R Package "httk"

John Wambaugh
National Center for Computational Toxicology
Office of Research and Development
U.S. Environmental Protection agency
wambaugh.john@epa.gov

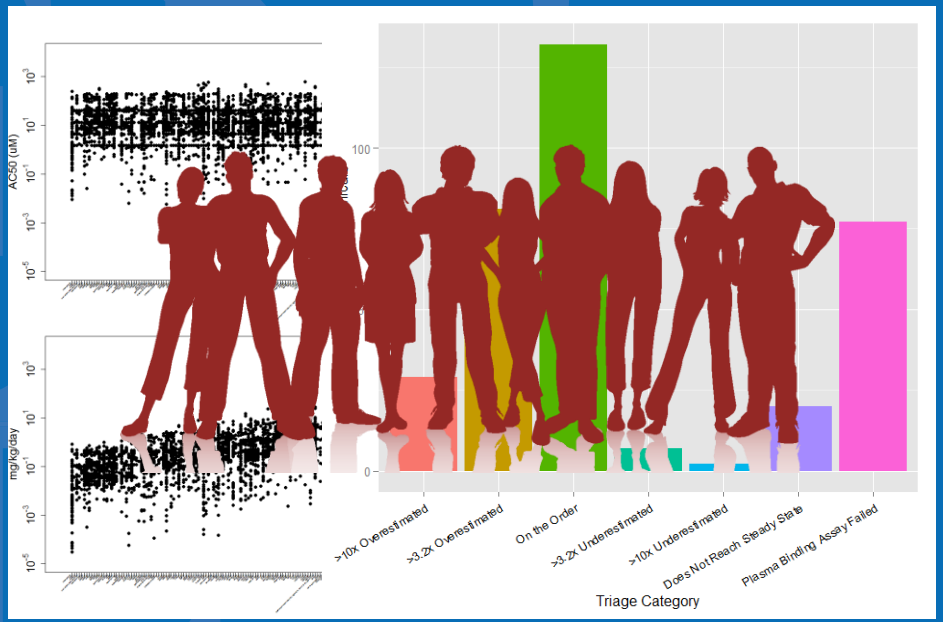


Figure includes image from Thinkstock

The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA

Introduction

- Toxicokinetics (TK) provides a bridge between HTS and HTE by predicting tissue concentrations due to exposure
  - Traditional TK methods are resource intensive

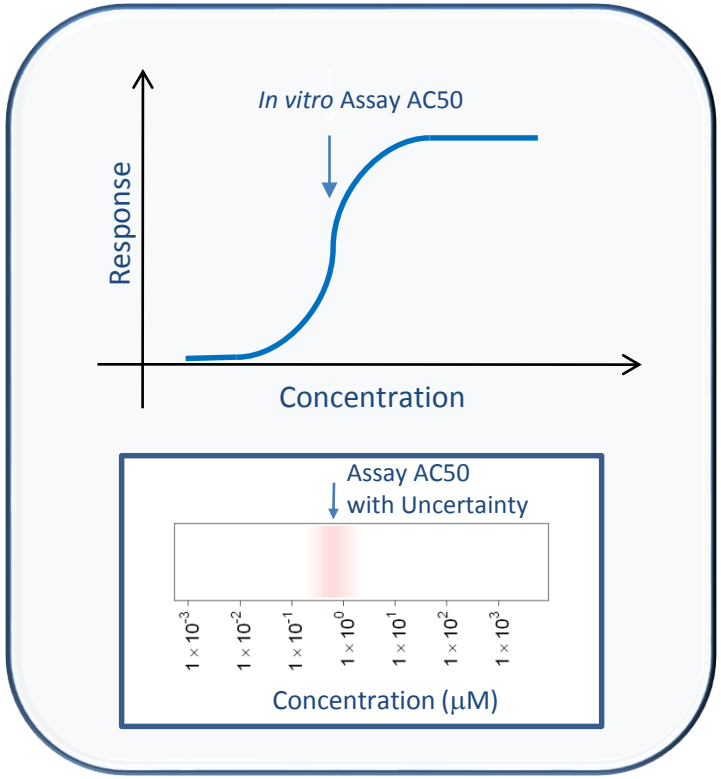
- Relatively high throughput TK (HTTK) methods have been used by the pharmaceutical industry to determine range of efficacious doses and to prospectively evaluate success of planned clinical trials (Jamei, *et al.*, 2009; Wang, 2010)

  - A key application of HTTK has been “reverse dosimetry” (also called Reverse TK or RTK)

