Application of PBPK Modeling and *In Vitro* Assays to Conduct Cumulative Risk Assessments for Environmental Exposures

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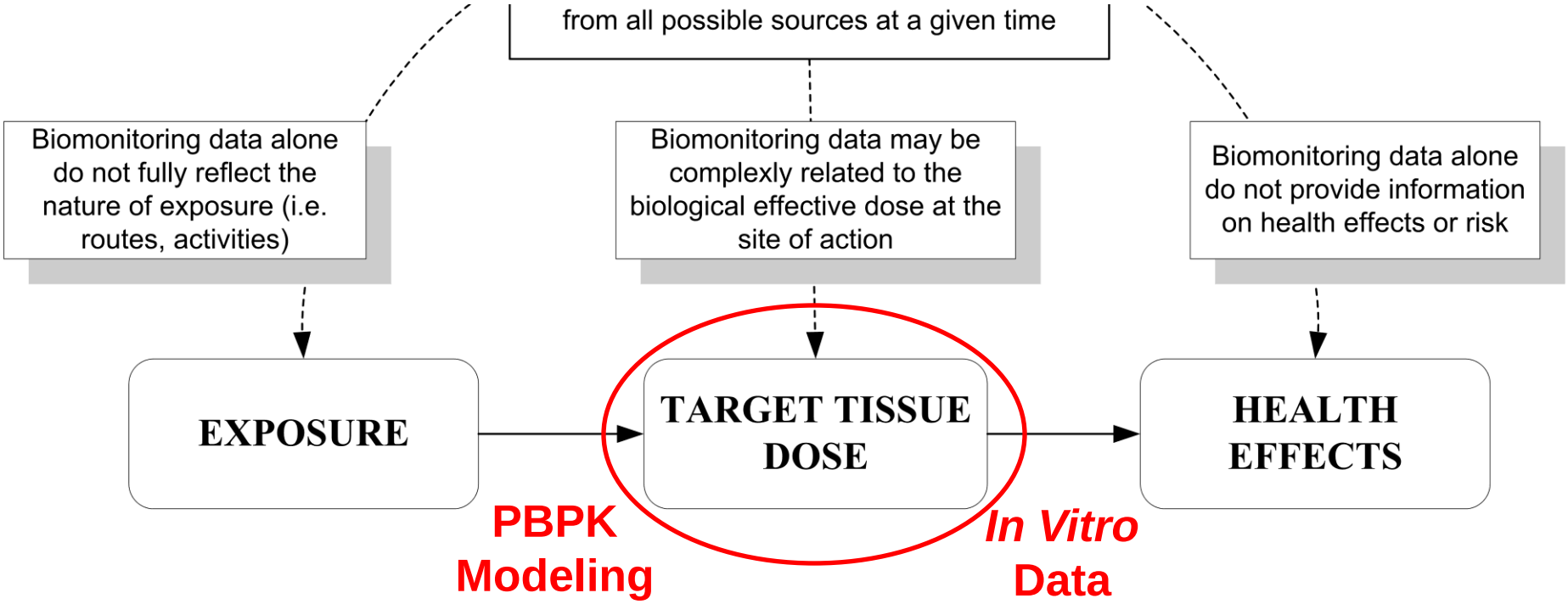
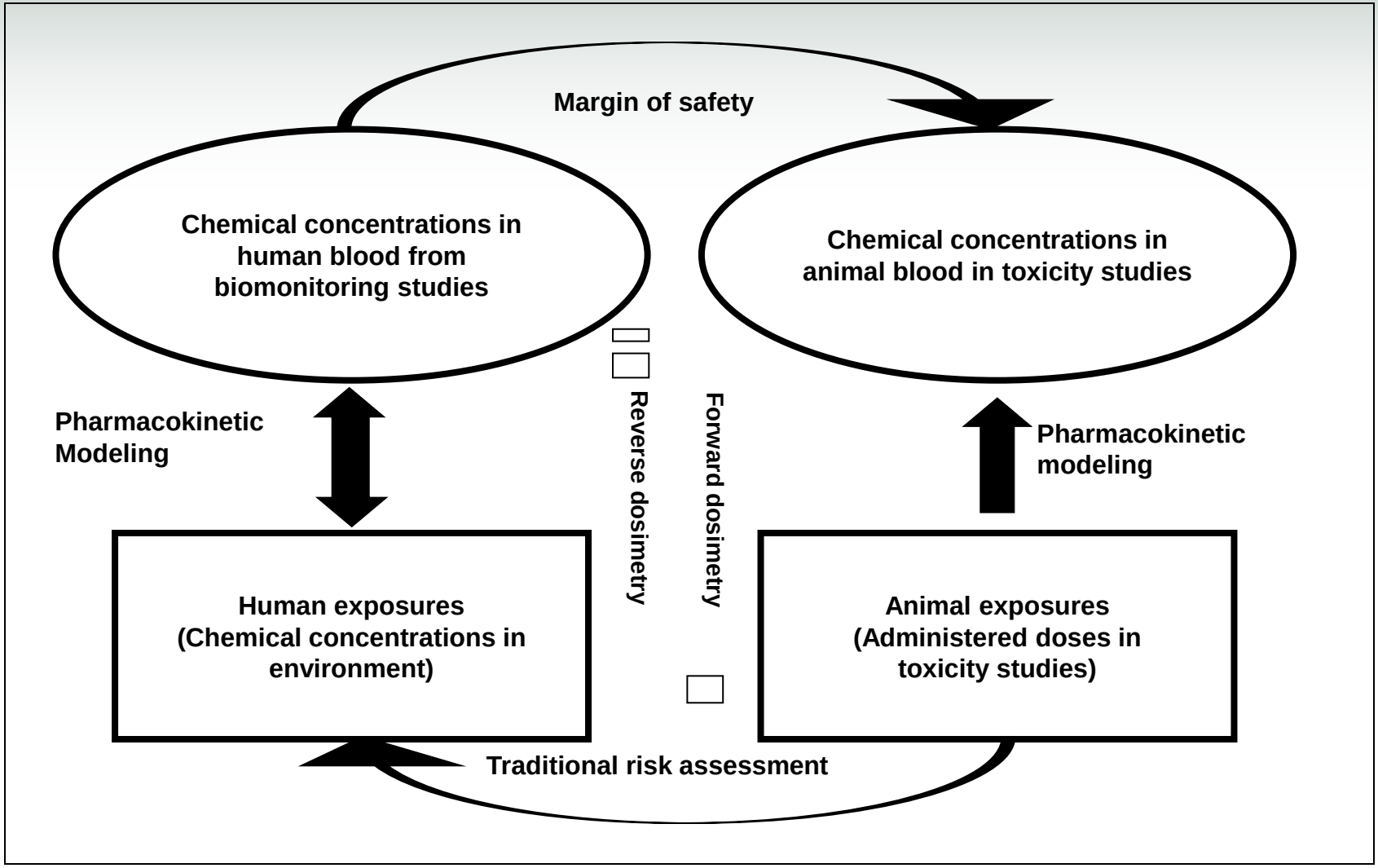
Relationship of Exposure, Biomonitoring Data, and Health Effects



