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INSTITUTES FOR HEALTH SCIENCES

WHERE GREAT MINDS & MEDICINE MEET

# **Using Pharmacokinetic Approaches Based on Animal and Human Datasets to Track and Allocate Cumulative Exposures and Aggregate Health Impacts**

Harvey Clewell

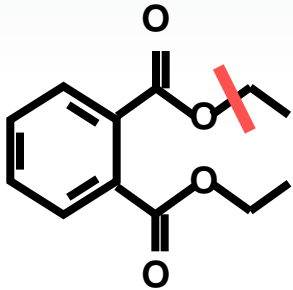
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The Hamner Institutes for Health Sciences  
Research Triangle Park, NC

# Humans are exposed to multiple phthalates

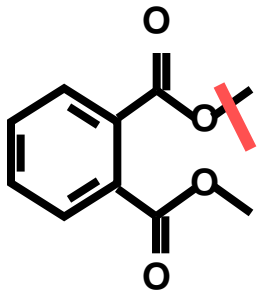
Structure affects activity – are not all the same

Question: Is this due to kinetic or dynamic differences?

No effect in animals

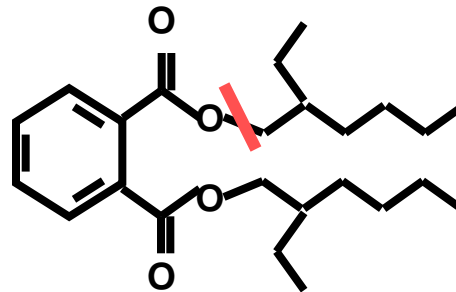


DEP

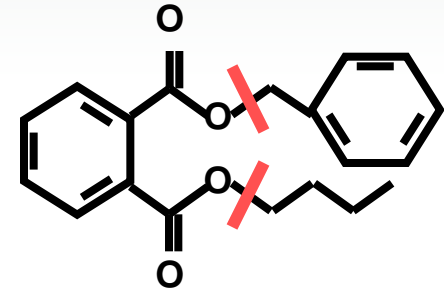


DMP

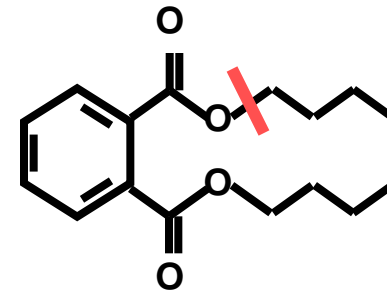
Adverse effects in animals



DEHP



BBP



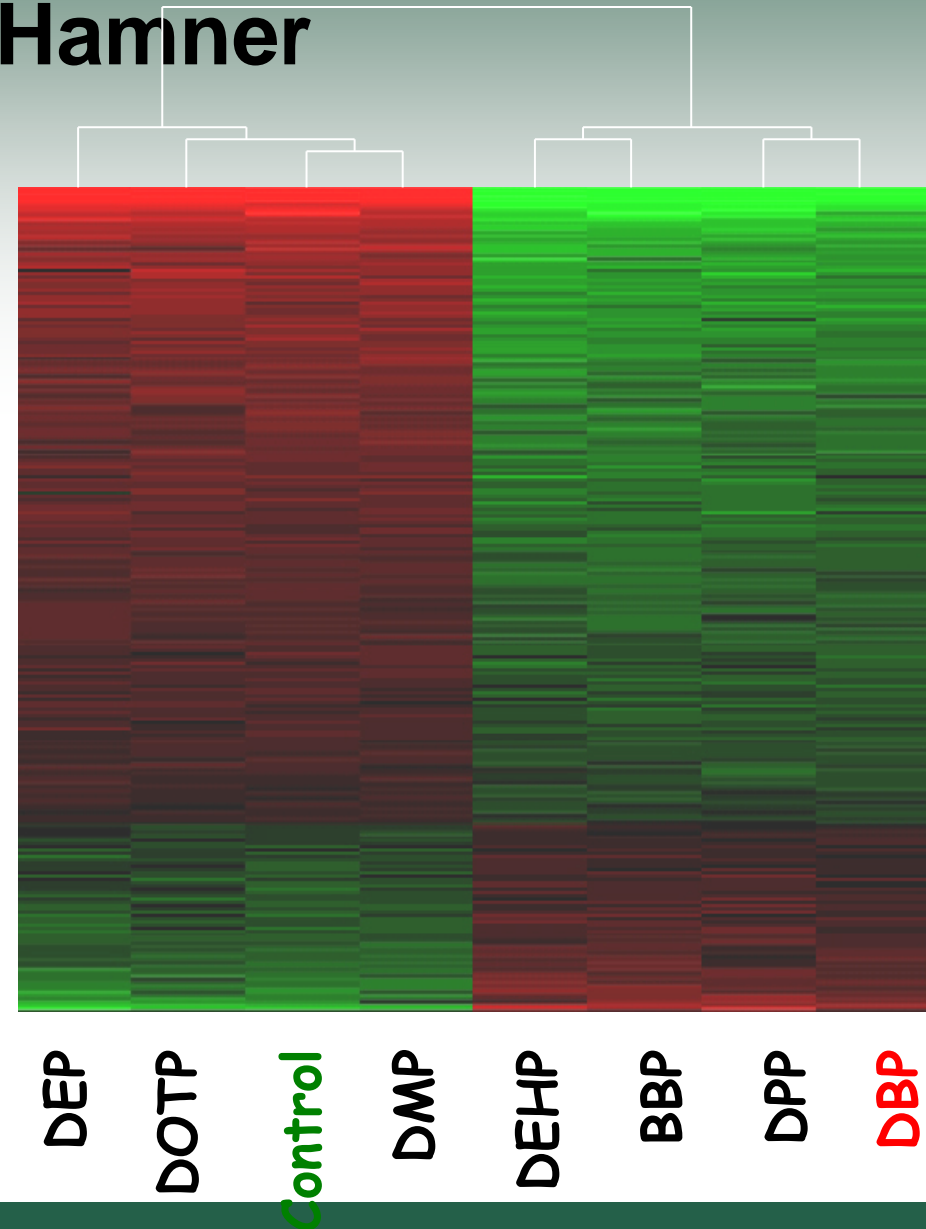
