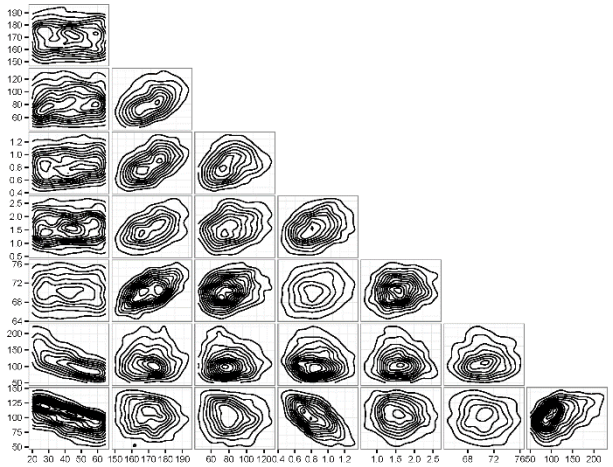
Inter-individual variability in high-throughput risk prioritization of environmental chemicals



Dr. Caroline Ring

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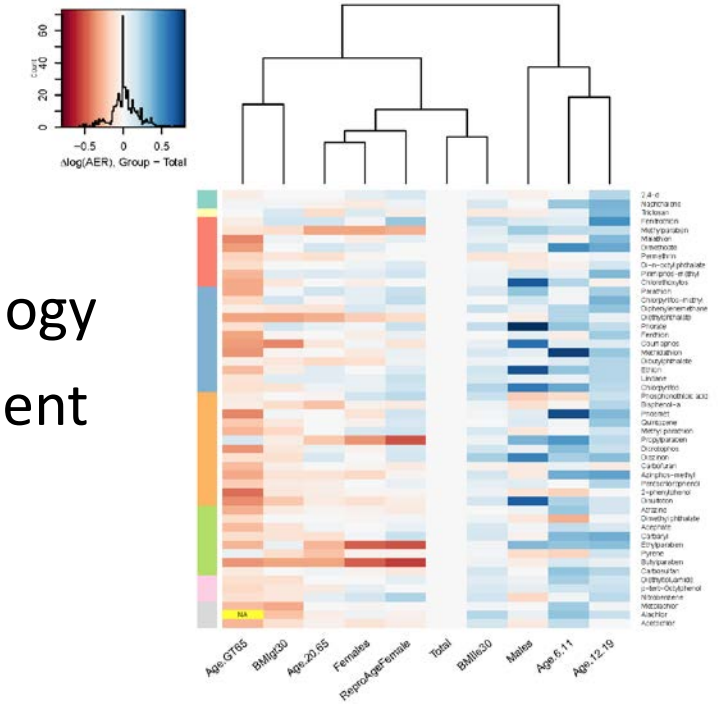
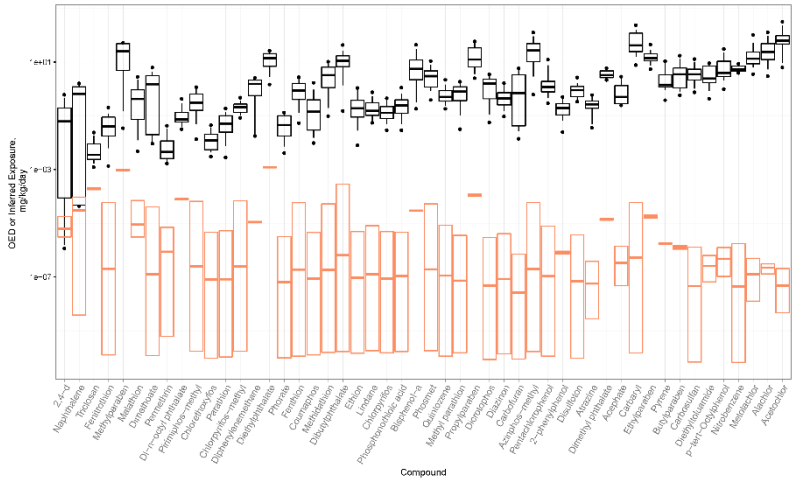
US EPA, Office of Research and Development





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Overview

- Risk prioritization: what and why
- Quick review: existing work on IVIVE for high-throughput risk prioritization with reverse TK
  - Quick overview of general reverse TK procedure
  - Activity-exposure ratio
- Our goals with this work
- HTTK-Pop: our population simulator for HT toxicokinetics
- Prioritization results using HTTK-Pop
- Areas for future work

Need for risk prioritization

- EPA authorized to assess risk of environmental chemicals [GAO 2005]
- Approx. 30,000 chemicals in wide commercial use [Judson *et al* 2009]
- Approx. 700-1000 new chemicals on the market every year [GAO 2005]
- Traditional *in vivo* approaches to tox characterization can cost $millions and take years per chemical [Judson *et al.* 2009]
- Need to triage: which chemicals should be prioritized for further testing? [Wambaugh *et al.* 2015]

Need low-cost, **high-throughput** methods of **risk prioritization**

High throughput risk prioritization

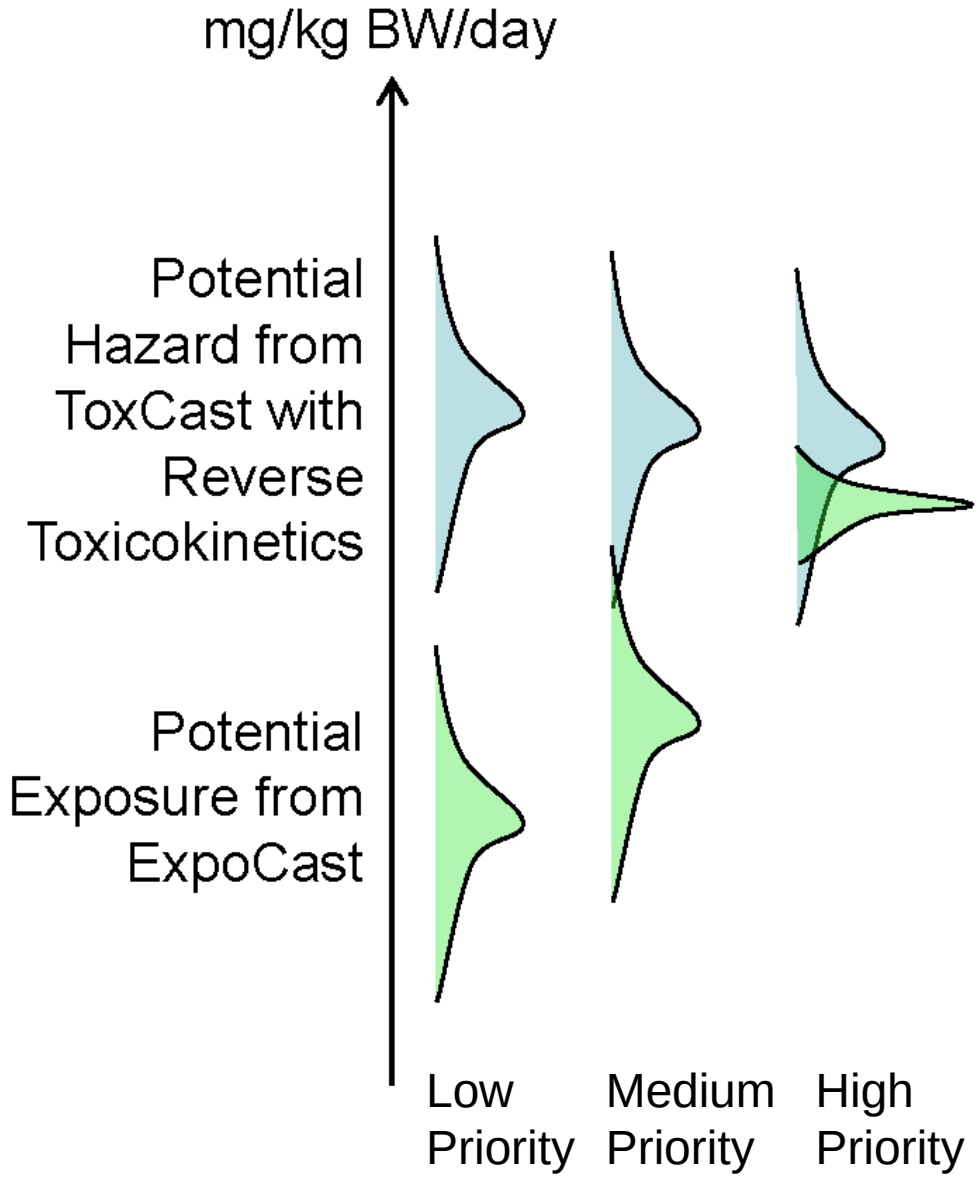
Potential **hazard** vs. potential **exposure**

- **Exposure**: HT model frameworks (*e.g.* ExpoCast) [Wambaugh et al. 2013, 2014]
  - Inferred/predicted based on biomonitoring data
- **Hazard**: *in vitro* HTS bioactivity assays (*e.g.* ToxCast) [Knudsen et al. 2015]
  - Dose-response data on >1800 chemicals for >800 assays (publicly available)

Relate *in vitro* bioactivity to *in vivo* toxicity and risk:

***In vitro-in vivo* extrapolation** (IVIVE) [Bois et al. 2010, Wetmore et al. 2012; Judson et al. 2014] —

using **reverse toxicokinetics** approach [Tan et al. 2006, 2007; Rotroff et al. 2010; Wetmore et al. 2012]

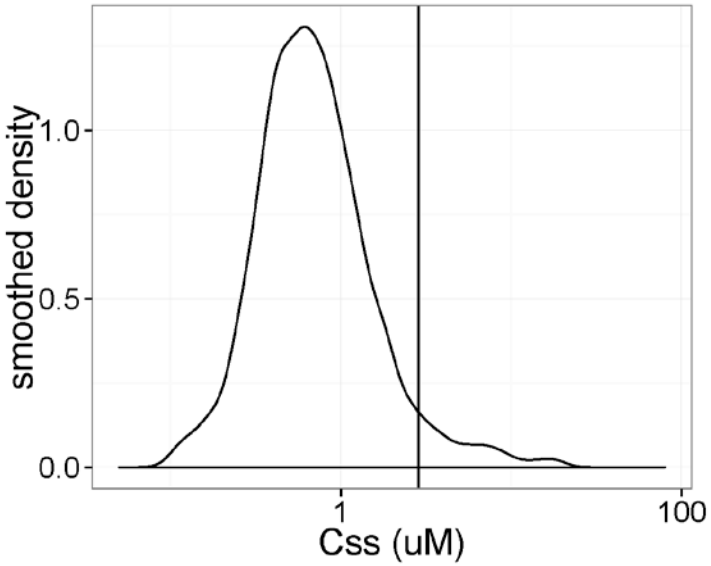


Reverse toxicokinetics: Convert *in vitro* bioactive concentration into equivalent dose

Summary: Reverse TK procedure

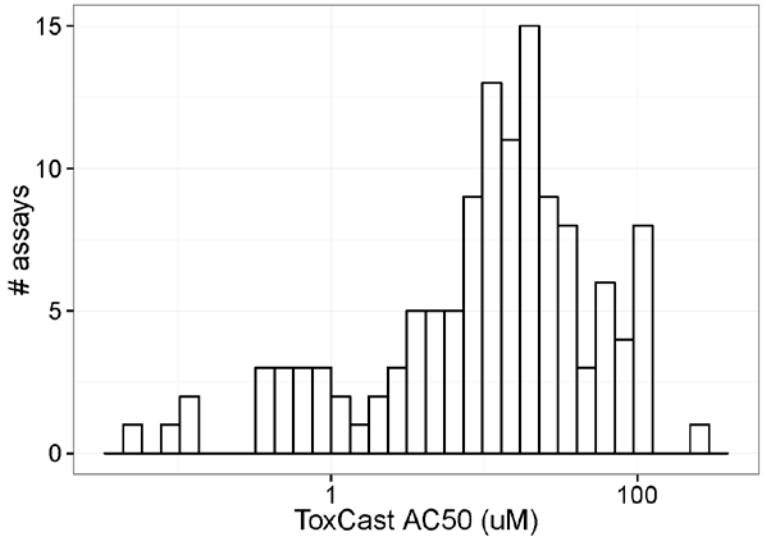
Fixed dose
(1 mg/kg/day)

