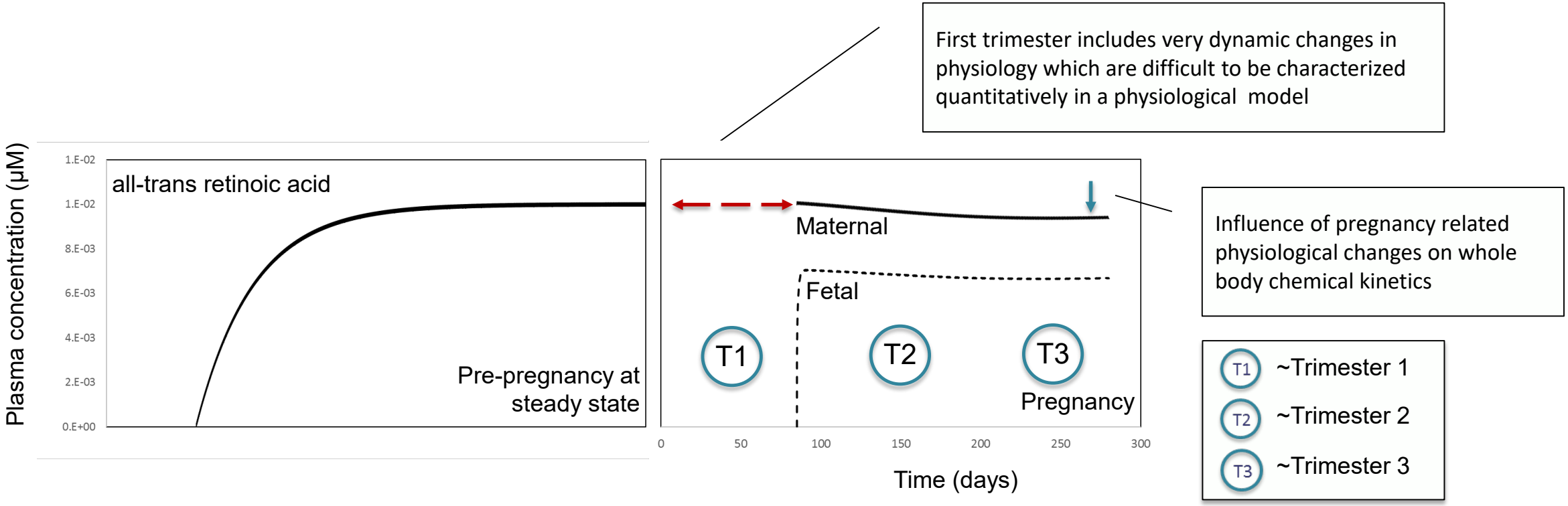
With minimal model inputs (Fup & CLint), the generic model:

- Well predicted the Area Under the Curve
- Underpredicted the Cmax by a factor of 10



Sample model outputs for ATRA following oral dosing

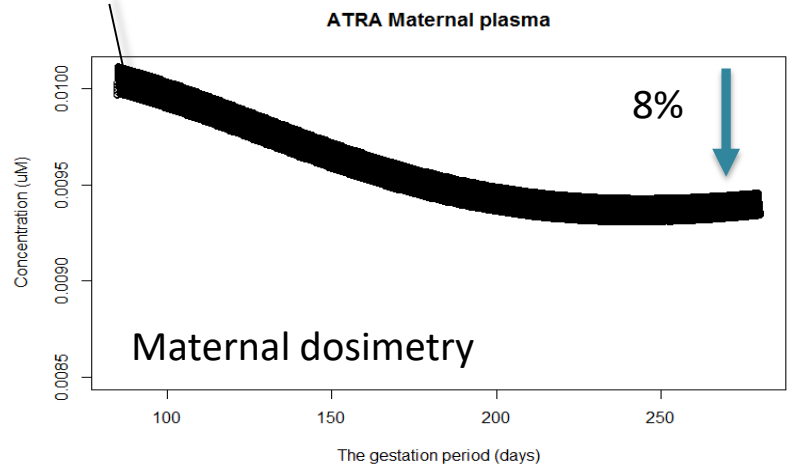
Forward Dosimetry Predictions during Pregnancy Generic Maternal/Fetal HTTK Model