DBP

# Long History of Research on Phthalates at CIIT / Hamner

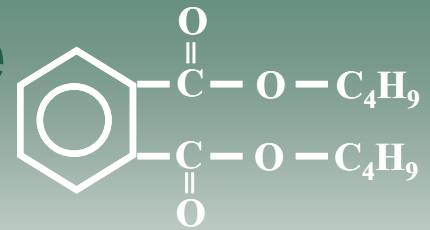
Kevin Gaido's lab: demonstrated similar mode of action (genomic signature) for developmental effects of active phthalate esters (e.g., DEHP, DBP), in contrast with inactive forms (e.g., DMP, DEP)

**Provides justification for  
cumulative assessment**

*(Liu et al. 2005)*



# Research to Support a Phthalate Cumulative Risk Assessment

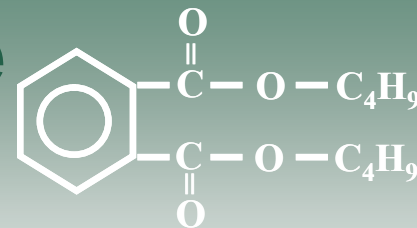


**Goal: Cumulative risk for exposures to all phthalates, including any active metabolites**

## **Challenges:**

- **Human exposure estimates** are based on **urinary concentrations** of unmetabolized **monoesters**
- Appropriate dose metric for **health effects** is **fetal concentrations of all active compounds** (monoesters and any other active metabolites)