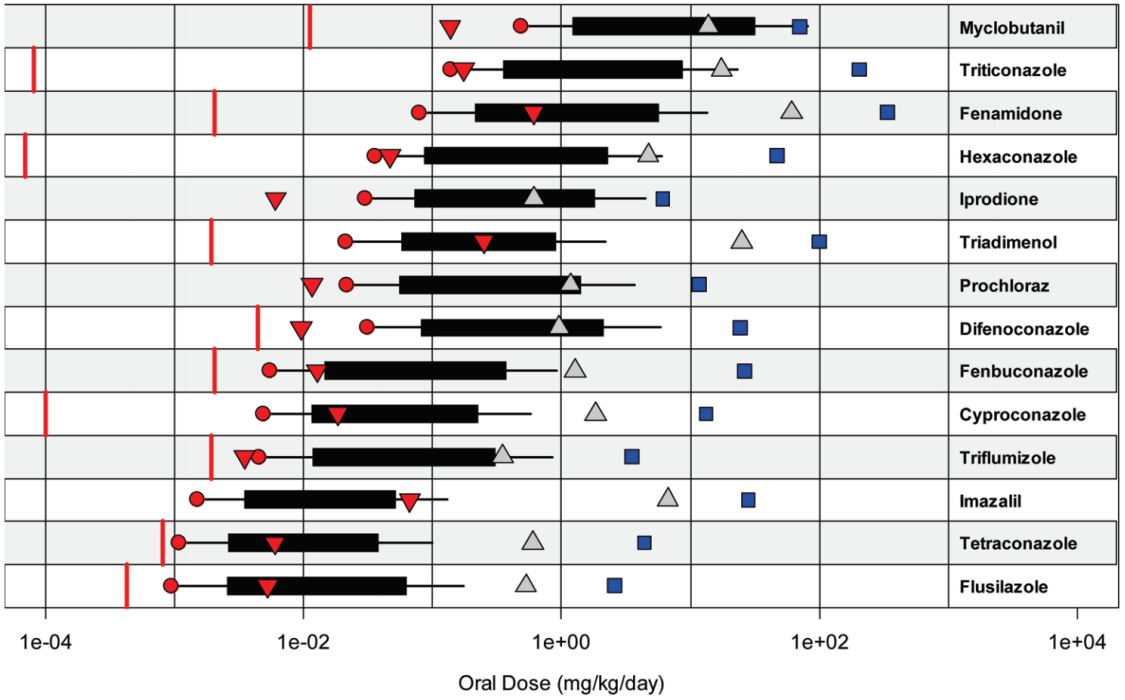
  - RTK can approximately convert *in vitro* HTS results to daily doses needed to produce similar levels in a human for comparison to exposure data (Wetmore, *et al.,* 2012)

High-Throughput Bioactivity

- **Tox21**: Examining >10,000 chemicals using ~50 assays intended to identify interactions with biological pathways (Schmidt, 2009)
- **ToxCast**: For a subset (>1000) of Tox21 chemicals ran >500 additional assays (Judson et al., 2010)
- Most assays conducted in dose-response format (identify 50% activity concentration – AC50 – and efficacy if data described by a Hill function)
- All data is public: http://actor.epa.gov/