Linking Exposure to Health Effects



Linking Exposure to Health Effects

Problems Vary with the Nature of the Chemical

- **Volatiles**
  - Complex household exposures
  - Rapid clearance
    - Blood levels highly sensitive to recent exposures
- **Highly persistent compounds**
  - Slow approach to steady state
  - Apparent clearance confounded by changes in body weight, fat content
- **Intermediate persistence compounds**
  - Interpretation depends on rate of clearance
    - Need to consider timing of exposures vs. sampling
  - May need to deal with active metabolites

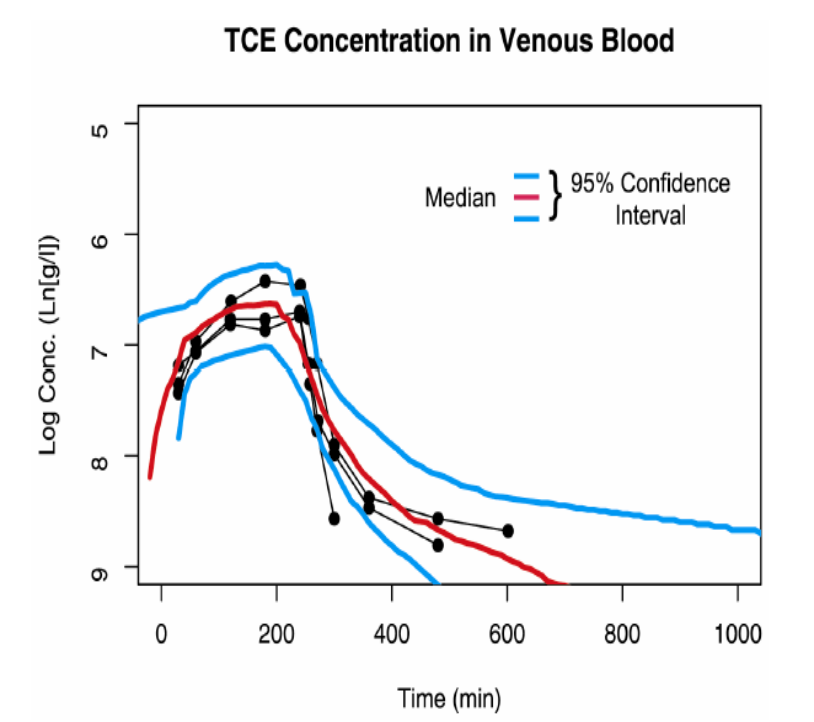
Linking Exposure to Health Effects

Problems Vary with the Nature of the Biomarker

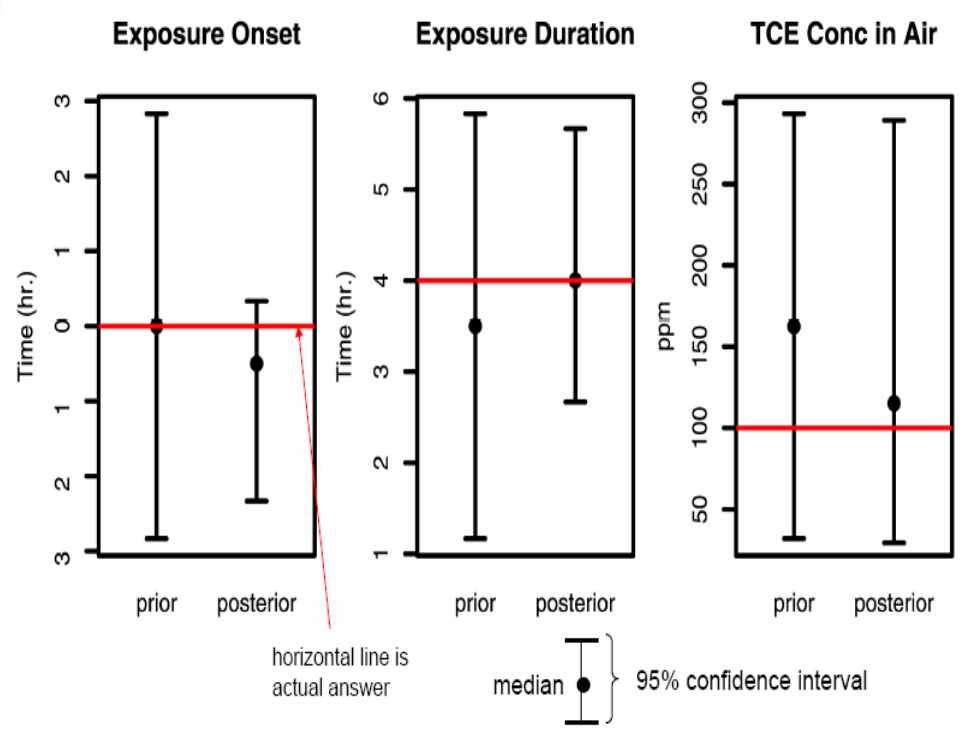
- **Active parent chemical / metabolite in blood**
  - Often a good surrogate for target tissue dose
  - Directly comparable to blood levels at NOAEL/LOAEL
  - Estimation of exposure requires PK modeling
- **Inactive parent chemical / metabolite in blood**
  - Any use requires PK modeling
  - Not directly comparable to blood levels at NOAEL/LOAEL
- **Parent chemical or metabolite in urine**
  - Estimation of exposure (uptake) depends on representativeness of sample
  - Estimation of blood levels requires PBPK modeling