Monte Carlo sampled
TK model parameters



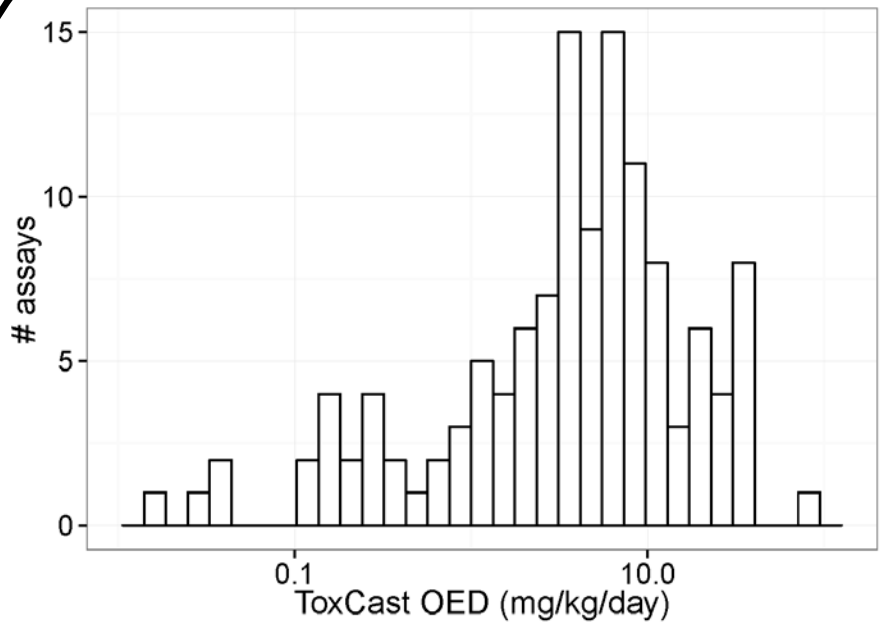
C_{ss} from fixed dose (uM) across "individuals"
Take 95th percentile (conservative)

ToxCast AC50s across assays (uM)



$$\text{Oral Equiv. Dose} = \text{Fixed dose} \times \frac{\text{ToxCast } AC_{50}}{C_{ss} \text{ from fixed dose}}$$

ToxCast OEDs across assays (mg/kg/day)



Compare to range of exposures

Activity-exposure ratio

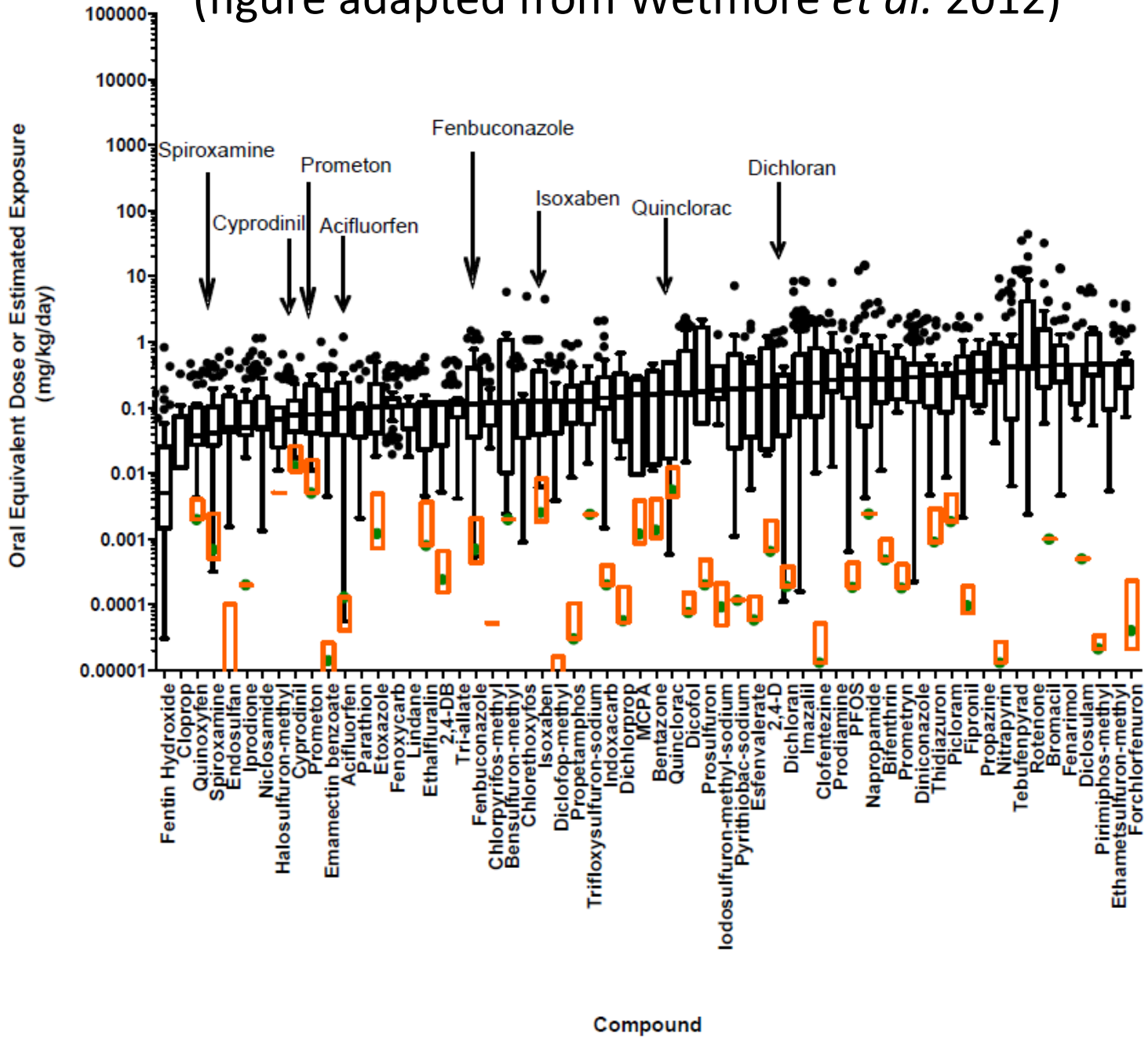
[Wetmore *et al.* 2012, 2014, 2015]

$$\mathrm{AER} = \frac{\text{Oral Equiv. Dose}}{\text{Estimated exposure}}$$

AER <=1 : Exposure potentially high enough to cause bioactivity

AER >> 1: Exposure less likely to be high enough to cause bioactivity

(figure adapted from Wetmore *et al.* 2012)



TK model: 3 compartment steady-state

$$C_{ss} = \frac{\text{dose}}{(GFR \times F_{ub}) + \frac{Q_{liver} \times F_{ub} \times CL_{int,hep}}{Q_{liver} + F_{ub} \times CL_{int,hep}}}$$

- Used in previous risk prioritization work:
  - Rotroff *et al.* 2010
  - Wetmore *et al.* 2012, 2014, 2015
  - Wambaugh *et al.* 2015
- "3 compartment": equiv. to steady-state liver concentration of a 3-compartment model (liver and gut) without partition coefficients
  - Also equiv. to steady-state concentration in 1-compartment model with infusion dosing
- Zero-order uptake of daily dose from gut; 100% bioavailability
- First-order hepatic metabolism
  - "Well-stirred" model to extrapolate $CL_{int,hep}$ from *in vitro* measurements
- Passive renal clearance
- Simple; can be parameterized for large number of chemicals

3 compartment steady-state model parameters

Chemical-specific parameters	Source of parameter values
Fraction unbound in plasma (Fub) Intrinsic clearance rate (CLint)	Measured in HT *in vitro* assays (Wetmore *et al.* 2012, 2014, 2015): pooled adult plasma samples and pooled adult hepatocytes
Physiological parameters	**Monte Carlo sampling to simulate population variability**
Body weight Tissue volumes & blood flows Glomerular filtration rate (GFR) Hepatocellularity	SimCYP [Jamei et al. 2009]: proprietary correlated Monte Carlo (used by Wetmore *et al.* 2012, 2014, 2015; typically N. Eur. Caucasian) *—Or—* Independent Monte Carlo: normal dist. about literature average values, typically for healthy adult Caucasian male (used by Wambaugh *et al.* 2015)

Our goals

- Open-source
  - R package `httk`, available on CRAN (Pearce *et al.*, *J Stat Soft* 2016)
  - General TK models can be parameterized for many chemicals
  - Currently includes independent Monte Carlo approach [Wambaugh et al. 2015]
  - Add **open-source correlated Monte Carlo** simulation approach
- Ability to simulate **modern U.S. population**
  - Compare directly to US population exposure estimates
  - Including potentially sensitive demographic subgroups
    - Identified as important issue in risk assessment framework [EPA 2006]

ExpoCast: Exposures inferred for US population groups, from CDC NHANES urine biomonitoring data [Wambaugh *et al.* 2012, 2014]

For 10 U.S. demographic groups:

1. Total
2. Age 6-11
3. Age 12-19
4. Age 20-65
5. Age >65
6. BMI <= 30
7. BMI > 30
8. Males
9. Females
10. Reproductive-Age Females (ages 16-49)

106 compounds; 50 HTTK compounds

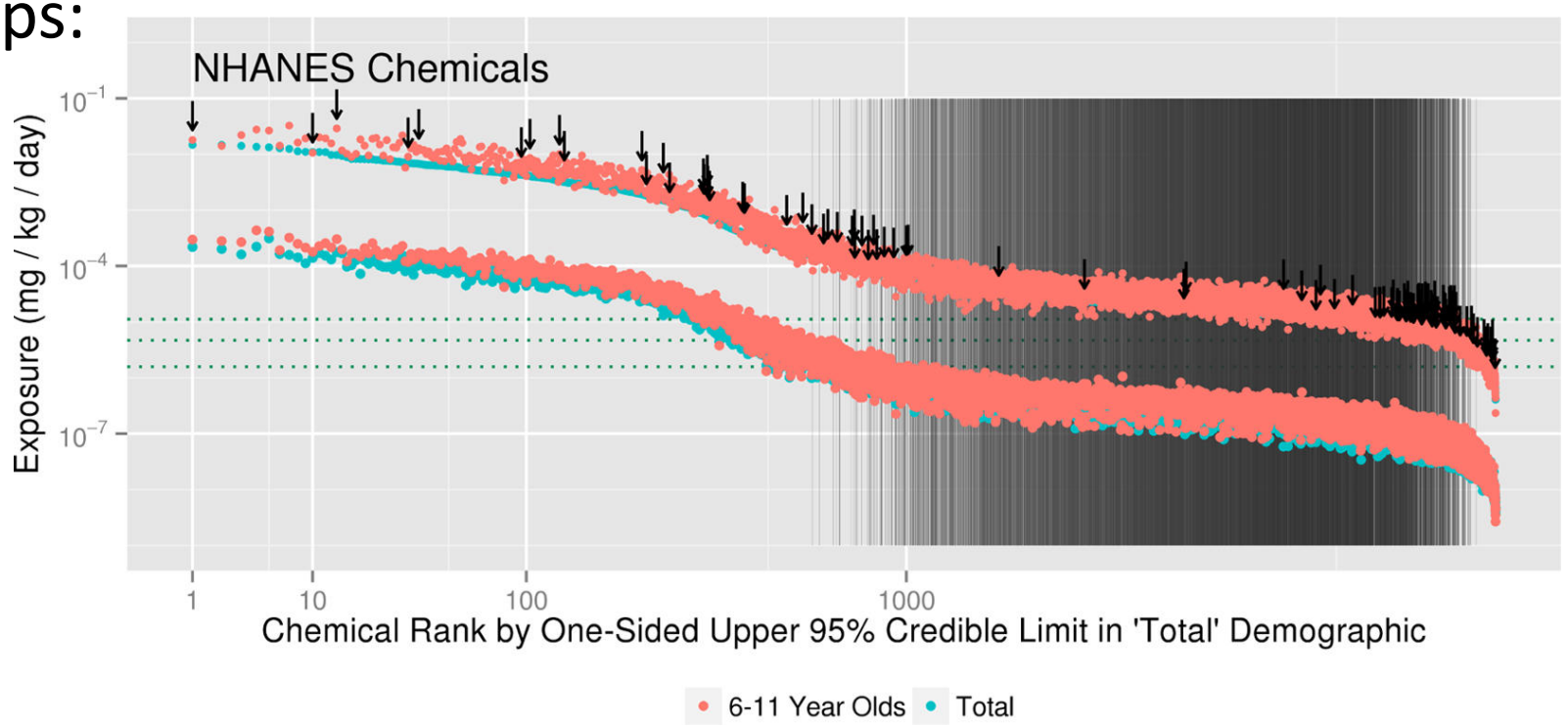


Figure adapted from Wambaugh *et al., Environ Sci Technol* 2014
See also Wambaugh *et al.*, *Environ Sci Technol* 2012

HTTK-Pop: Population simulator for HTTK

Correlated Monte Carlo sampling of physiological model parameters

Body weight
Tissue masses
Tissue blood flows
GFR
Hepatocellularity

Source of data:
Centers for Disease Control,
National Health and Nutrition Examination Survey

Large, ongoing survey of US population: demographic, body measures, medical exam, biomonitoring (health and exposure), ….