In vitro Bioactivity, HTTK, and *in Vivo* Toxic Doses



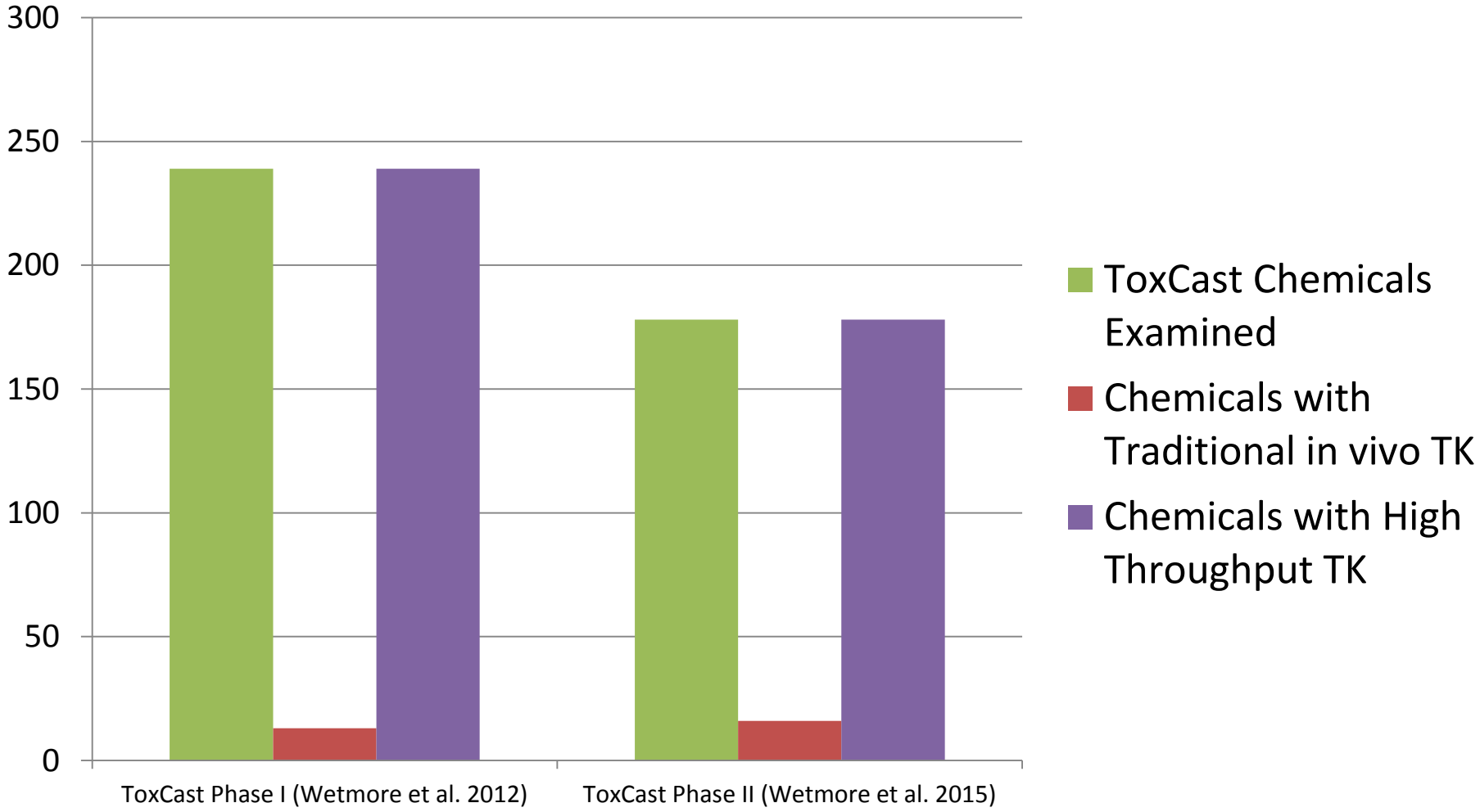
Judson *et al*. (2011)

Comparison of HTTK predicted oral equivalent doses (box and whisker plots in mg/kg/day) with doses for no effect and low effect groups in animal studies

- **Lowest Observed Effect Level**
- **No Observed Effect Level (NEL)**
- **NEL/100**

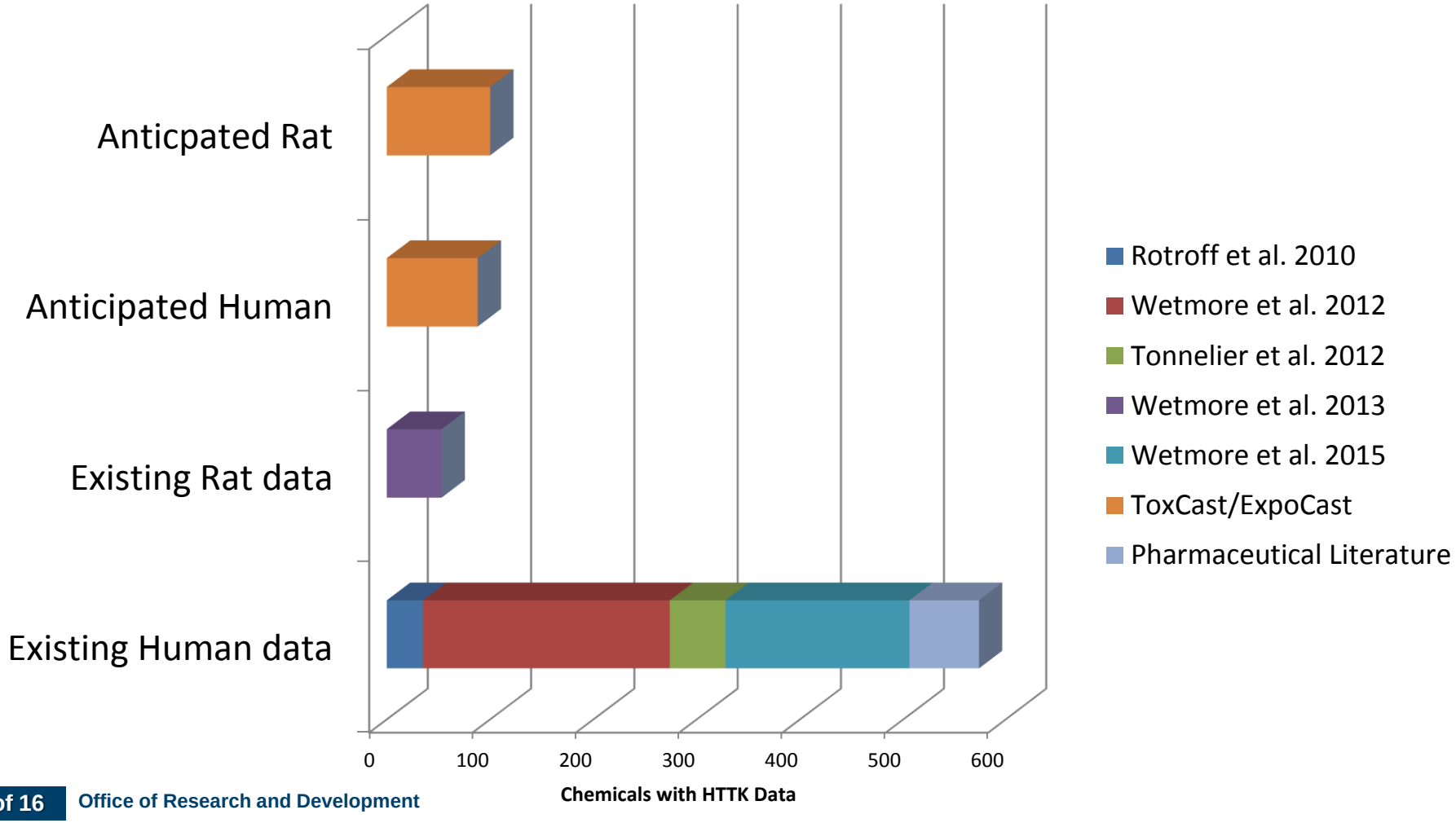
Estimated chronic exposure levels from food residues are indicated by vertical red lines. All values are in mg/kg/day.