Exposure Reconstruction: An "ILL-Posed Problem"
(Many possible solutions)

Comparison of PBBK Predicted Blood Concentrations with Experimental Data



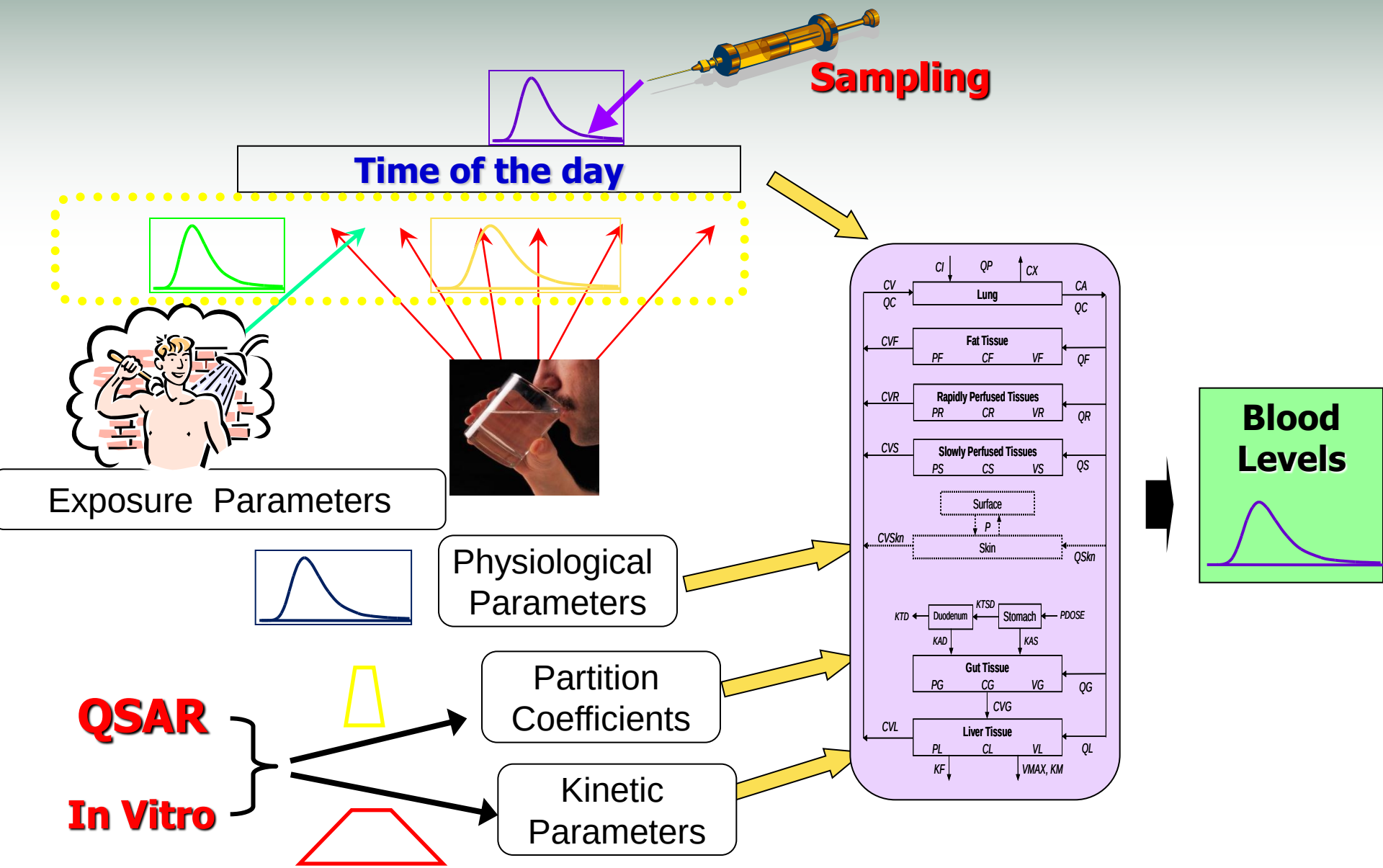
Comparison of Reconstructed Exposure Conditions with Actual Exposure Conditions



(Sohn et al., 2004)