Designed to be representative of US population according to census data

Data sets publicly available
(http://www.cdc.gov/nchs/nhanes.htm)

HTTK-Pop: Population simulator for HTTK

Sample NHANES quantities

Sex
Race/ethnicity
Age
Height
Weight
Serum creatinine

Regression equations from literature (+ residual marginal variability)

Predict physiological quantities

Tissue masses
Tissue blood flows
GFR (kidney function)
Hepatocellularity

(Similar approach used in SimCYP [Jamei et al. 2009], GastroPlus, PopGen [McNally et al. 2014], P3M [Price et al. 2003], physB [Bosgra et al. 2012], etc.)

HTTK-Pop: Generating demographic subgroups

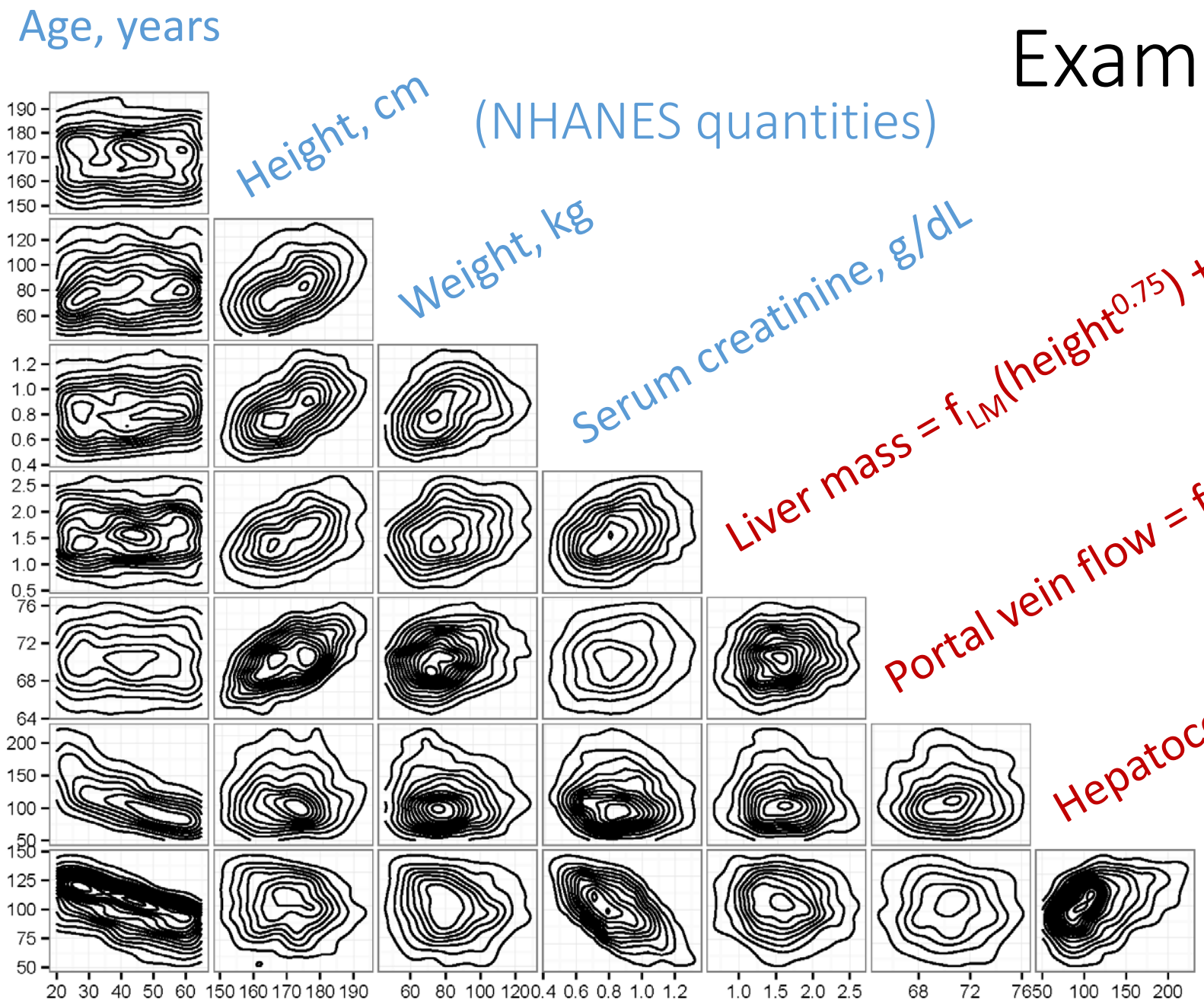
User can specify….	Default if not specified
Age limits	0-79 years
Sex (# males, # females)	NHANES proportions
Race/ethnicity (5 NHANES categories)	NHANES proportions
BMI/weight categories	NHANES proportions

NHANES quantities sampled from appropriate *conditional* distribution (given specifications)

Physiological parameters predicted accordingly

Simulated populations matching the 10 ExpoCast demographic groups (N=1000 in each)

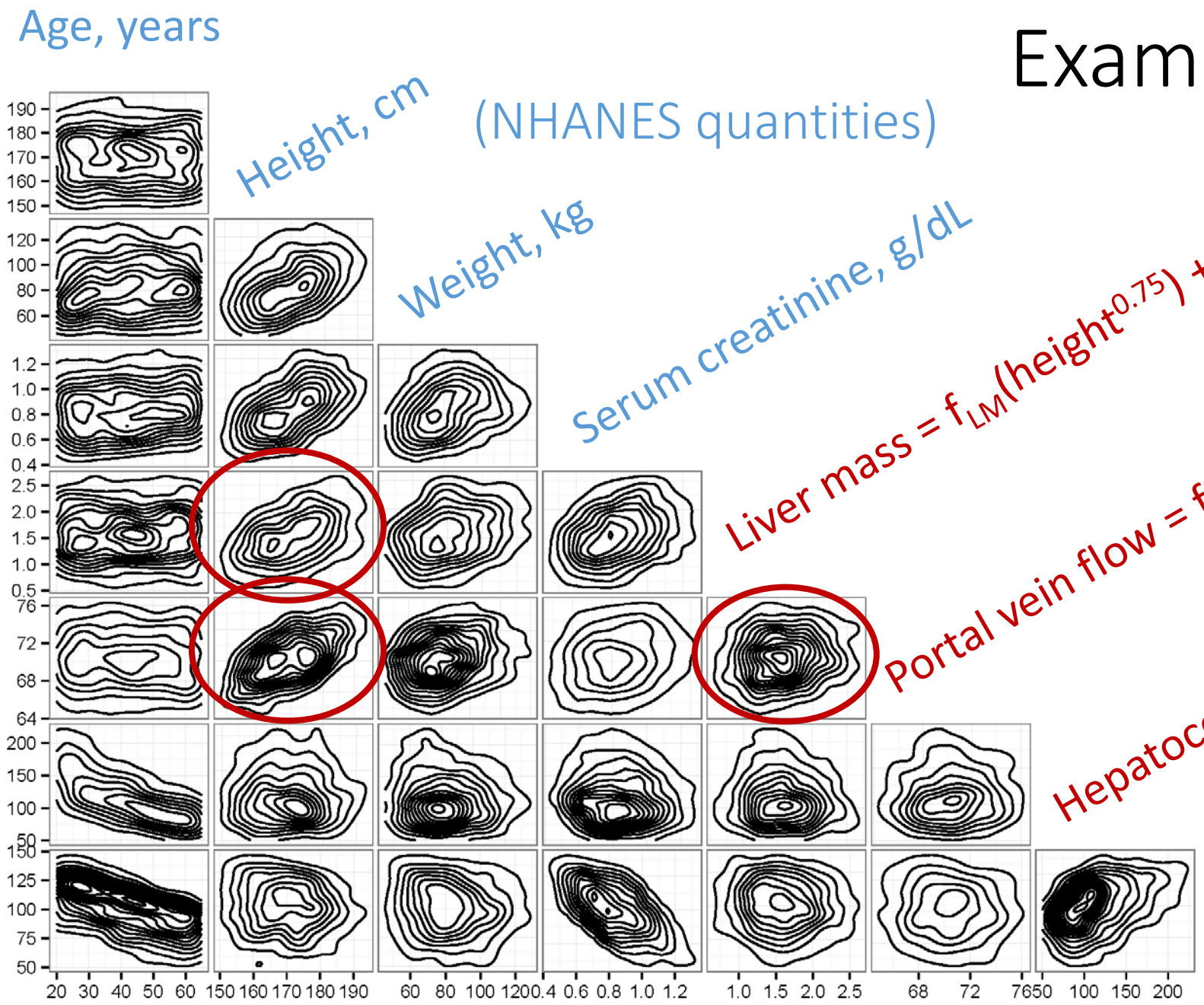
Example: Age 20-65



(Predicted with literature regressions)

GFR = $f_{CKD\text{-}EPI}$(Scr, age, race, sex)

Example: Age 20-65



(NHANES quantities)

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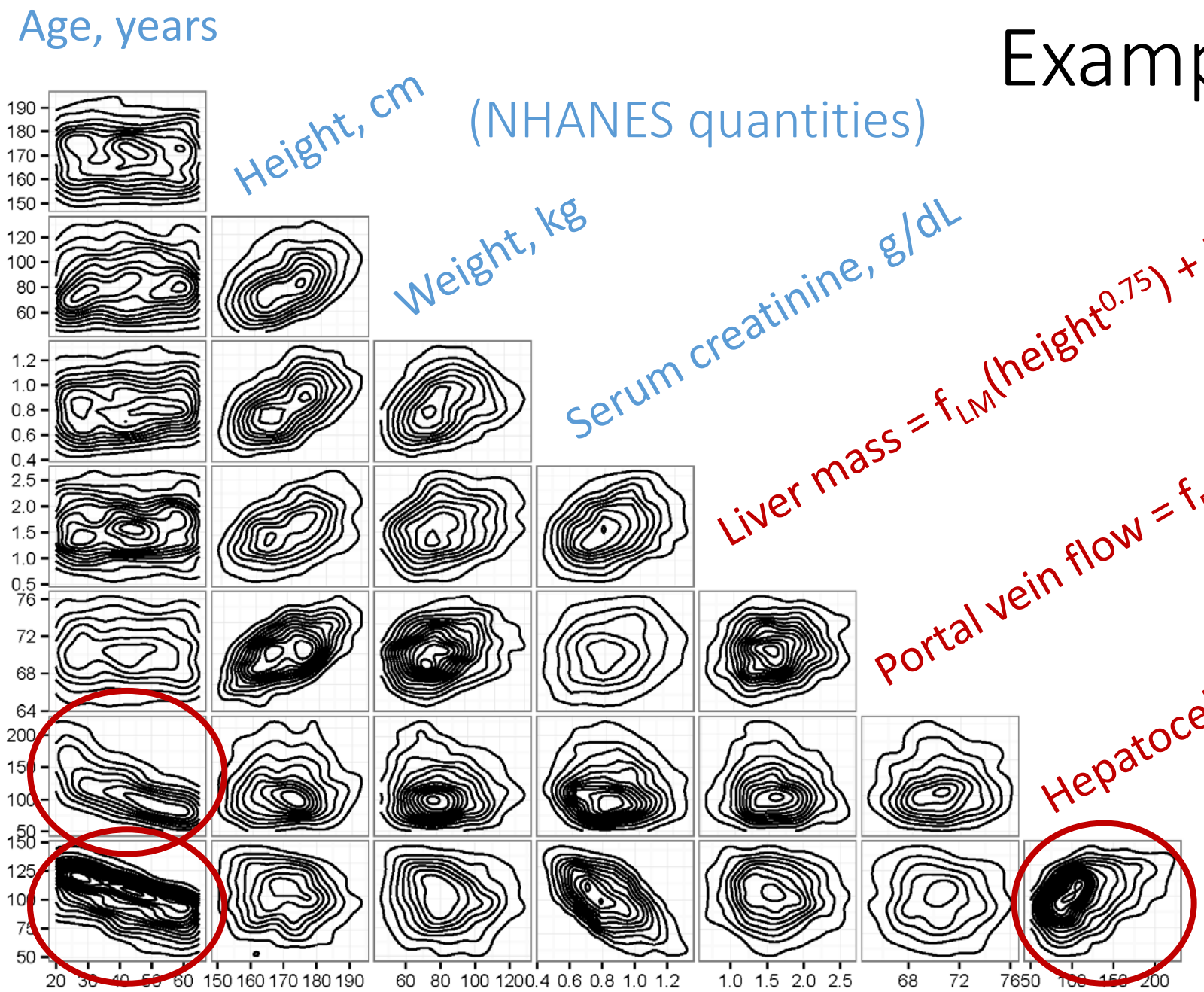
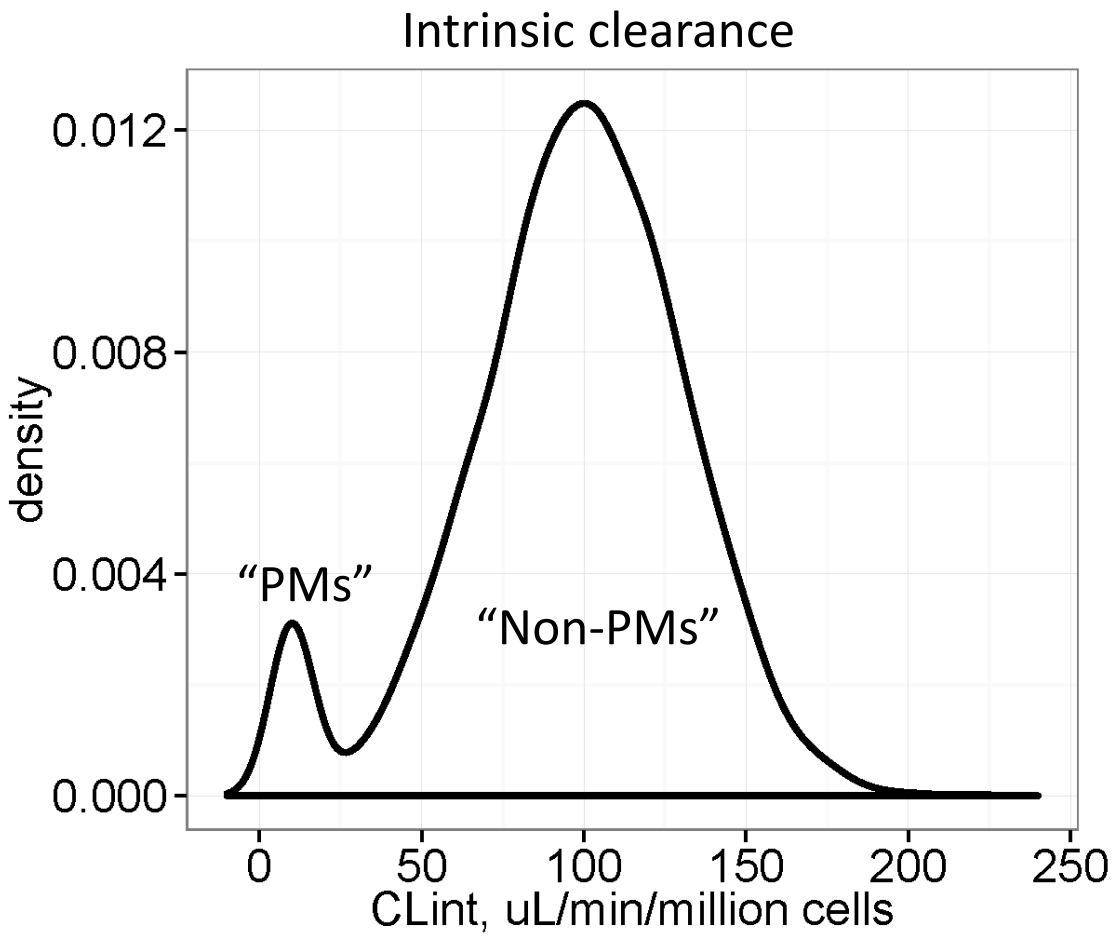