# Research to Support a Phthalate Cumulative Risk Assessment

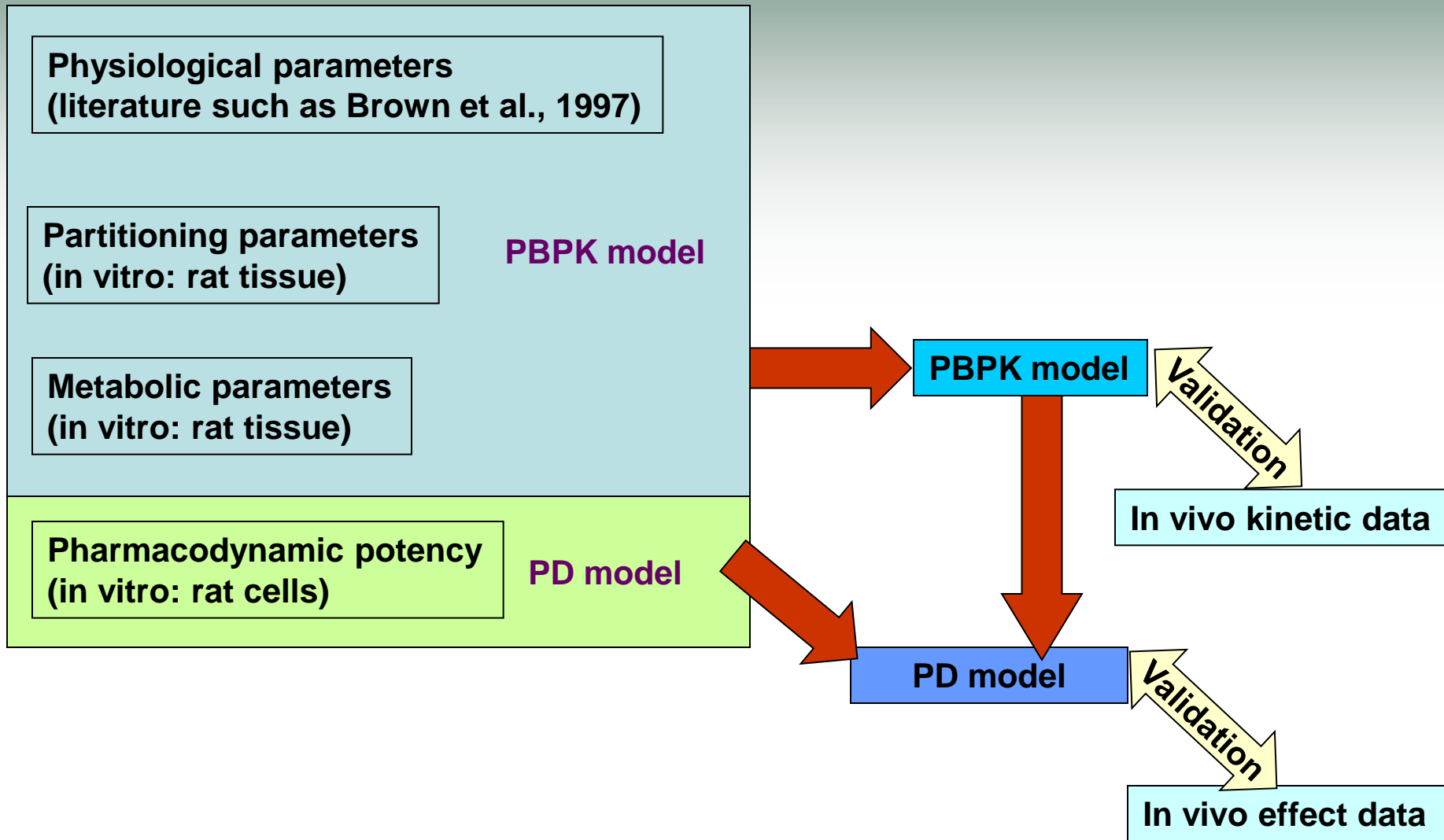


**Goal: Cumulative risk for exposures to all phthalates, including any active metabolites**

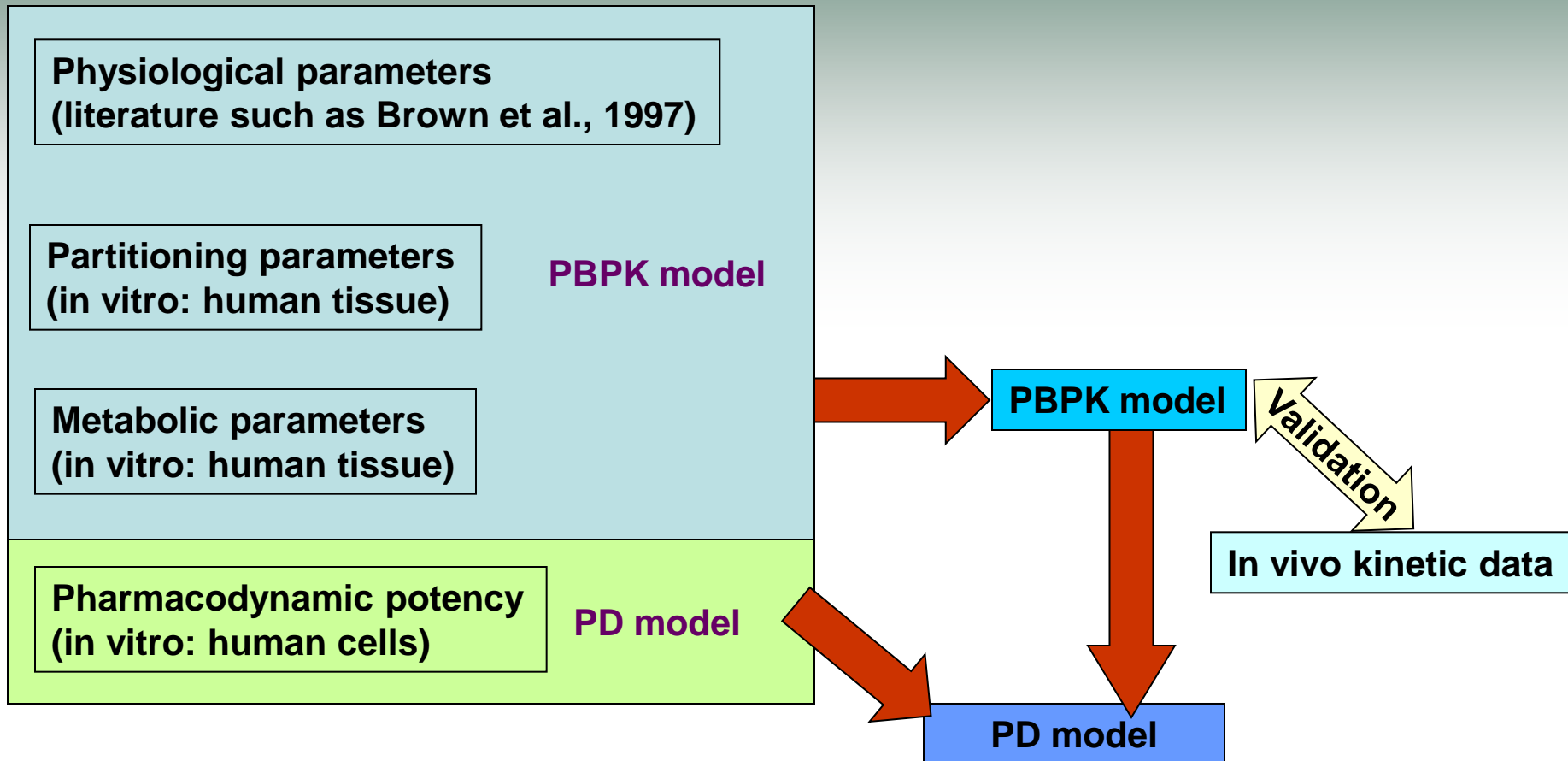
## Research approach

1. PBPK prediction of blood concentrations of active metabolites associated with a given exposure (dose-rate)
2. Comparison of model predictions with urinary phthalate measurements from CDC
3. In vitro estimation of activity of diesters, monoesters, and oxidative metabolites
4. Initial cumulative risk assessment for phthalates and comparison with results of epidemiological studies