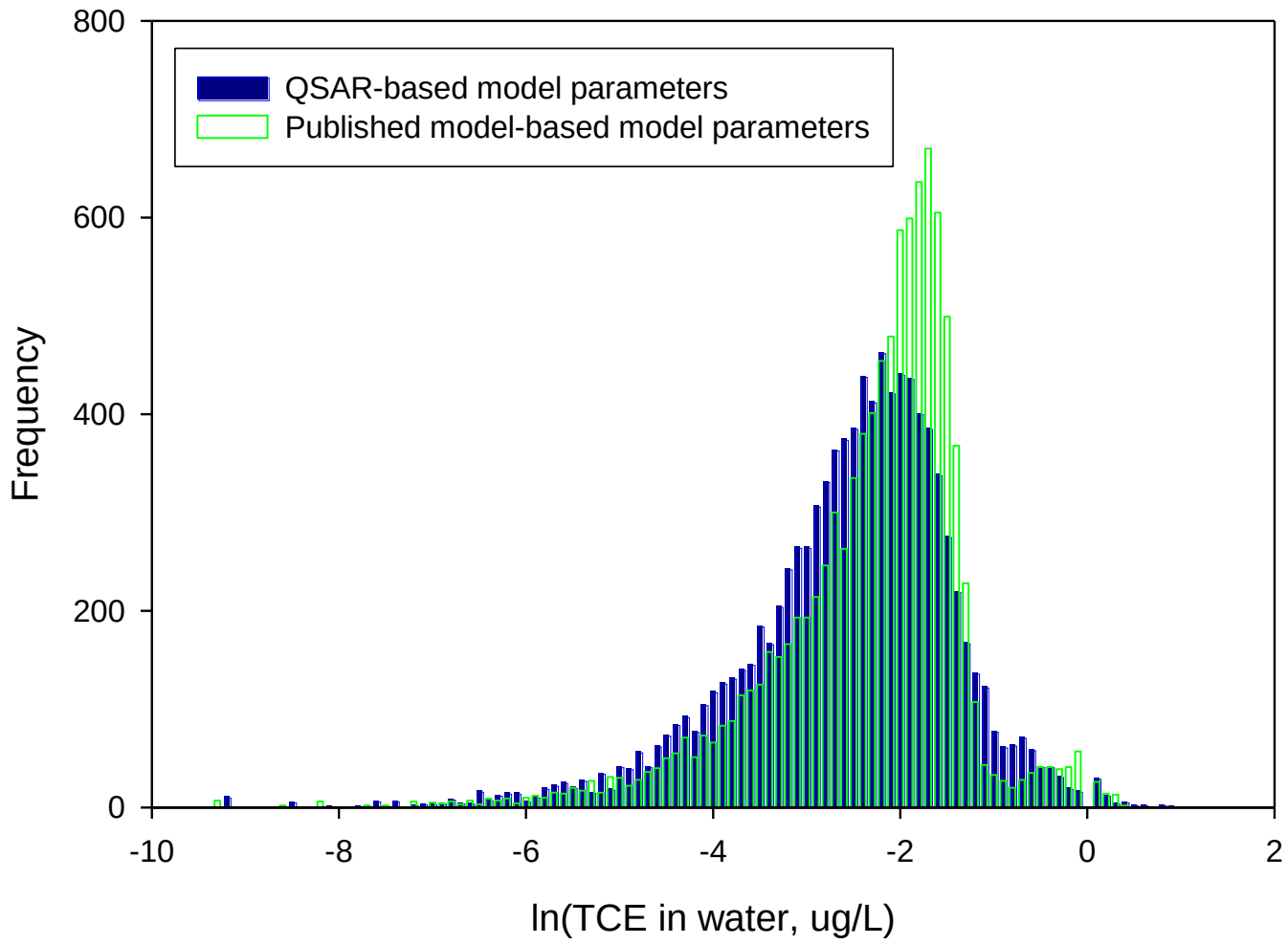
Requires population-level, probabilistic approach

Monte Carlo Analysis to Simulate Population Exposures
(Liao et al. 2007)



Monte Carlo Simulation for TCE Concentrations in Blood Across a Population (Liao et al., 2007)

Impact of Uncertainty in QSAR-derived PBPK Model

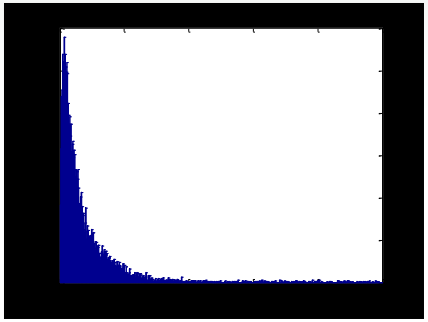


Probabilistic Reverse Dosimetry Approach

(Tan et al., 2006, 2007)

ppb of chemical in air
or
µg/L of chemical in water

Monte Carlo analysis



Estimated distribution of chemical in blood

÷ *Invert Distribution*

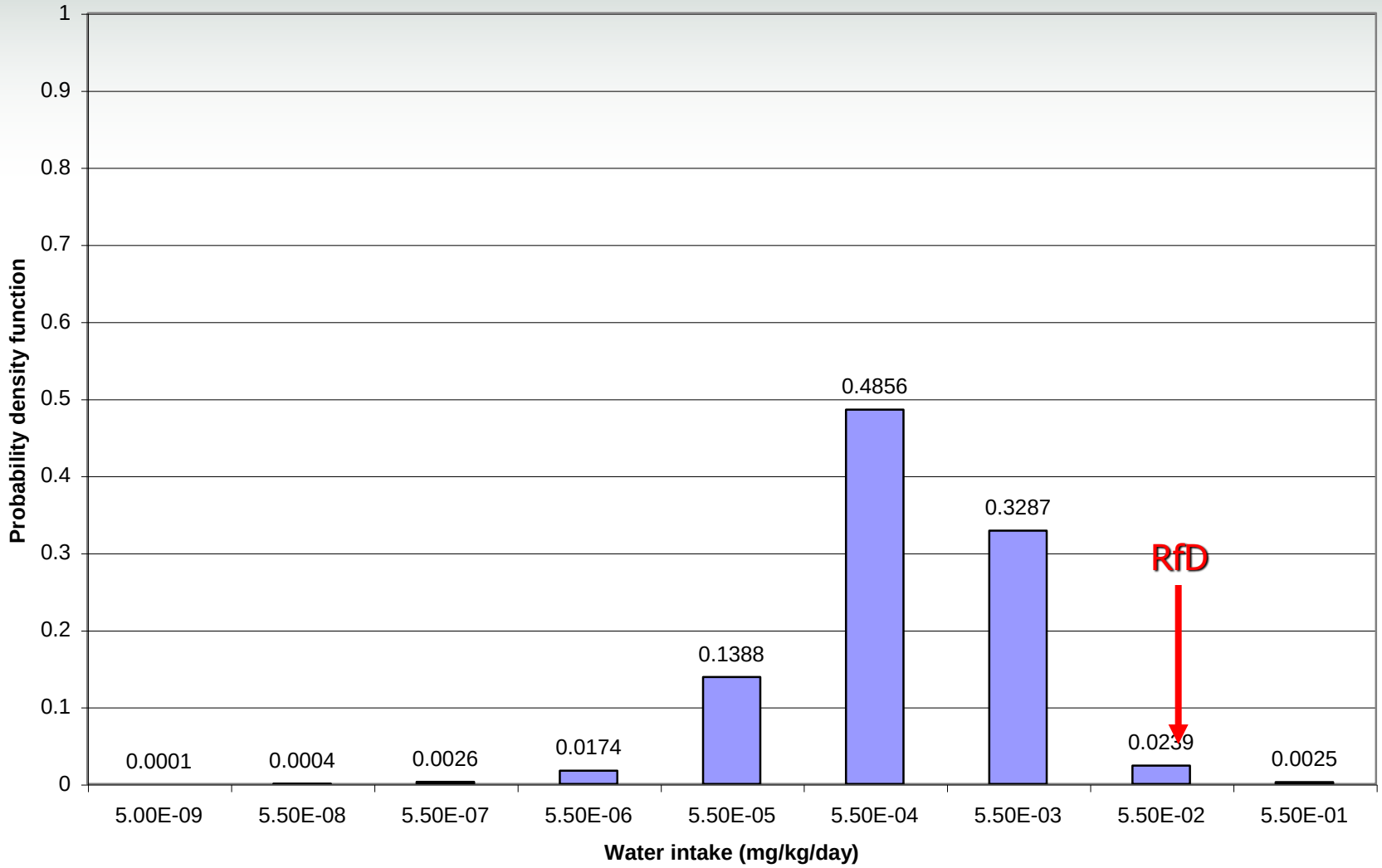
"Exposure Conversion Factor" distribution