(NHANES quantities)

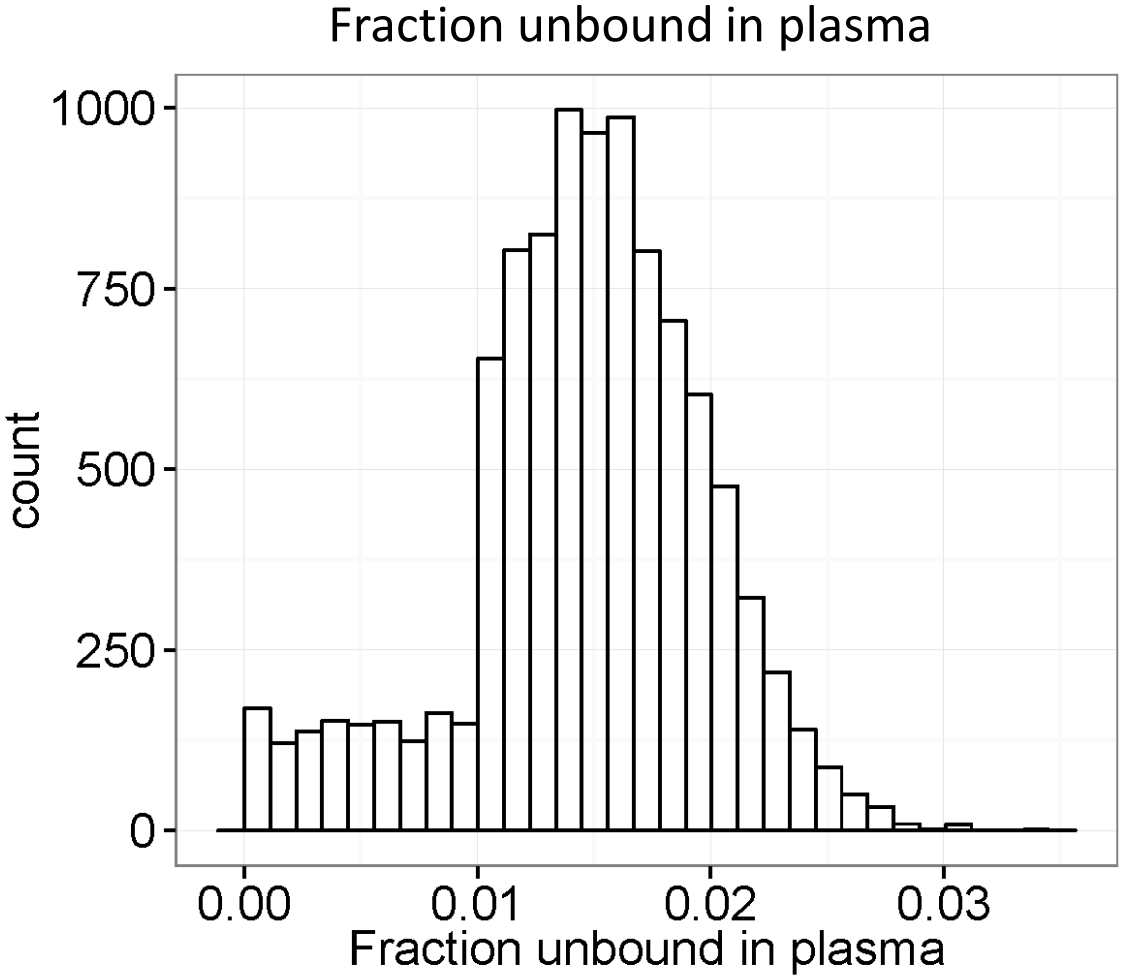
(Predicted with literature regressions)

GFR = $f_{CKD\text{-}EPI}$(Scr, age, race, sex)

Chemical-specific parameters: assume independent distributions about *in vitro* measured values



Assume 5% of population are poor metabolizers



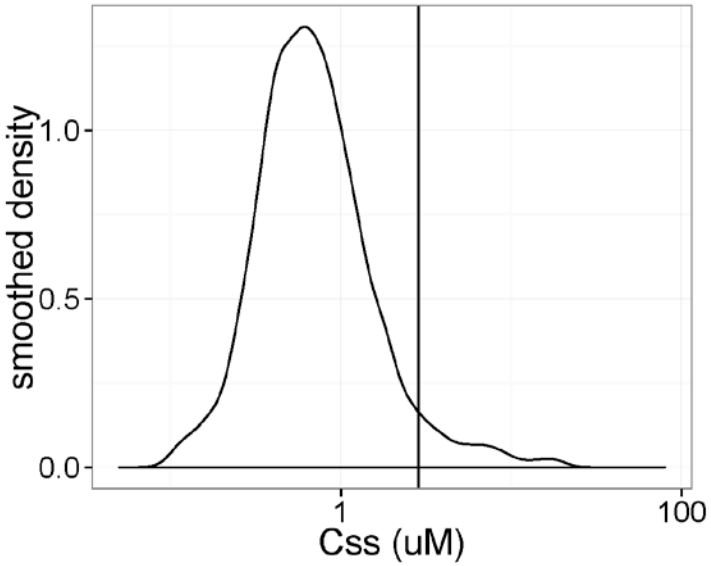
Assume Fub distribution censored below average LOD (0.01)

See: Wambaugh *et al. Toxicol Sci* 2015

Reverse TK: 50 chemicals, 10 ExpoCast demographic groups

Fixed dose
(1 mg/kg/day)

HTTK-Pop



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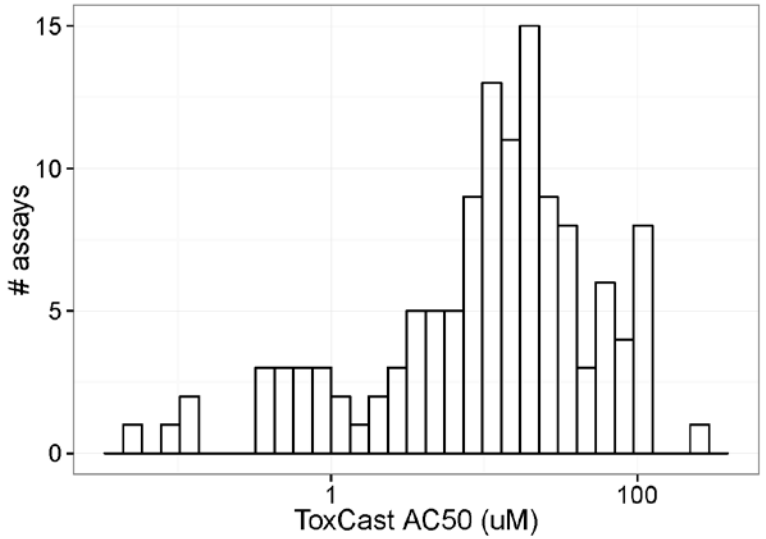

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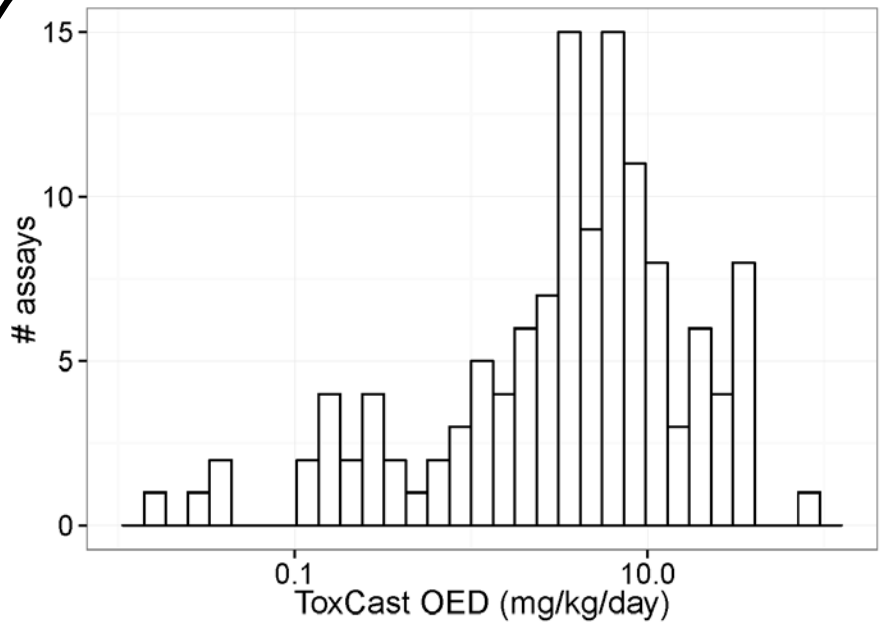
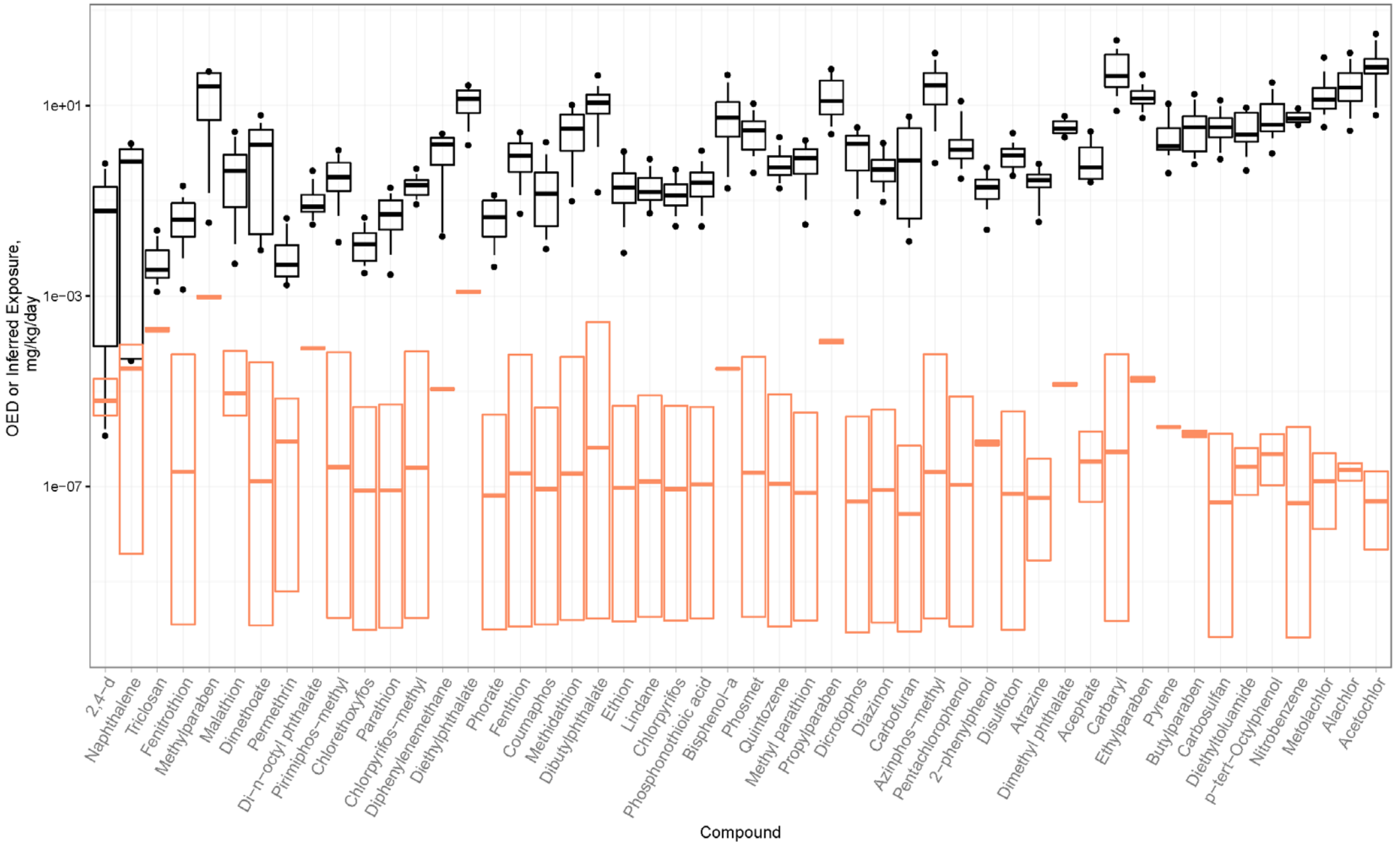