The Need for *In Vitro* Toxicokinetics



- Studies like Wetmore et al. (2012),addressed the need for TK data using *in vitro* methods

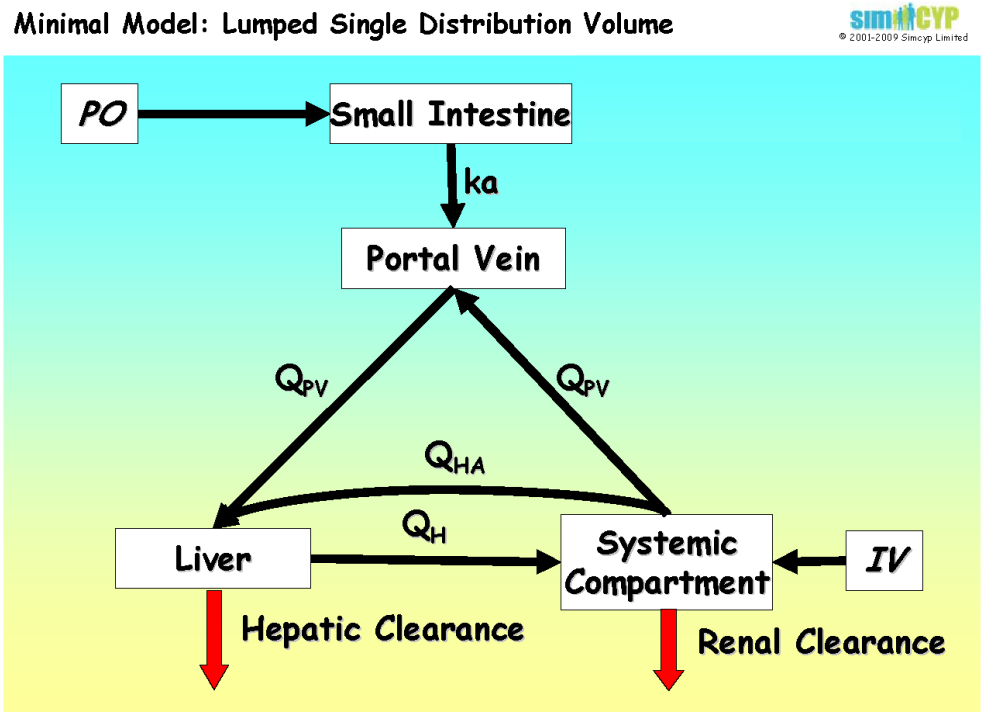
Chemicals with HTTK Data



High Throughput Toxicokinetics (HTTK)

Jamei *et al*. (2009)