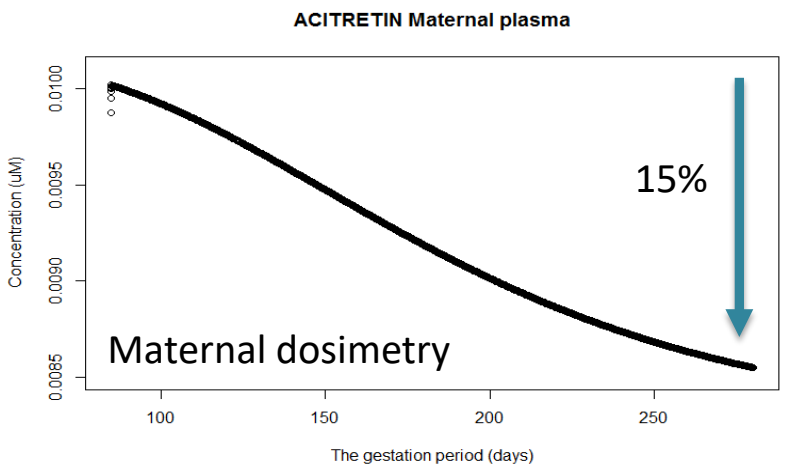


- Pregnancy related physiological changes for ATRA results in a dilution effect of chemical internal dosimetrics

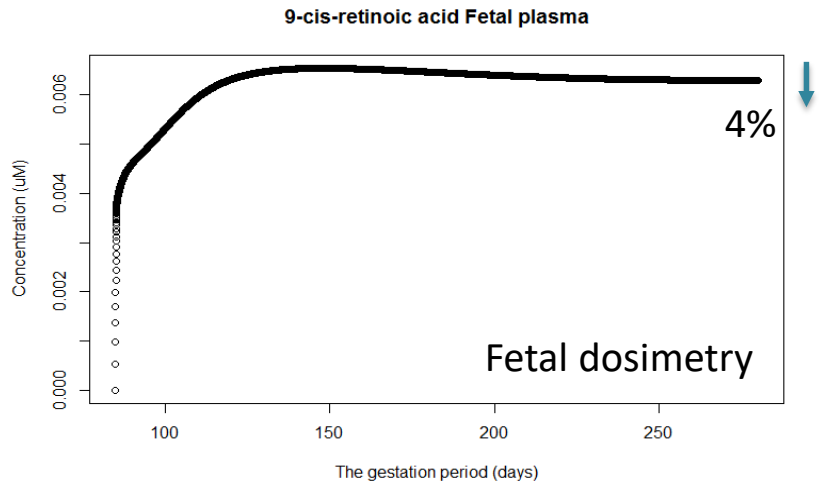
Maternal/Fetal HTTK Model Predictions for Retinoid Analogues

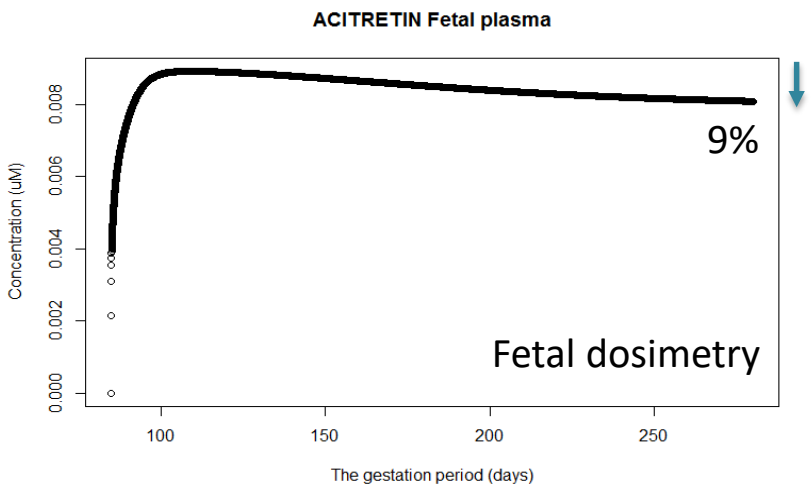
Normalized initial plasma concentration for each retinoid analogue