Compare to ExpoCast exposures

OEDs and inferred exposures for total U.S. population

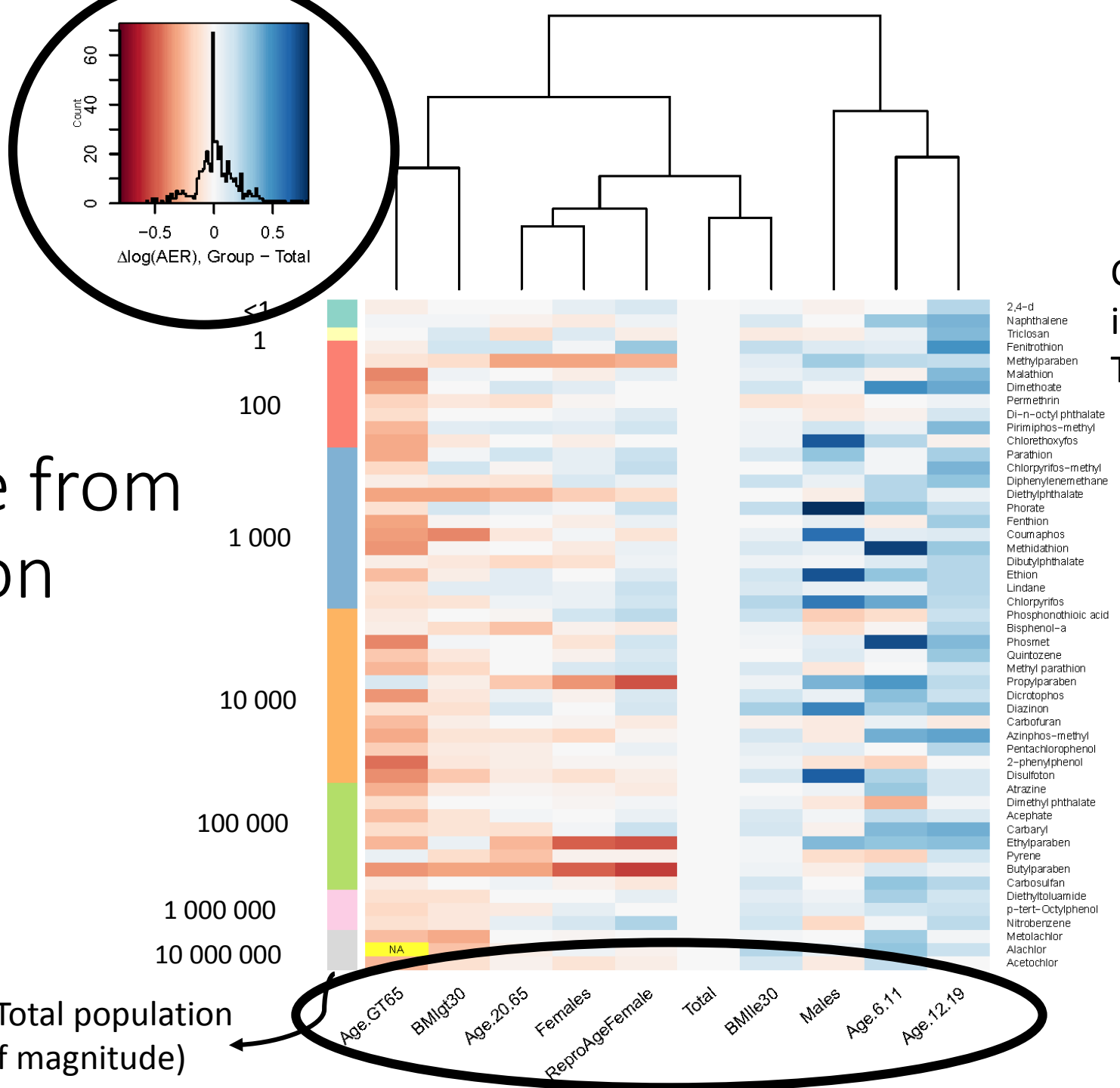


Range of OEDs

AER

Range of inferred exposures

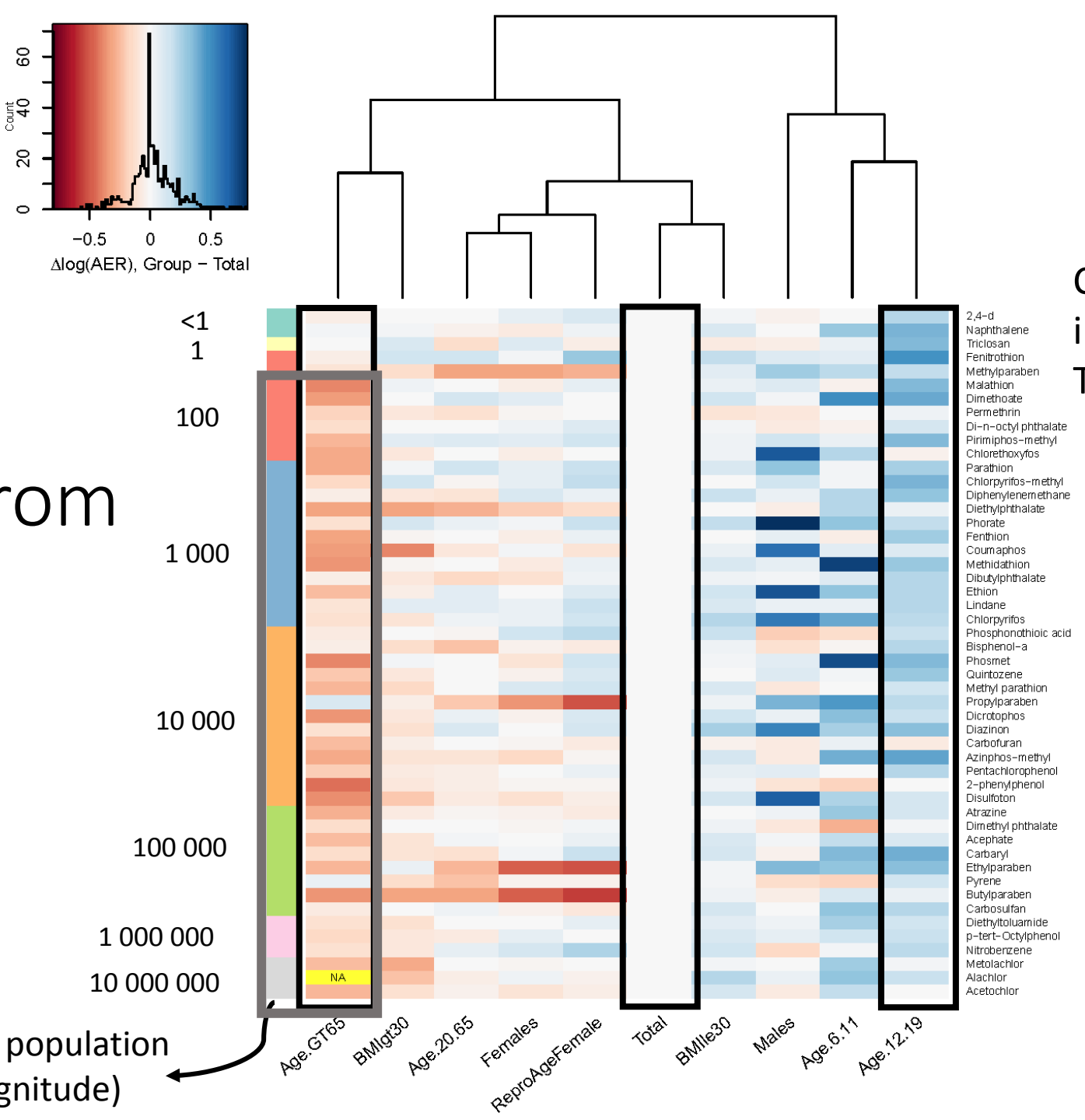
Subgroups: AER difference from total population (order-of-magnitude)



Chemicals by increasing AER for Total population

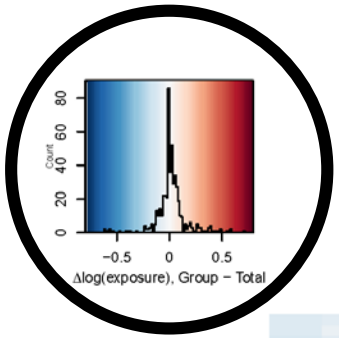
AER for Total population (order of magnitude)

Subgroups: AER difference from total population (order-of-magnitude)

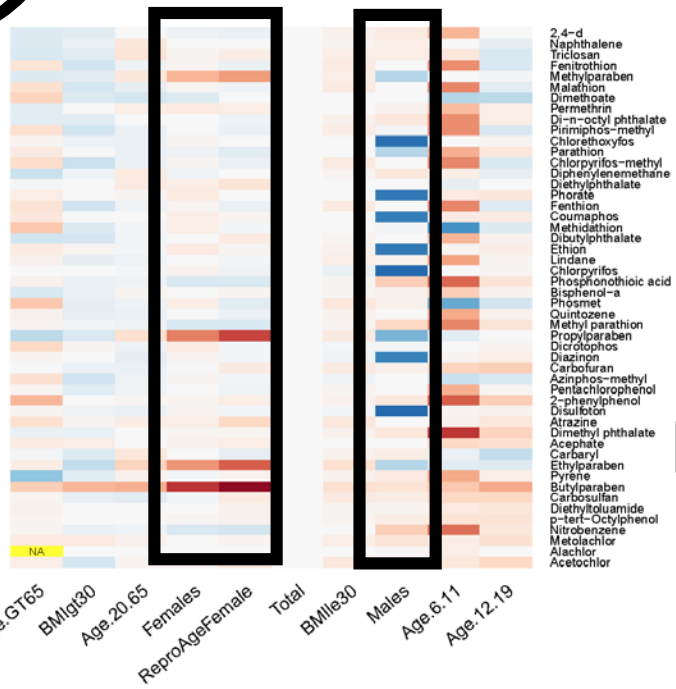


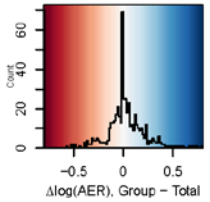
AER for Total population (order of magnitude)