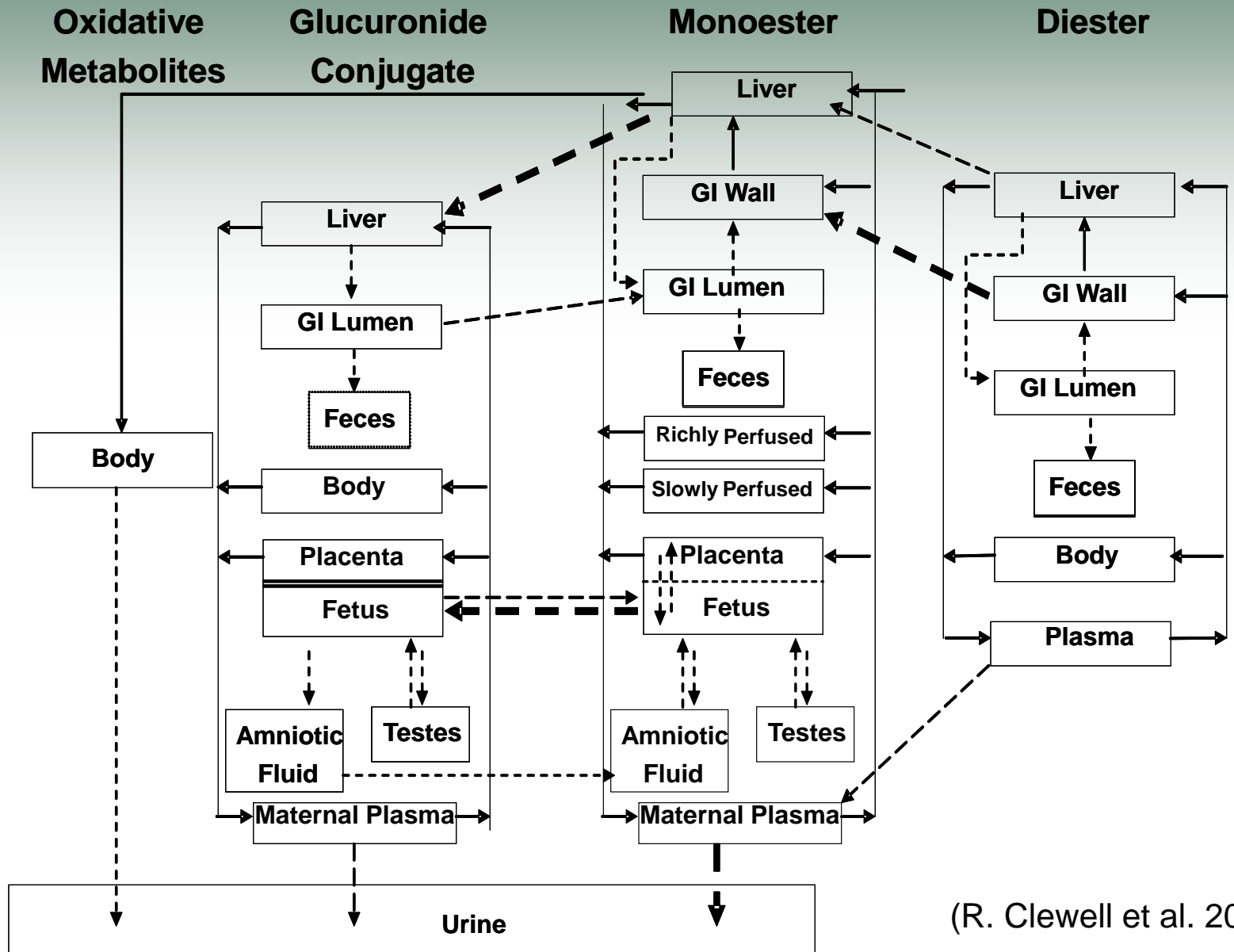
# Estimation of parameters for rat model