Convolute Distributions ✕

Estimated population exposure distribution

Distribution of measured blood concentrations

Predicted Distribution of Exposures to Chloroform in the Population Reported in NHANES III (mg/kg/day)

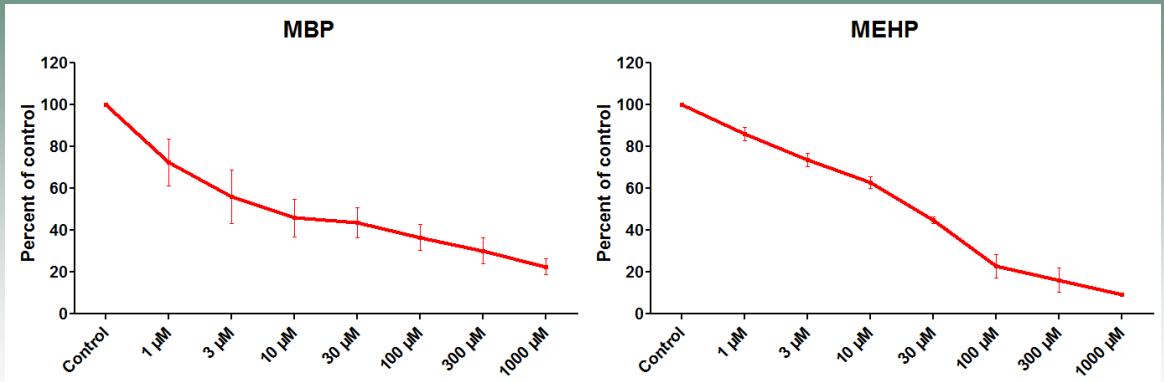


Cumulative Risk Assessment for Phthalates Using *In Vitro* Potency Estimates

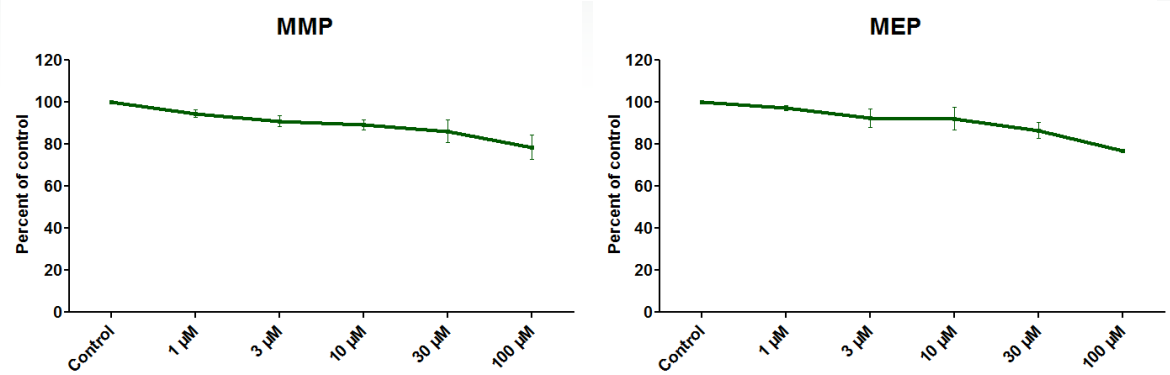
- **Goal: Cumulative risk for combined exposures to all phthalates, including any active metabolites**
- **Problem: *In vivo* data not available on all phthalates / metabolites reported in NHANES**
- **Risk assessment approach**

1. Estimate daily intake (Di) of each phthalate ester from NHANES urinary excretion data
2. Estimate relative potency (Pi) of phthalate monoesters and oxidative metabolites using *in vitro* assay for testosterone inhibition, using MBP as the basis for comparison
3. Calculate cumulative risk: $Rcum = \Sigma (Pi * Di)$
4. Determine MoE vs. RfD (0.1 mg/kg/d)

In Vitro Assay for Testosterone Inhibition (R2C rat Leydig cells)



Known Endocrine Active



Known Inactive

TESTOSTERONE INHIBITION IN VITRO VS. IN VIVO		
	IC_{50} (µM)	
	In vivo (fetal rat testes)[a]	In vitro
MBP	3	3
MEHP	6	7
MEP	>306[b]	205
MMP	>>409 [b]	713.9

Comparison of in vitro and in vivo IC_{50} values.

[a]Calculated from PBPK models (Clewell et al., 2008; Gentry et al., 2011) and data in rat fetal testes (Clewell et al., 2009; Kurata et al., 2012).

[b]No testosterone reduction measured at highest tested doses (750 mg/kg/day) in vivo. Fetal testes monoester concentrations at 750 mg/kg/day DEP and DMP maternal dose (Clewell et al., 2010).