Chemicals by increasing AER for Total population

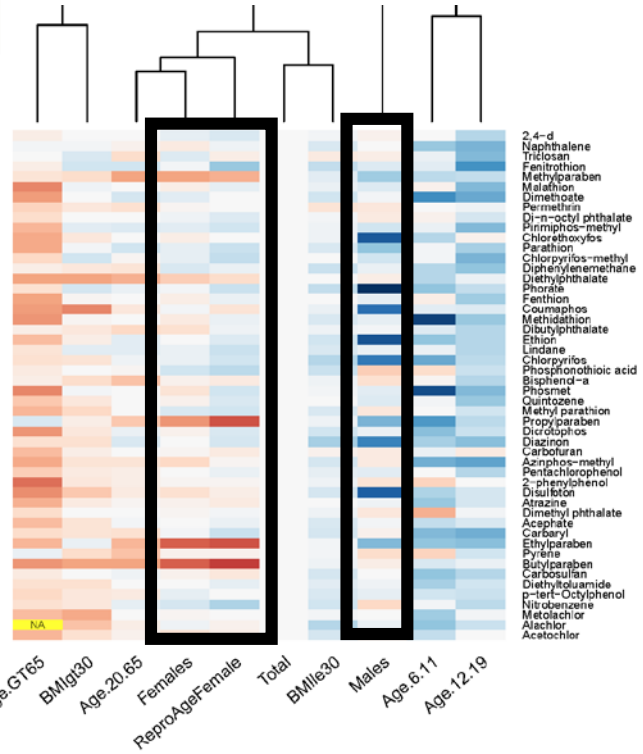


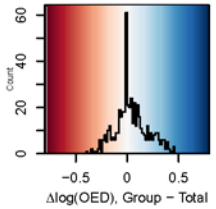
Exposure



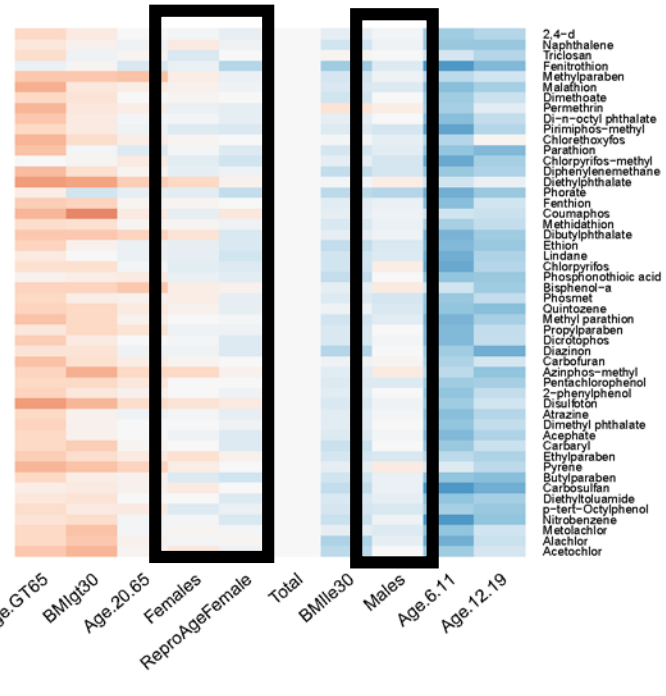


AER





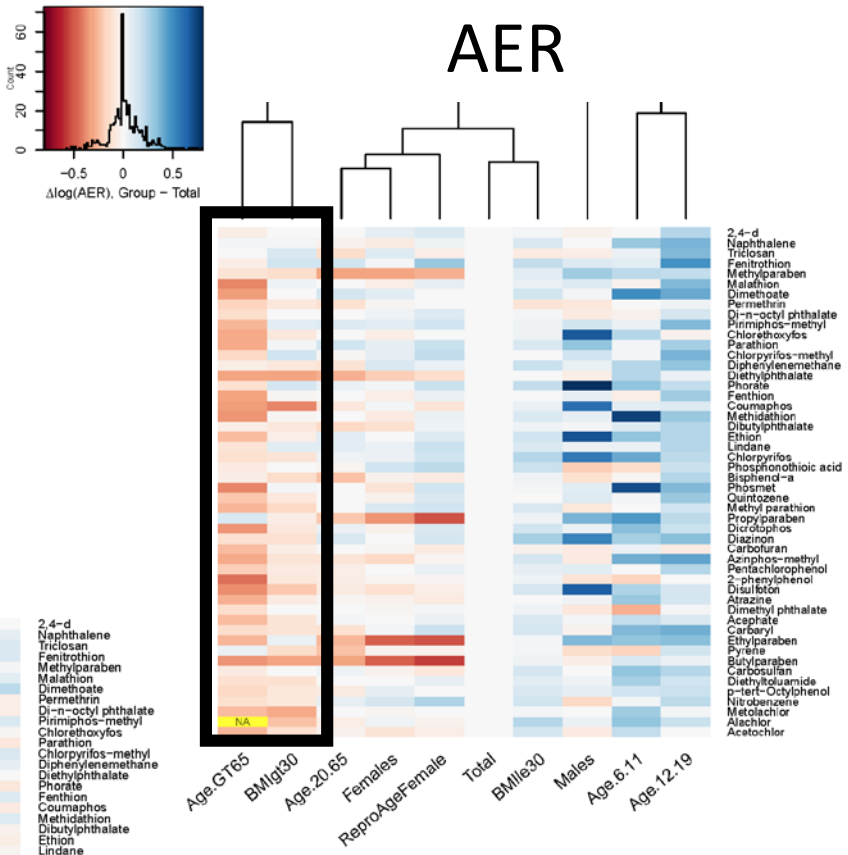
Oral equiv. dose

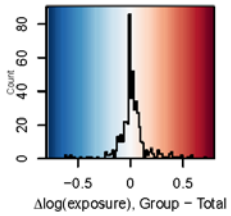


Females

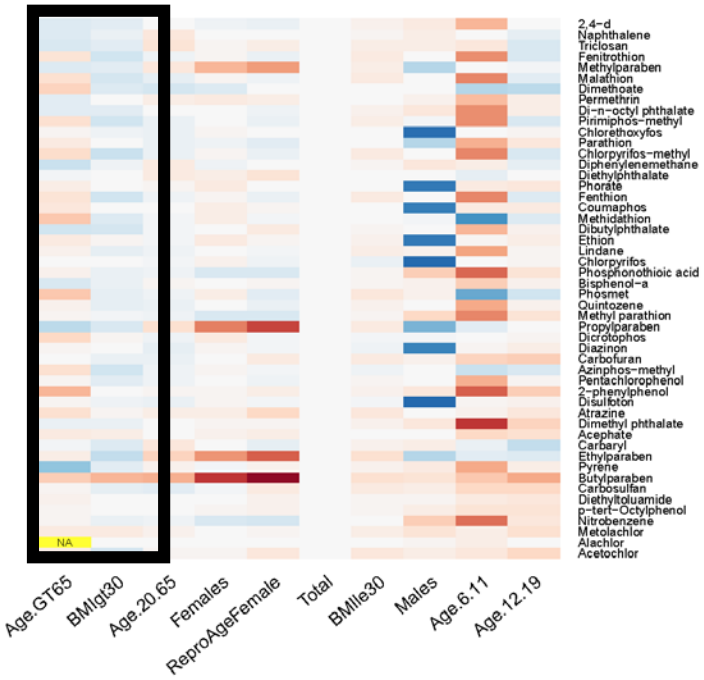
Reproductive-Age Females

Males

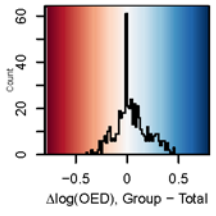




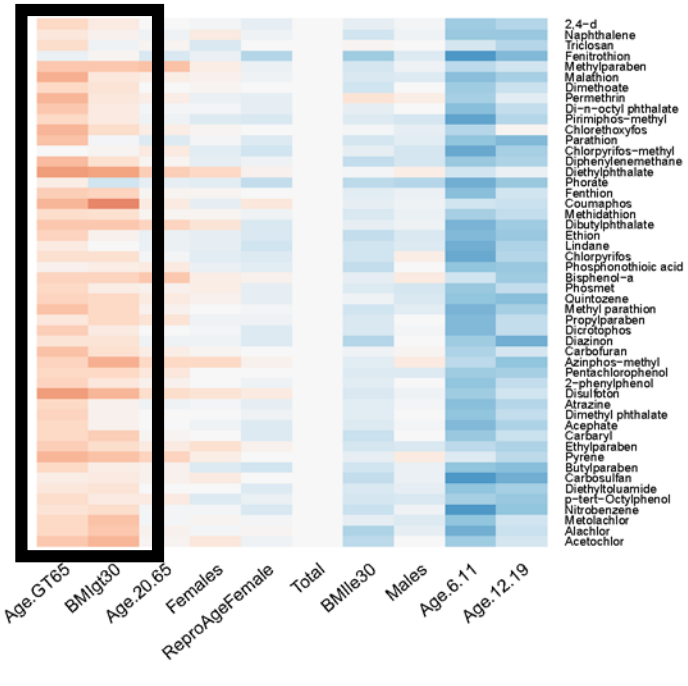
Exposure

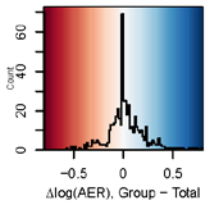


Age GT 65
BMI GT 30

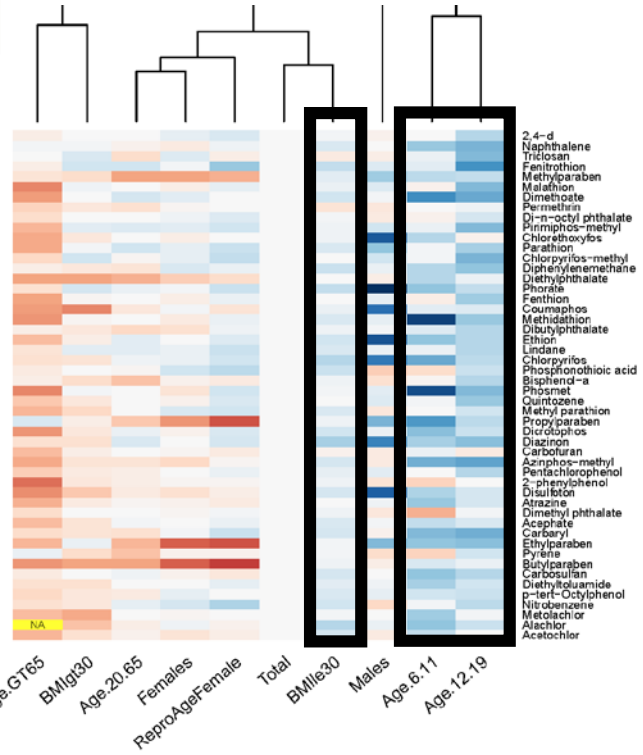


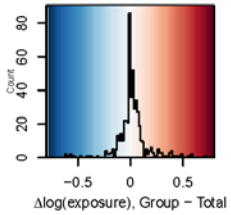
Oral equiv. dose



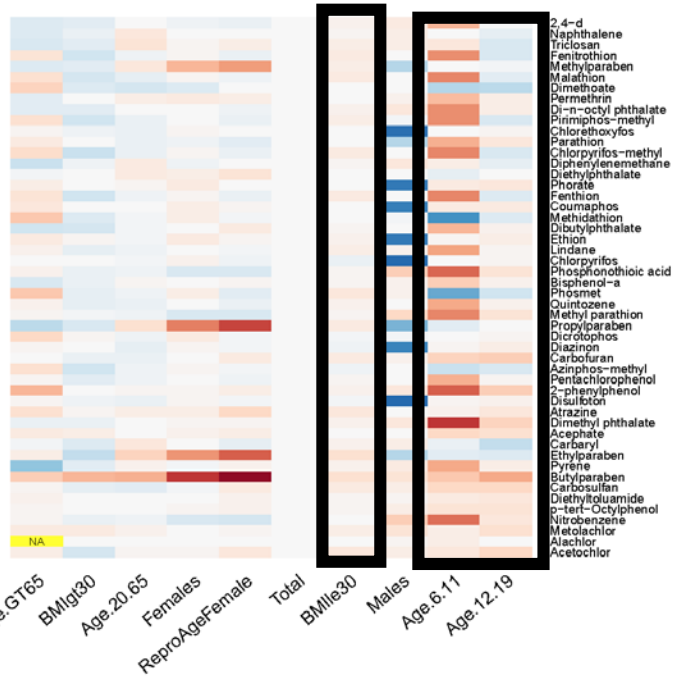


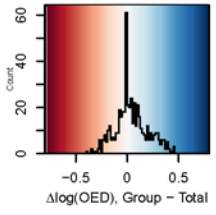
AER



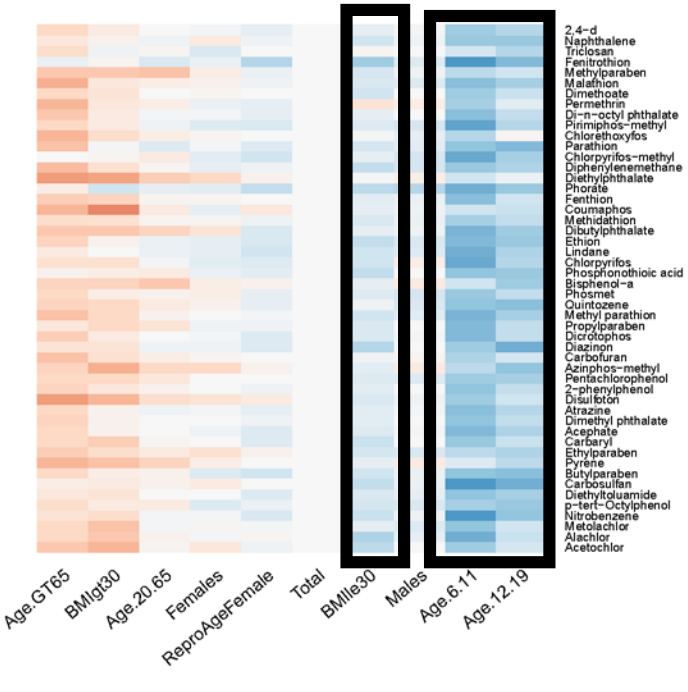


Exposure





Oral equiv. dose



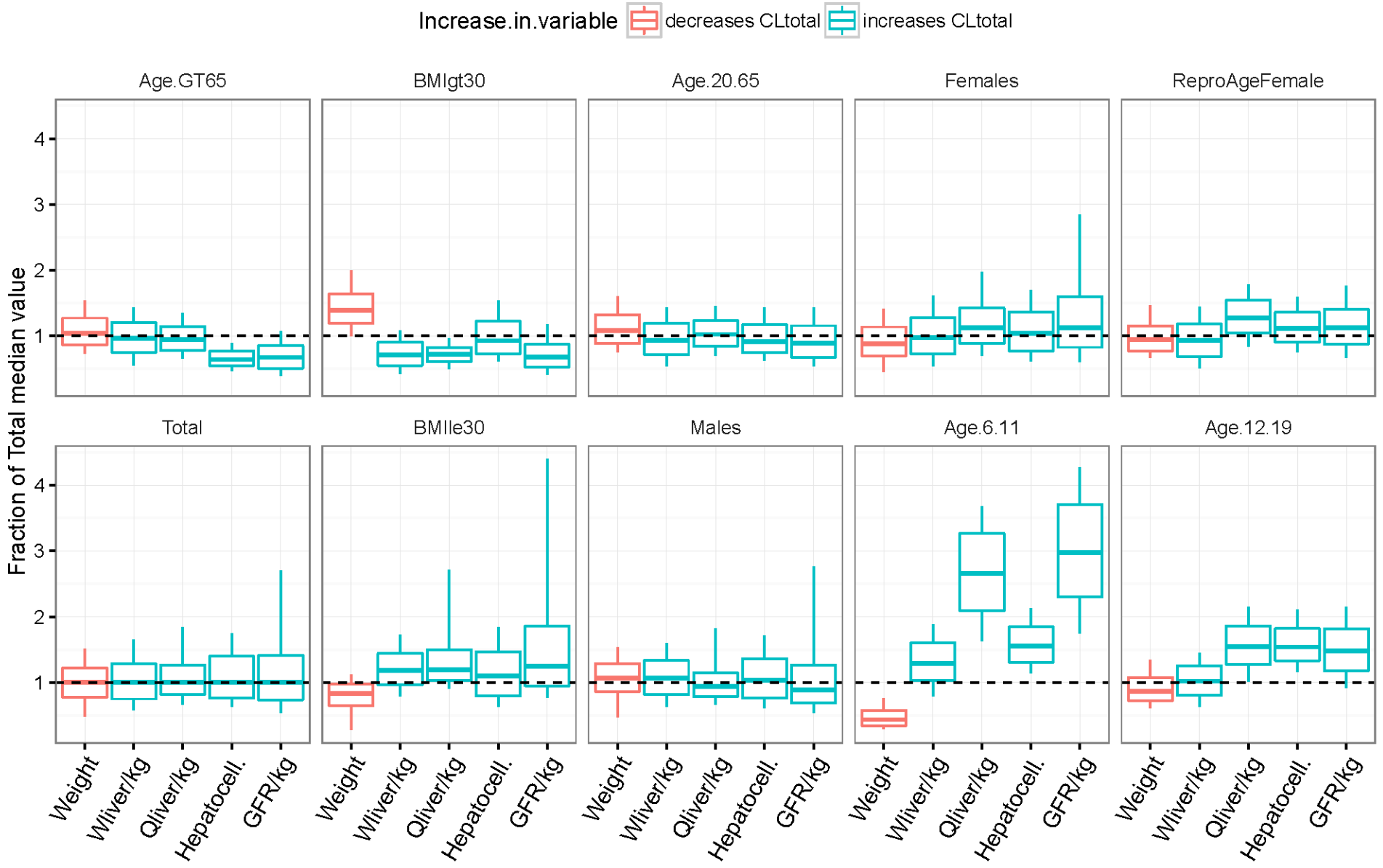
BMI LE 30

Age 6-11

Age 12-19

Q. Why the consistent differences in oral equiv. dose for some groups? A. Consistent differences in physiology.

- Oral equiv. dose is linearly related to steady-state plasma concentration (C_{ss}), which depends on total clearance *per kg body weight*
- Total clearance per kg depends on CLint, body weight, Vliver, Qliver, hepatocellularity, GFR
- CLint is drawn from same distribution for all groups (*in vitro* data from pooled adult hepatocytes)
- Others: see figure at right



Evaluating predicted clearance differences between demographic groups

Ginsberg et al. 2002: *in vivo* PK database in infants, children, and adults

Summary of *in vivo* clearance/kg body weight in various age groups compared to adults (for 27 chemicals):

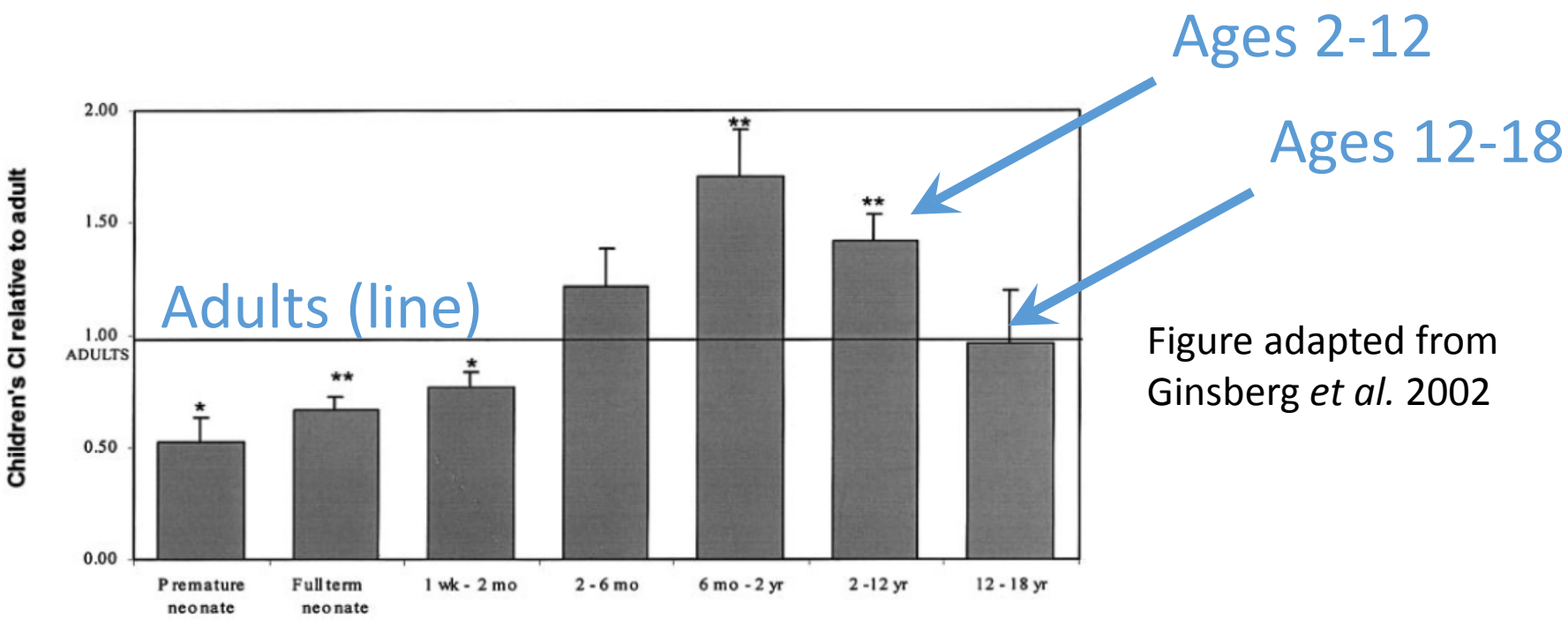


Figure adapted from Ginsberg *et al.* 2002

Why?

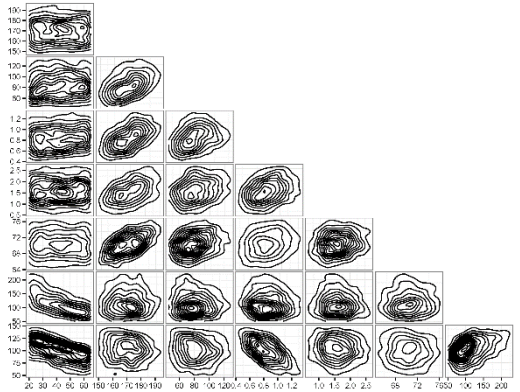