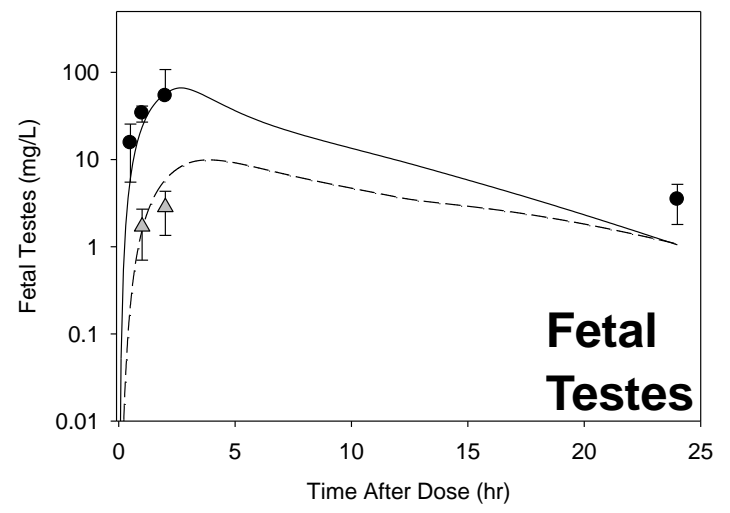
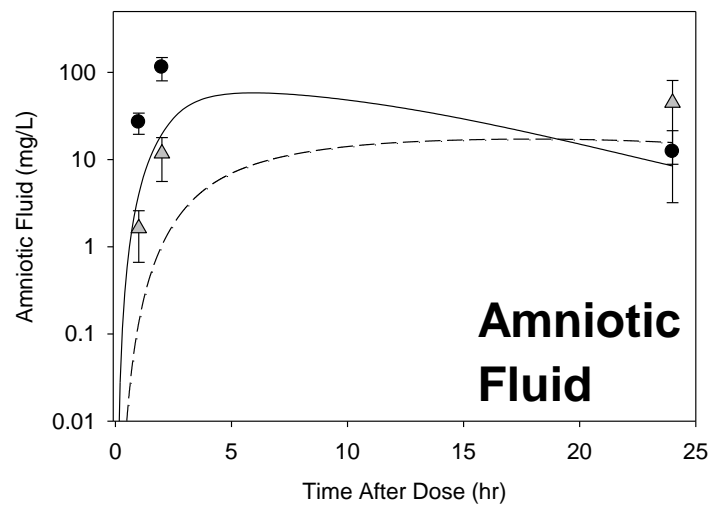
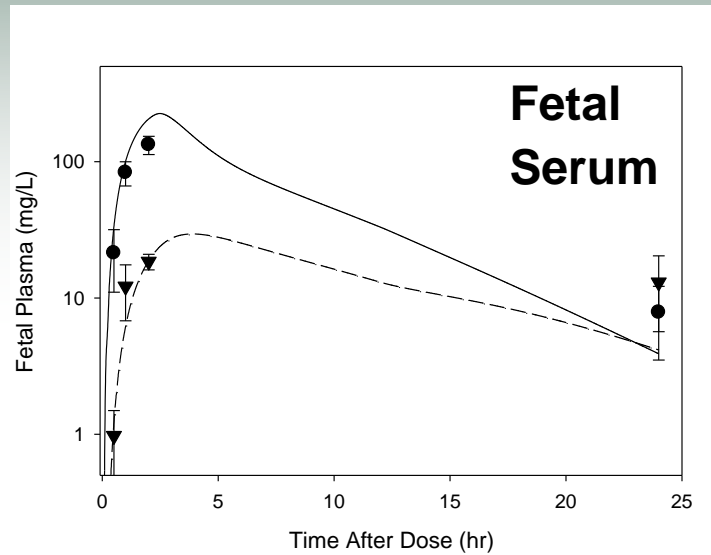
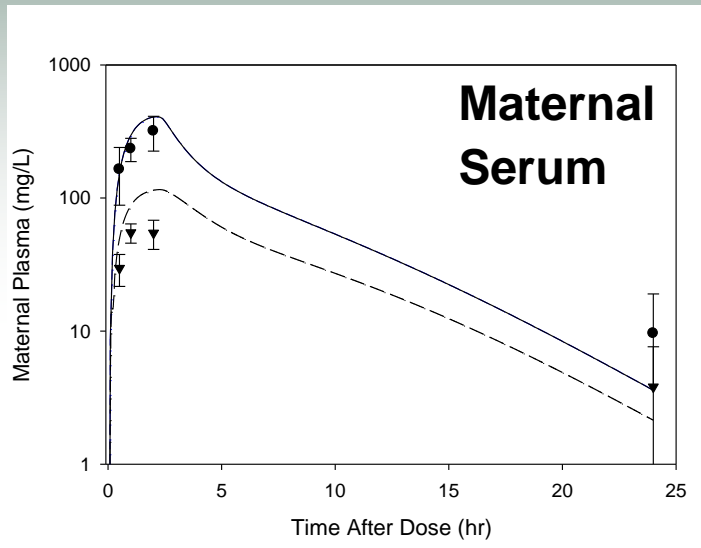
# Estimation of parameters for human model