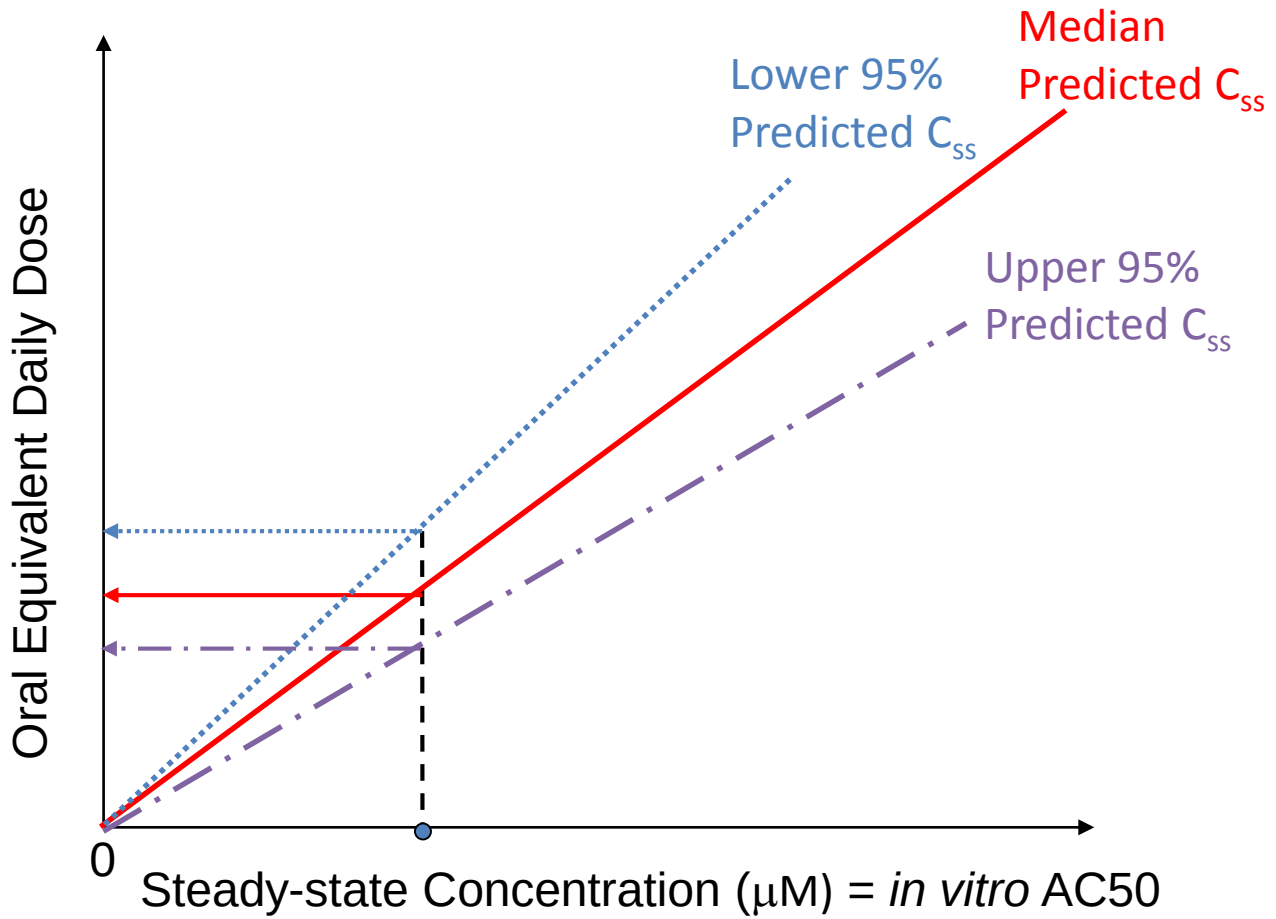
- *In vitro* plasma protein binding and metabolic clearance assays allow approximate hepatic and renal clearances to be calculated
- At steady state this allows conversion from concentration to administered dose
- 100% bioavailability assumed



$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{ub}) + \left(\text{Q}_l * \text{F}_{ub} * \frac{Cl_{int}}{\text{Q}_l + \text{F}_{ub} * Cl_{int}}\right)}$$

Oral dose in (mg/kg/day) → Sum of hepatic and renal clearance (mg/kg/day)