CYP enzymes reach near-adult abundances and activity before 1 year of age, plus greater liver blood flow and liver size/kg body weight (Ginsberg et al. 2003)

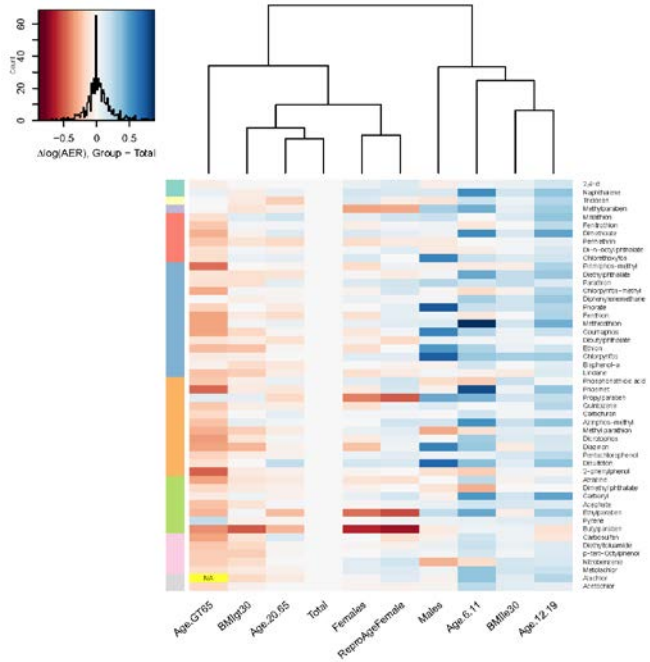
Future improvements

- More realistic Fub distribution?
  - Plasma protein concentration variability: age, gender, disease state…? [Johnson et al. 2006, Israili et al. 2001]
  - Albumin or AAG binding? [Routledge 1986]
- More realistic CLint distribution?
- Isozyme abundances and activity: varies with age, ethnicity (at least) [Yasuda et al. 2008, Howgate et al. 2006, Johnson et al. 2006]
- Isozyme-specific data & modeling [Wetmore et al. 2014]
  - Isozyme-specific metabolism assays not HT
  - *In silico* predictions of isozyme-specific metabolism? Not easy!
    - Existing data is mostly for pharmaceuticals [Peach et al. 2014]
  - Other sources of HT metabolism variability data?

Conclusions

- HTTK-Pop: population physiology simulator
  - Open-source
  - Correlated Monte Carlo approach
  - Based on NHANES data: Modern US population
  - Can be used to simulate various demographic subgroups
- Use HTTK-Pop to do IVIVE of ToxCast *in vitro* bioactivity data for different groups
- Range of oral equivalent doses to compare with estimated potential exposures for each group
  - Differences in physiology between groups → differences in oral equiv. doses
  - Differences in exposure between groups inferred from NHANES exposure biomonitoring (ExpoCast)
  - AERs up to 6-fold different from total population
- HTTK-Pop + ToxCast + ExpoCast = HT AER prioritization for potentially sensitive subpopulations





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  - Woody Setzer (EPA/ORD/NCCT)
  - Robert Pearce (EPA/ORD/NCCT)
  - John Wambaugh (EPA/ORD/NCCT)

Thank you!