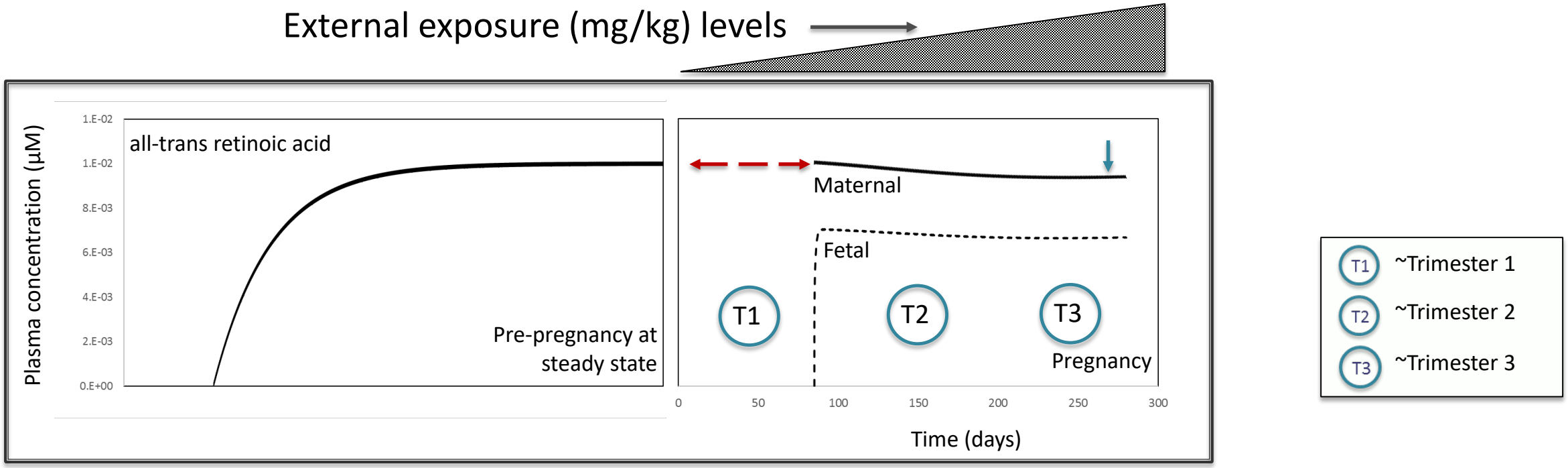
- Decrease in maternal plasma concentrations for retinoid analogues ranged from 8-15%





- Decrease in Fetal plasma concentrations for retinoid analogues ranged from 4-9%

Reverse Dosimetry Predictions during Pregnancy [Scales Linearly]