Estimating Cumulative Phthalate Risk

COMPOUND	Percentile	Concetration in Urine (µg/g creatinine)	Daily Intake (µMol/day)	Relative spotency factor	MBP Equivalent Intake (µMol/day)	DBP Equivalent Intake (mg/kg/day)	% Total Risk that results directly from DBP	MoE vs RfD = 0.1 (mg/kg/d)
Mono butyl phthalate (MBP)	50th %	21.5	0.1016	1	0.1016			
	95th %	91.5	0.4323	1	0.4323			
Mono-2-ethylhexyl phthalate (MEHP)	50th %	4.43	0.0167	0.53	0.0089			
	95th %	35.1	0.1324	0.53	0.0702			
5-hydroxy MEHP	50th %	17.6	0.0628	0.194	0.0122			
	95th %	160	0.5708	0.194	0.1107			
5-oxo MEHP	50th %	12.5	0.0449	0.029	0.0013			
	95th %	92.3	0.3315	0.029	0.0096			
Mono-ethyl phthalate (MEP)	50th %	171	0.9255	0.0145	0.0134			
	95th %	1430	7.7397	0.0145	0.1122			
Mono-methyl phthalate (MMP)	50th %	1.45	0.0085	0.0042	0.0000			
	95th %	10	0.0583	0.0042	0.0002			
Mono (2-octyl) phthalate (MnOP)	50th %	< LOD		0.125	0.0000			
	95th %	3.1	0.0117	0.125	0.0015			
Mono benzyl phthalate (MBzP)	50th %	15.1	0.0714	0.109	0.0078			
	95th %	95.8	0.4531	0.109	0.0494			
Total:	**50th %ile**				**0.1452**	**0.00067**	**70%**	**148**
	95th %ile				**0.7861**	**0.00365**	**55%**	**28**

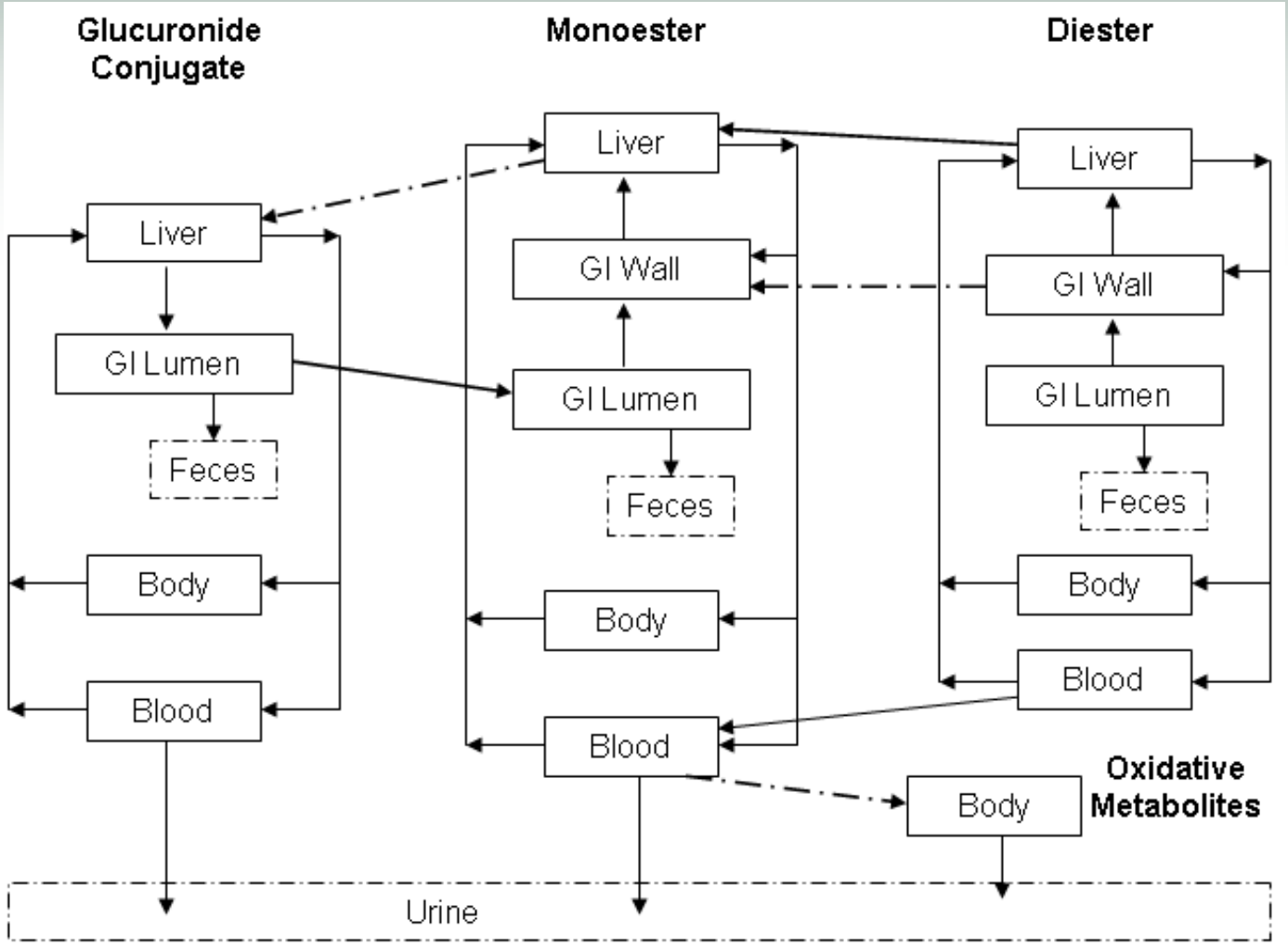
Cumulative Risk Assessment for Phthalates Using PBPK Modeling and Reverse Dosimetry

- **Goal: Cumulative risk for exposures to all phthalates, including any active metabolites**

- **Difficulty:**
  - Exposure estimates are based on urinary concentrations of total monoester (free plus glucuronide)
  - Appropriate dose metric for health effects is free blood concentrations of all active compounds (monoesters and their active metabolites)