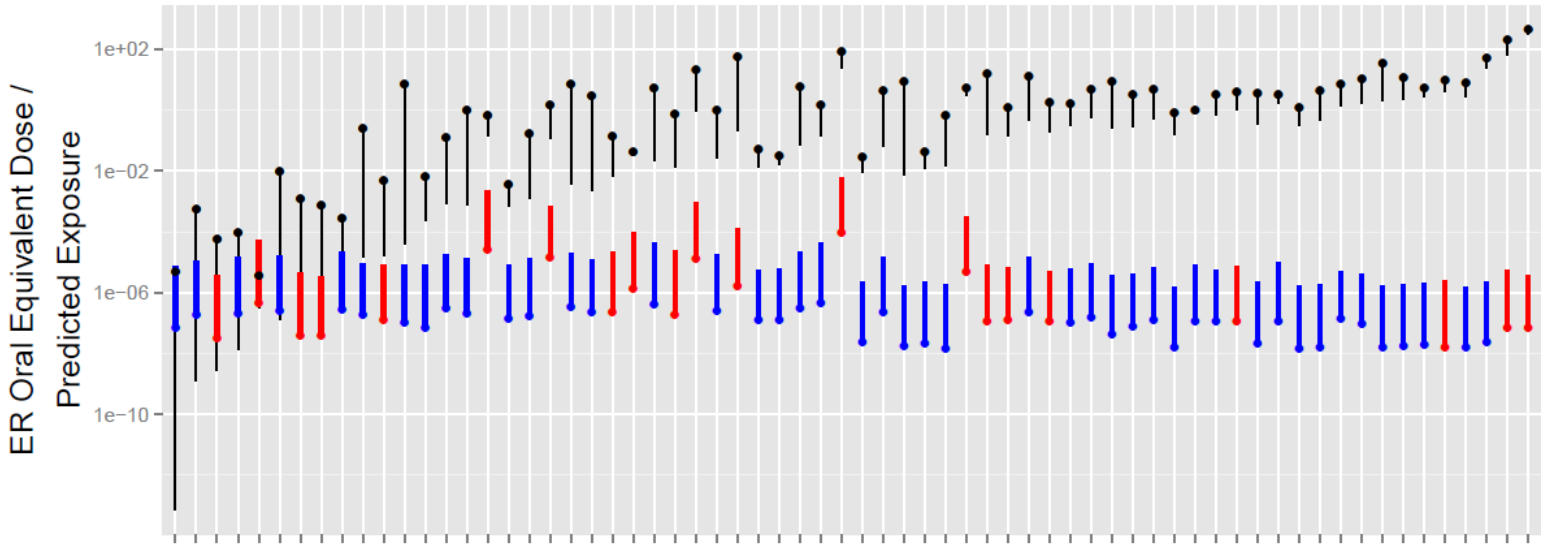
Steady-State In Vitro-In Vivo Extrapolation (IVIVE)



- The higher the predicted C_{ss}, the lower the oral equivalent dose, so the upper 95% predicted C_{ss} from the MC has a lower oral equivalent dose

Dosimetry and Exposure Provides Context for HTS

Endocrine disruption AOP *(Judson et al., in prep.)*



ToxCast Bioactivity Converted to mg/kg/day with HTTK (Wetmore et al., 2012)

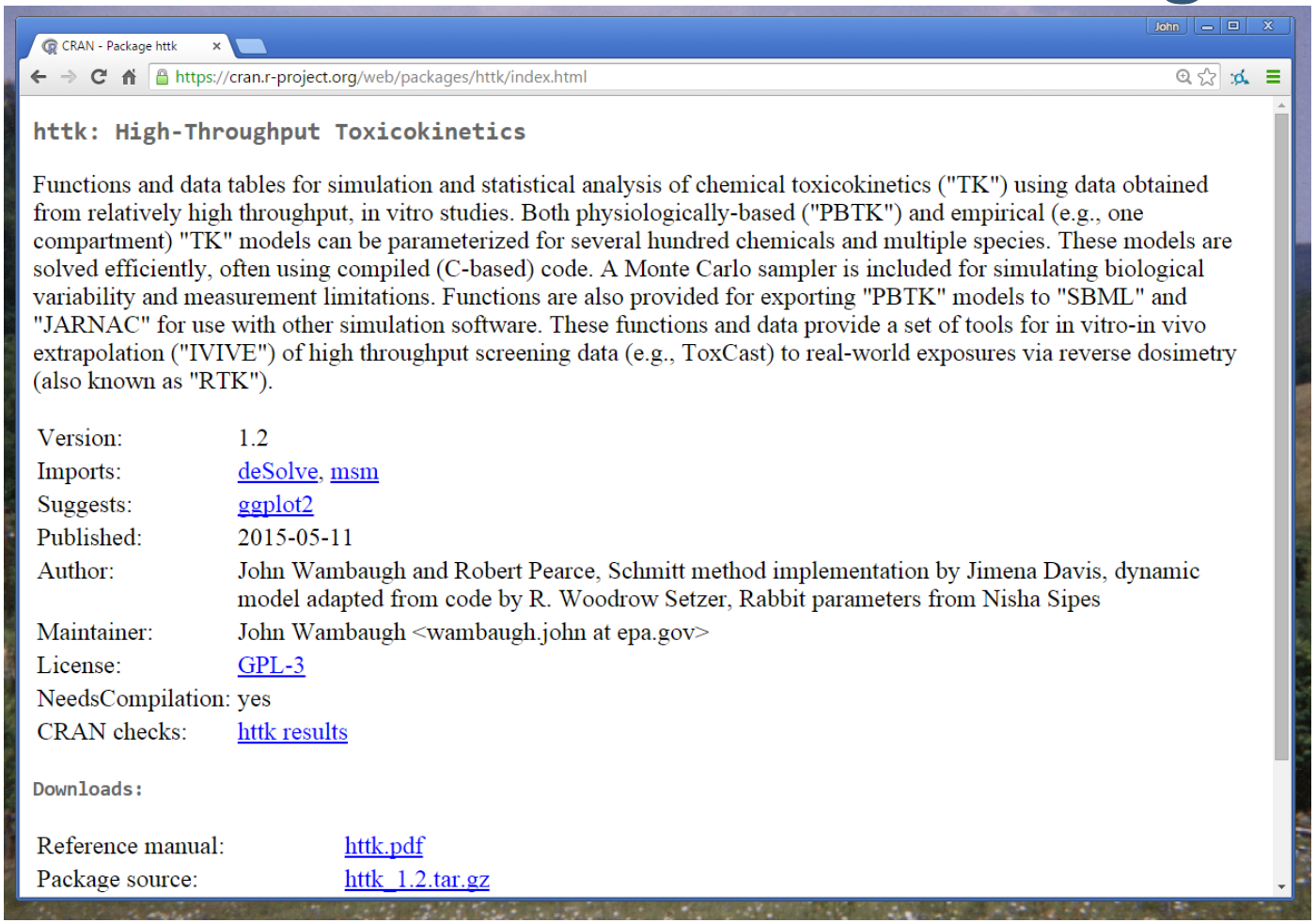
ExpoCast Exposure Predictions (Wambaugh *et al.*, 2014)