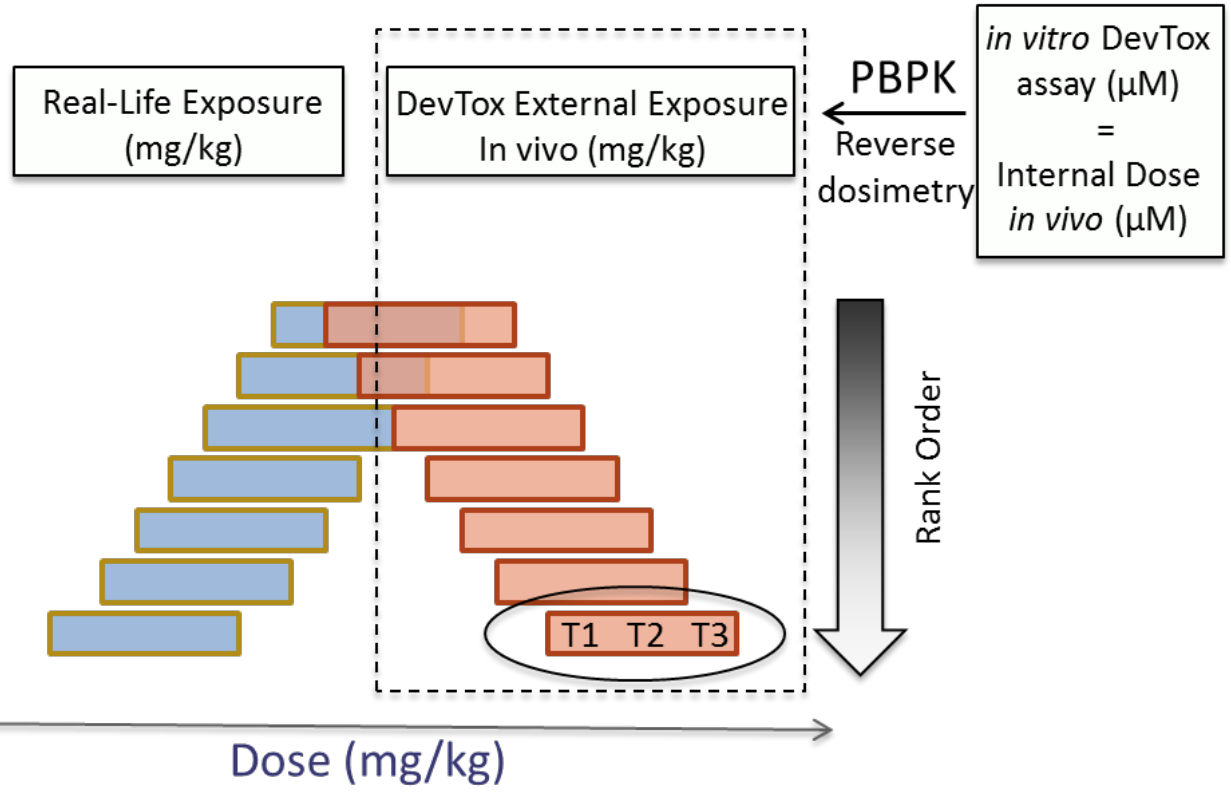


- Over the course of pregnancy it takes higher *in vivo* exposure doses to yield the same *in vitro* measured developmental toxicity potential estimates - depending on the extent of decrease in maternal plasma concentration during pregnancy

Model Predicted External Exposures Associated with Developmental Toxicity

Retinoid analogs	in vitro Developmental toxicity potential (dTP, nM) [Palmer et al. 2017]	Corresponding HTTK predicted lowest external exposure in vivo (mg/kg/day)
all-trans retinoic acid	19 (±15)	2.20E-03
13-cis-retinoid acid	65 (±35)	6.34E-03
9-cis-retinoic acid	36 (±9)	3.51E-03
Etretinate	1694 (±1537)	9.59E-02
Acitretin	ND	-
Retinol	191536 (±108464)	4.05E+01
TTNPB	62 (±38)	NA*

*chemical-specific model does not reach steady state for the given inputs



Project Summary and Next Steps

- *In vivo* external exposure doses associated with developmental toxicity (as measured in vitro) for retinoid analogues were determined using HTTK modeling platform
- HTTK pregnancy model allowed for the study of the effects of physiological changes on chemical kinetics.
- HTTK pregnancy model implications stands to have more confidence for chemicals that have physiological parameters as the most influential determinant of maternal-fetal disposition
- Future efforts include gathering available environmental exposure levels for activity-to-exposure ratio determinations
- In-progress pregnancy PBYK models when characterized fully will serve to be an invaluable tool for understanding pregnancy related changes on chemical kinetics

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