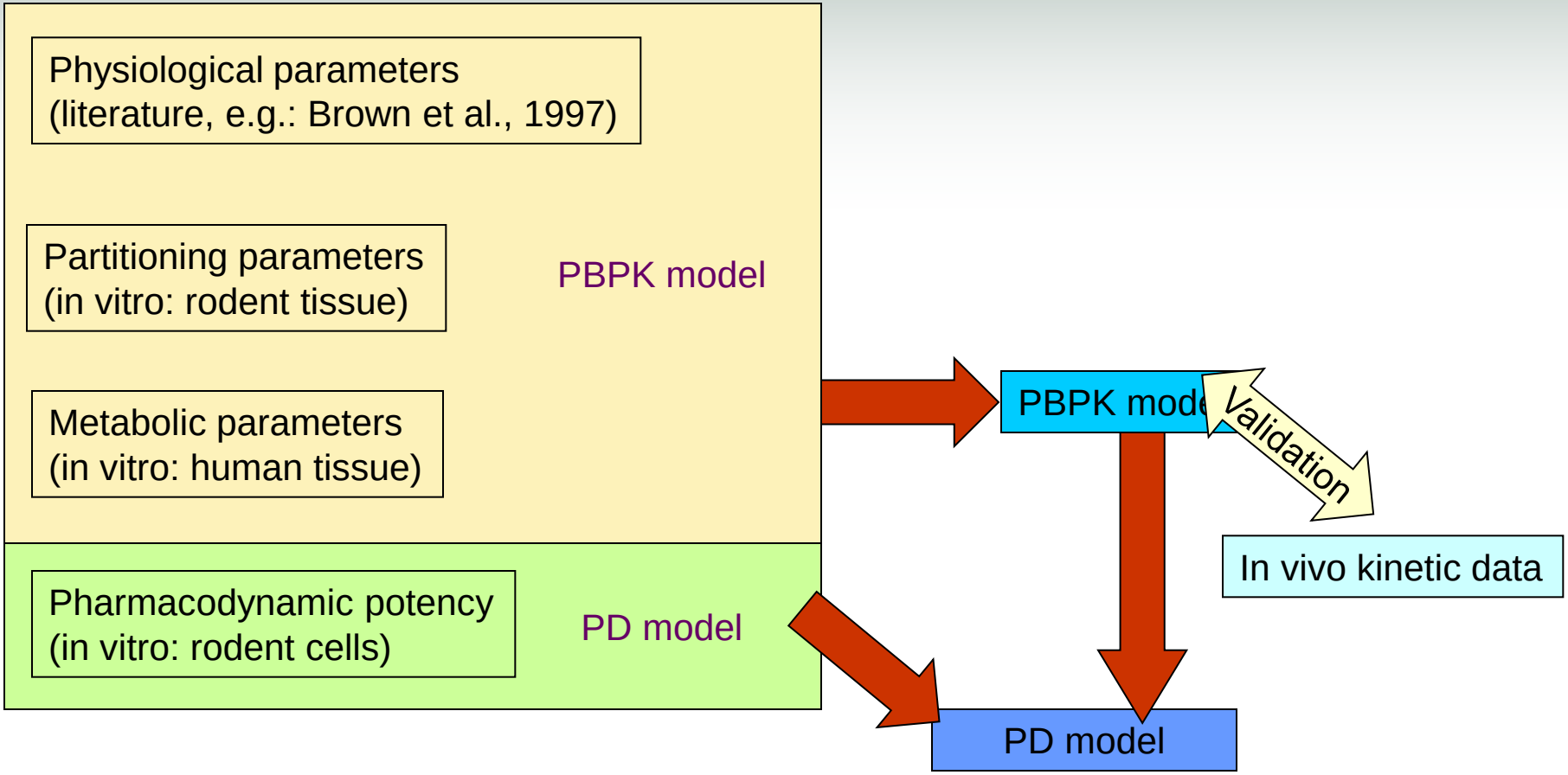
- **Role of PBPK model:**
  - Relate of total monoester urine concentrations to blood concentrations of free monoester
  - Estimate maternal exposure from NHANES data (reverse dosimetry) and predict associated fetal exposure (forward dosimetry)