ToxCast Chemicals

December, 2015 Panel:
"Scientific Issues Associated with Integrated Endocrine Bioactivity and Exposure-Based Prioritization and Screening"

DOCKET NUMBER: EPA–HQ–OPP–2014–0614

Steady State Concentrations with httk R Package



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

Can access this from the R GUI: “Packages” then “Install Packages”

Steady State Concentrations with httk R Package

```
library(httk)

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for human for Acetochlor (published value):
get_wetmore_css(chem.cas="34256-82-1")

# Should produce error:
get_wetmore_css(chem.name="34256-82-1")

#Capitalization shouldn't matter:
get_wetmore_css(chem.name="acetochlor")
get_wetmore_css(chem.name="Acetochlor")

# What's going on?
help(get_wetmore_css)

# What chemicals can I do?
get_wetmore_cheminfo()
```

Version history for the "httk" R Package

The publicly available R package contains code and data that has been part of peer-reviewed publications

- Version 1.1 accompanied "Toxicokinetic Triage for Environmental Chemicals" Wambaugh et al. (2015) Tox. Sci.
- Version 1.2 accompanied "httk: R Package for High-Throughput Toxicokinetics" Pearce et al., submitted to Journal of Statistical Software
- Version 1.3 is in development and will be released to accompany "Incorporating High-Throughput Exposure Predictions with Dosimetry-Adjusted *In Vitro* Bioactivity to Inform Chemical Toxicity Testing" Wetmore et al., Tox. Sci. *in press*

While we develop research we maintain internal versions containing data and code that has yet to be peer reviewed.

Released version

```
sessionInfo()$otherPkgs$httk$Version
[1] "1.2"
all.data <-
get_cheminfo(info=c("CAS","Compound","Clint","Funbound.plasma"))
all.data[sapply(all.data$Compound,function(x) regexpr("conazole",x)!=-1),]
CAS       Compound Human.Clint Human.Funbound.plasma
53   94361-06-5  Cyproconazole    1.537980           0.108556089
69  119446-68-3 Difenoconazole   24.210384           0.004255109
75   83657-24-3   Diniconazole    2.586969           0.019467923
93  114369-43-6  Fenbuconazole   11.191873           0.005000000
119  79983-71-4   Hexaconazole   16.220043           0.040999740
214 112281-77-3  Tetraconazole    0.000000           0.024838039
234 131983-72-7  Triticonazole    6.117502           0.137256517
304  65277-42-1   Ketoconazole   55.000000           0.062000000
```

Development version

```
sessionInfo()$otherPkgs$httk$Version
[1] "1.2-4"
all.data <- get_cheminfo(info=c("CAS","Compound","Clint","Funbound.plasma"))
all.data[sapply(all.data$Compound,function(x) regexpr("conazole",x)!=-1),]
           CAS       Compound Human.Clint Human.Funbound.plasma
53   94361-06-5  Cyproconazole    1.537980           0.108556089
69  119446-68-3 Difenoconazole   24.210384           0.004255109
75   83657-24-3   Diniconazole    2.586969           0.019467923
93  114369-43-6  Fenbuconazole   11.191873           0.005000000
119  79983-71-4   Hexaconazole   16.220043           0.040999740
215 112281-77-3  Tetraconazole    0.000000           0.024838039
235 131983-72-7  Triticonazole    6.117502           0.137256517
242  60207-90-1  Propiconazole   18.400000           0.029491801
356  65277-42-1   Ketoconazole   55.000000           0.062000000
```