Questions?

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AER−based rank
10
20
30
40
50
Age.GT65
BMIgt30
Age.20.65
Females
ReproAgeFemale
Total
BMIle30
Males
Age.6.11
Age.12.19
Demographic group
Rank in Total pop.
1
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20
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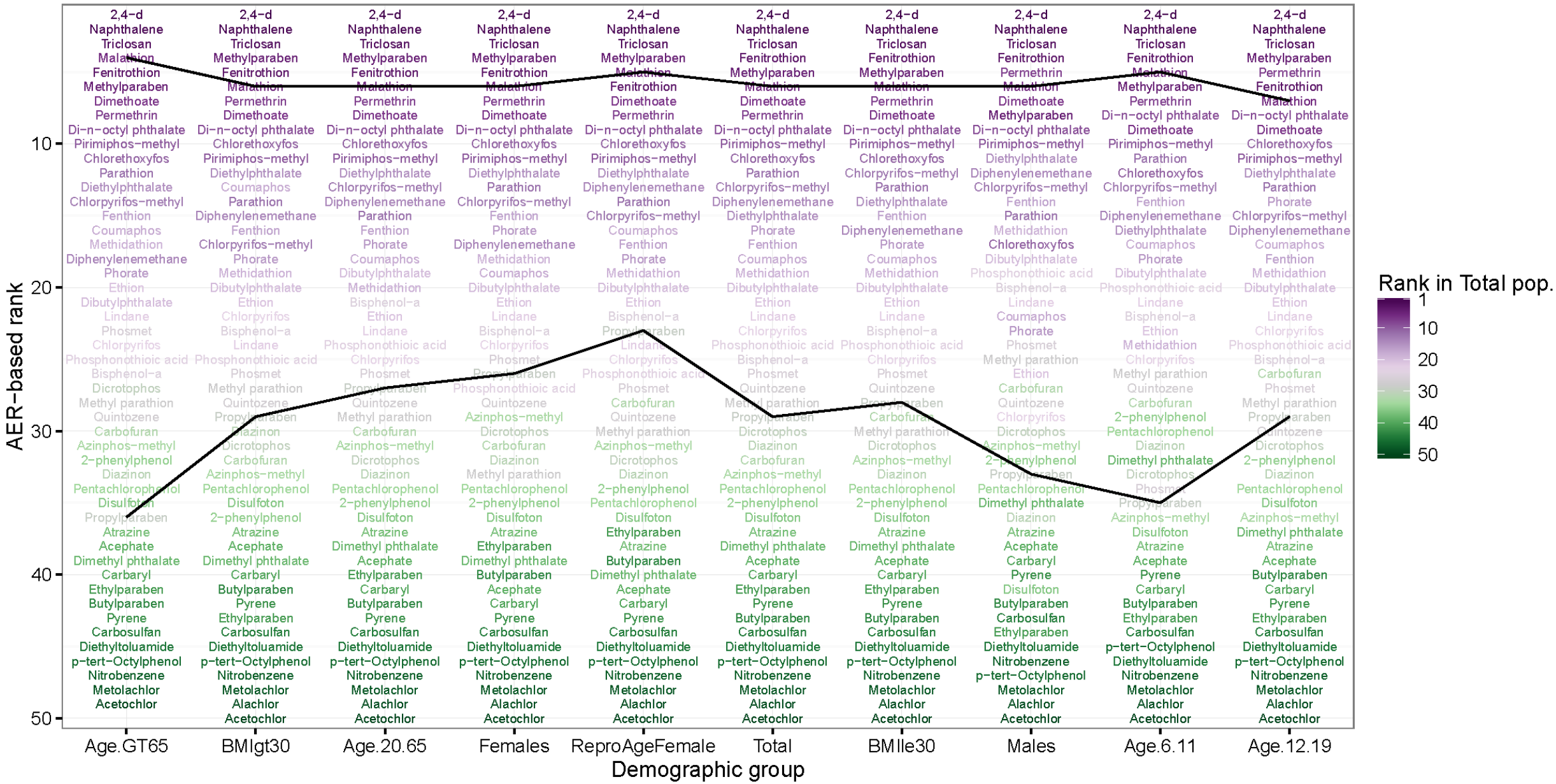
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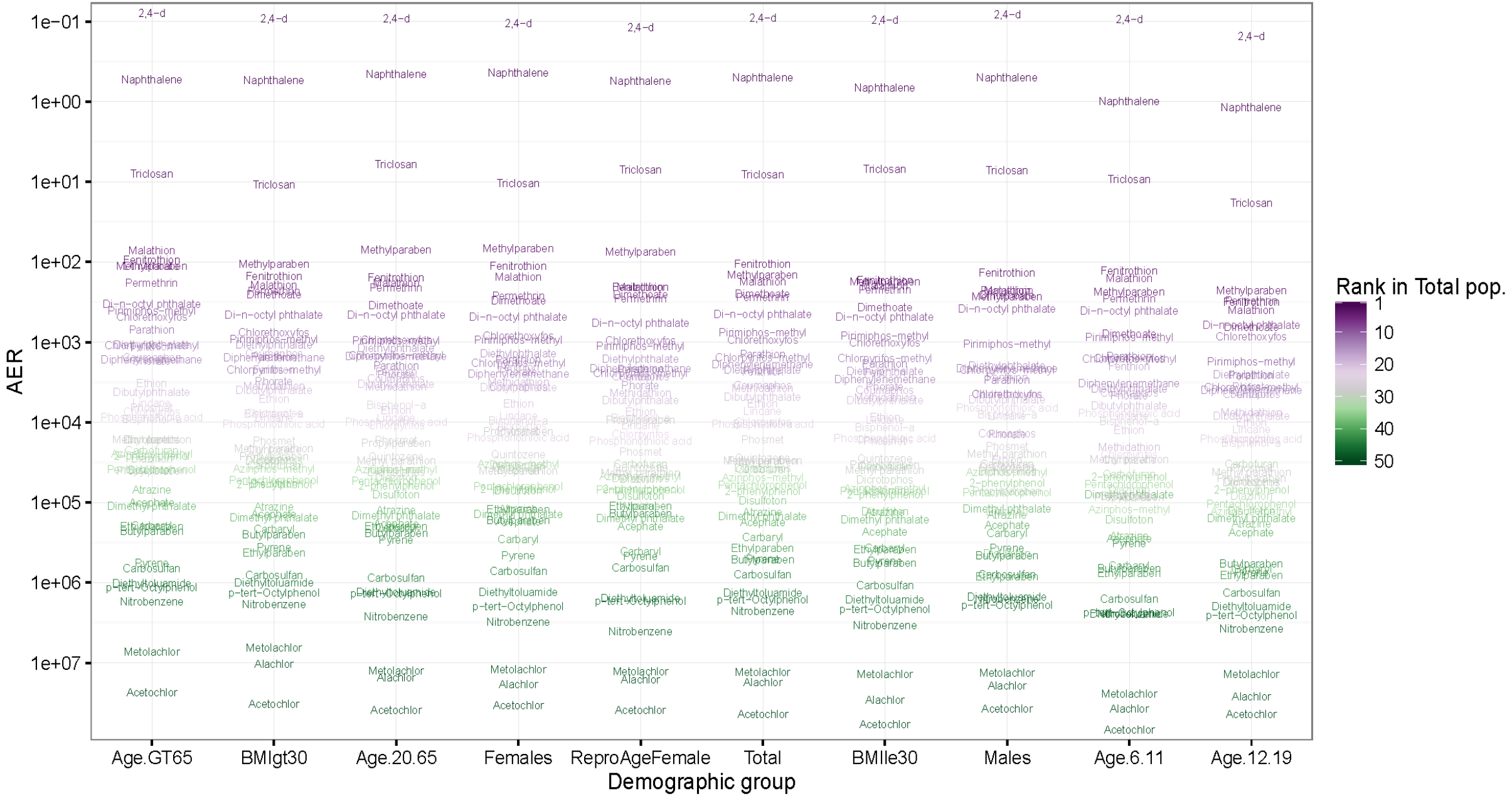
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Females
ReproAgeFemale
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Age.6.11
Age.12.19
Demographic group
Rank in Total pop.
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Triclosan
Methylparaben
Malathion
Fenitrothion
Permethrin
Dimethoate
Di−n−octyl phthalate
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Chlorethoxyfos
Parathion
Diphenylenemethane
Diethylphthalate
Chlorpyrifos−methyl
Phorate
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Ethion
Lindane
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Phosmet
Quintozene
Methyl parathion
Azinphos−methyl
Pentachlorophenol
2−phenylphenol
Disulfoton
Atrazine
Dicrotophos
Dimethyl phthalate
Acephate
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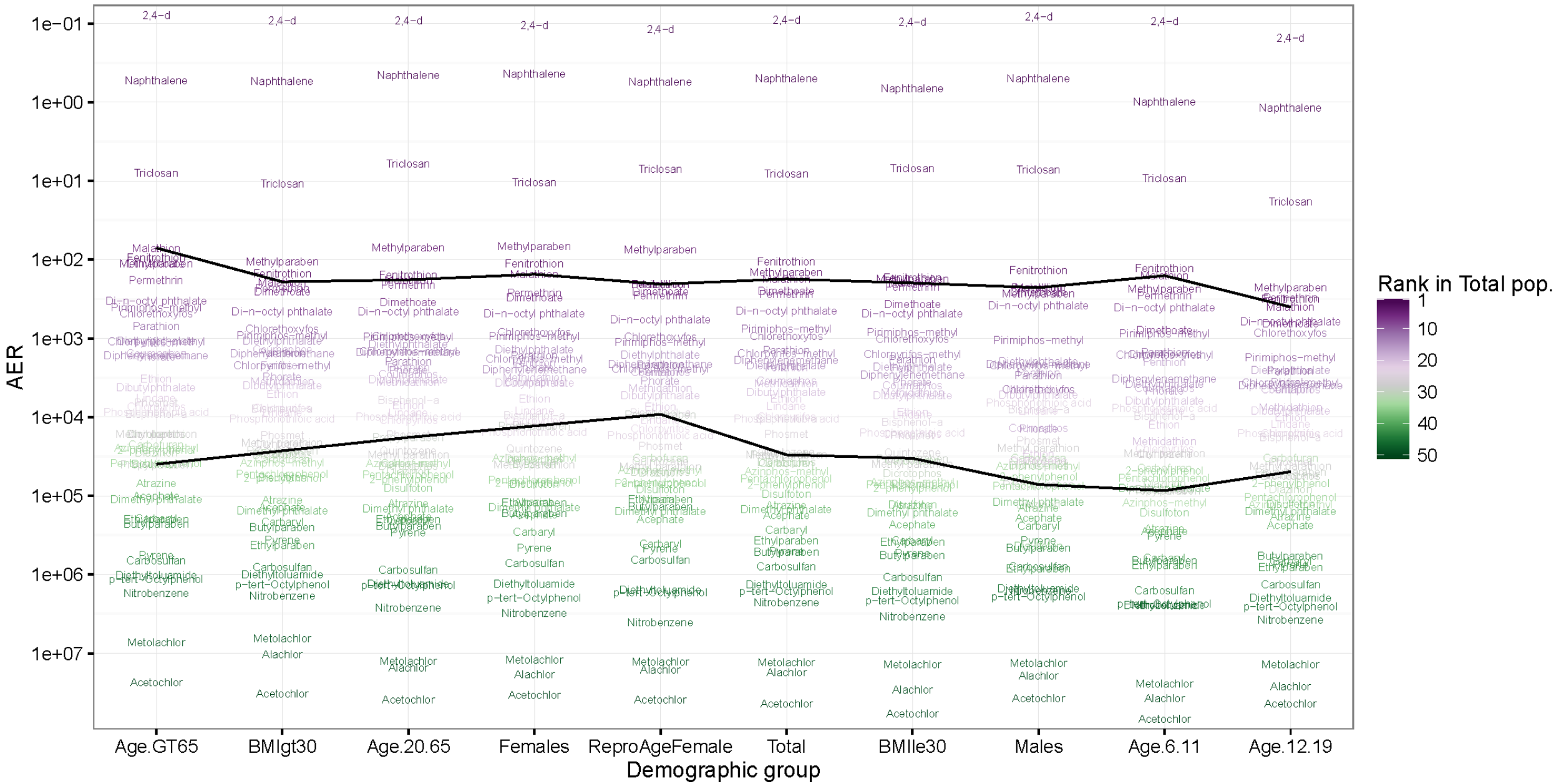
Acetochlor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