DBP/DEHP Model Structure

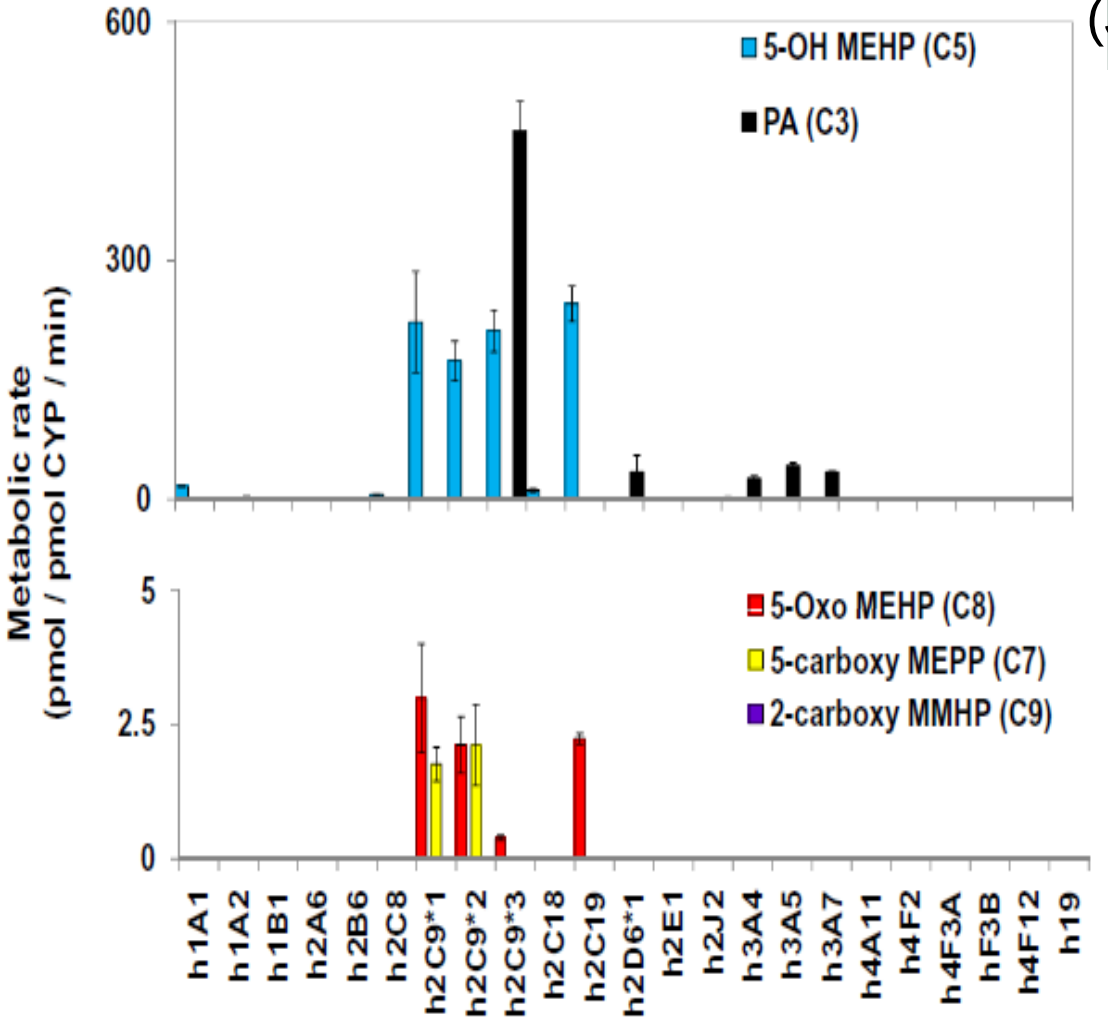


R. Clewell et al. 2008

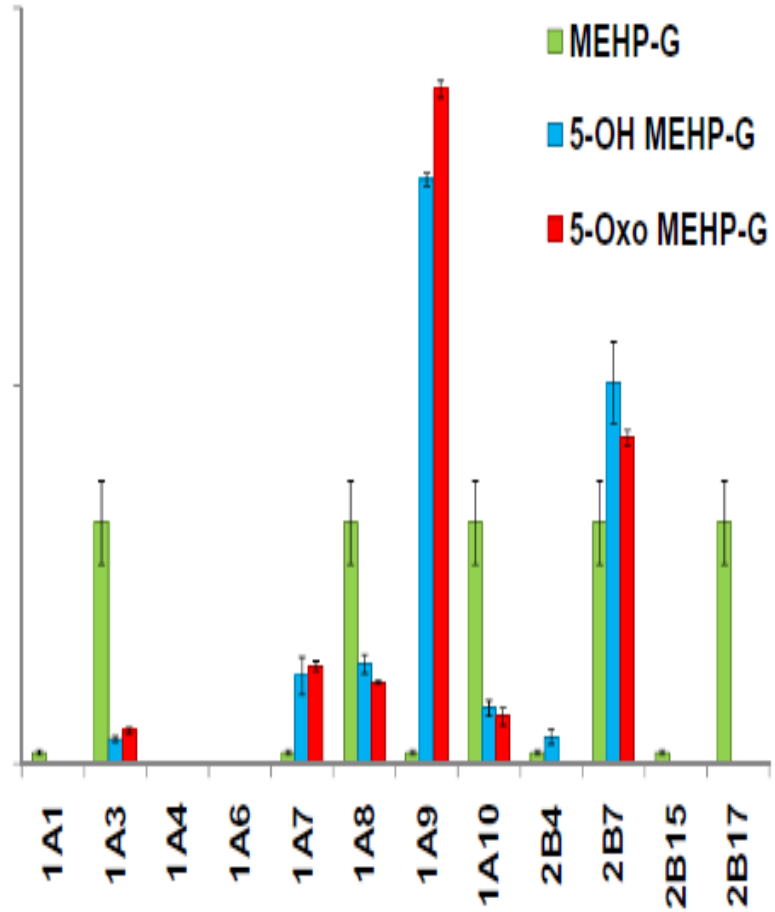
Estimation of parameters for human phthalate model



Metabolism of MEHP by 23 selected human CYP isoforms (Choi et al., 2011)



Percentage of human UGT isoforms involved in phase II conjugation.
(Joo et al., 2011)



Reverse Dosimetry

- Simulations of 60 exposure concentrations - 0.01 to 10 µg/kg
- Exposures - oral bolus (i.e., ingestion of food) occurring over 15 min
- Urine and amount of MBP accumulated for random lengths of time beginning just prior to ingestion of meal to 3 h post exposure
- Sampling times varied from 0.5 to 3 h post last elimination event
- 1,000 iterations were accumulated at each exposure concentration

Exposure Reconstruction for NHANES III

Data on Di-n-Butyl Phthalate

Percentile	NHANES III Concentration in Urine (µg/L)	Predicted Intermittent Exposure* (µg/kg/day)	Predicted Continuous Exposure (µg/kg/day)
10%	-	0.30	
25%	-	0.45	0.25
50%	20.4	1.17	0.8
75%	40.4	3.78	1.4
90%	73.6	5.46	3.0
95%	108	7.74	4.2

*Assumed to be sum of three ingestion events per day

Compares well with 24-hour urine data in German study (Wittassek et al., 2007): average = 1.9 µg/kg/day, 95% = 5.3 µg/kg/day

Forward dosimetry analyses with the human pregnancy PBPK model for DBP

- Based on 100,000 iterations using the reverse dosimetry output

	Daily Exposure (µg/kg/day)	Maternal Blood	Maternal Blood Total	Fetal Blood	Fetal Testes	Amniotic Fluid	Urine
Mean	1.885	0.32	0.52	0.21	0.073	2.02	44.8
Std. Deviation	2.038	0.34	0.55	0.27	0.096	6.87	61.7
CV %	108.1%	108.2%	106.8%	125.8%	131.2%	340.2%	137.7%
Confidence Interval of the Mean							
Lower 95%	1.872	0.316	0.51	0.21	0.073	1.98	44.5
Upper 95%	1.897	0.32	0.52	0.22	0.074	2.06	45.2

Comparison of mono-butyl phthalate concentration in maternal and fetal tissues in the rodent at the NOAEL (10 mg/kg/day, Lehmann et al., 2004) and the human at 95th percentile determined in the forward dosimetry.

Acknowledgements

Hamner colleagues

Rebecca Clewell – phthalate PBPK model development

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CCC – trihalomethane reverse dosimetry

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