Steady State Concentrations with httk R Package

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for human for Acetochlor (calculated value):

```
calc_mc_css(chem.cas="34256-82-1")
```

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for rat for Acetochlor (should produce errors since there is no published value, 0.5 quantile only):

```
get_wetmore_css(chem.cas="34256-82-1",species="Rat")
```

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for rat for Acetochlor (calculated value):

```
calc_mc_css(chem.cas="34256-82-1",species="Rat")
```

#Steady-state concentration (uM) for 1 mg/kg/day for 0.5 quantile for rat for Acetochlor (published value):

```
get_wetmore_css(chem.cas="34256-82-1",species="Rat",which.quantile=0.5)
```

#Steady-state concentration (uM) for 1 mg/kg/day for 0.5 quantile for rat for Acetochlor (calculated value):

```
calc_mc_css(chem.cas="34256-82-1",species="Rat",which.quantile=0.5)
```

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for mouse for Acetochlor (should produce error since there is no published value, human and rat only):

```
get_wetmore_css(chem.cas="34256-82-1",species="Mouse")
```

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for mouse for Acetochlor (calculated value):

```
calc_mc_css(chem.cas="34256-82-1",species ="Mouse")
```

Oral Equivalent Doses with with httk R Package

#State-state oral equivalent dose (mg/kg BW/day) to produce 0.1 uM serum concentration for human, 0.95 quantile, for Acetochlor (published value):

get_wetmore_oral_equiv(0.1,chem.cas="34256-82-1")

#State-state oral equivalent dose (mg/kg BW/day) to produce 0.1 uM serum concentration for human, 0.95 quantile, for Acetochlor (calculated value):

calc_mc_oral_equiv(0.1,chem.cas="34256-82-1")

#State-state oral equivalent dose (mg/kg BW/day) to produce 0.1 uM serum concentration for human, 0.05, 0.5, and 0.95 quantile, for Acetochlor (published values):

get_wetmore_oral_equiv(0.1,chem.cas="34256-82-1",which.quantile=c(0.05,0.5,0.95))

#State-state oral equivalent dose (mg/kg BW/day) to produce 0.1 uM serum concentration for human, 0.05, 0.5, and 0.95 quantiles, for Acetochlor (calculated value):

calc_mc_oral_equiv(0.1,chem.cas="34256-82-1",which.quantile=c(0.05,0.5,0.95))

#State-state oral equivalent dose (mg/kg BW/day) to produce 0.1 uM serum concentration for rat, 0.95 quantile, for Acetochlor (calculated value):

calc_mc_oral_equiv(0.1,chem.cas="34256-82-1",species="Rat")

Summary

- Toxicokinetics (TK) provides a bridge between HTS and HTE by predicting tissue concentrations due to exposure
- HTTK methods developed for pharmaceuticals have been adapted to environmental testing
- A primary application of HTTK is “Reverse Dosimetry” or RTK
- New R package “httk” freely available on CRAN allows statistical analyses
  - Analysis has been submitted

EPA United States Environmental Protection Agency

Chemical Safety for Sustainability (CSS) Rapid Exposure and Dosimetry (RED) Project

NCCT
Chris Grulke
Richard Judson
Thomas Knudsen
Chantel Nicolas*
Robert Pearce*
James Rabinowitz
Caroline Ring*
Woody Setzer
Imran Shah
Rusty Thomas
Antony Williams

NRMRL
Yirui Liang*
Xiaoyu Liu

NHEERL
Jane Ellen Simmons
Marina Evans
Mike Hughes

***Trainees**

NERL
Craig Barber
Derya Biryol*
Dalizza Colon
Kathie Dionisio*
Marc Duchatelier*
Peter Egeghy
Kim Gaetz
Brandall Ingle*
Kristin Isaacs
Haluk Ozkaynak
Katherine Phillips*
Mark Strynar
Jon Sobus
Mike Tornero-Velez
Dan Vallero

High Throughput Toxicokinetics Researchers

Collaborators

Arnot Research and Consulting
Jon Arnot
Chemical Computing Group
Rocky Goldsmith
Environmental Protection Agency
Alicia Frame
Hamner Institutes
Barbara Wetmore
Cory Strope
Indiana University
James Sluka
Michigan State University
Jade Mitchell
National Institute for Environmental Health Sciences (NIEHS)
Mike Devito
Nisha Sipes
Kyla Taylor
Kristina Thayer
Netherlands Organisation for Applied Scientific Research (TNO)
Sieto Bosgra
North Carolina State University
Anran Wang
Research Triangle Institute
Timothy Fennell
Silent Spring Institute
Robin Dodson
University of California, Davis
Deborah Bennett
University of Michigan
Olivier Jolliet
University of North Carolina, Chapel Hill
Alexander Sedykh*
Alex Tropsha