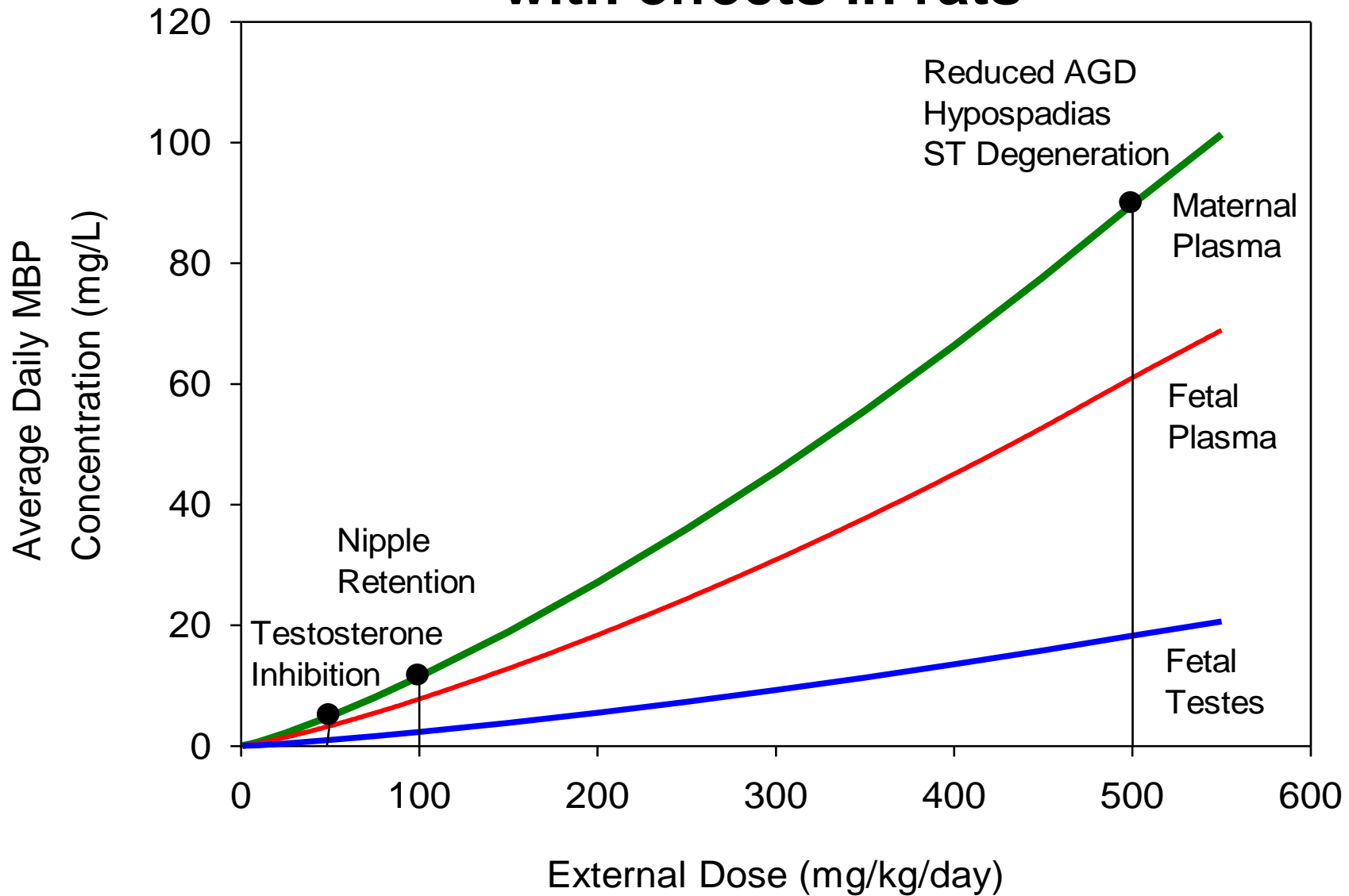
# PBPK Model of DBP in Rat Gestation



# Monoester kinetics after single DBP dose - 500 mg/kg, GD19 rats

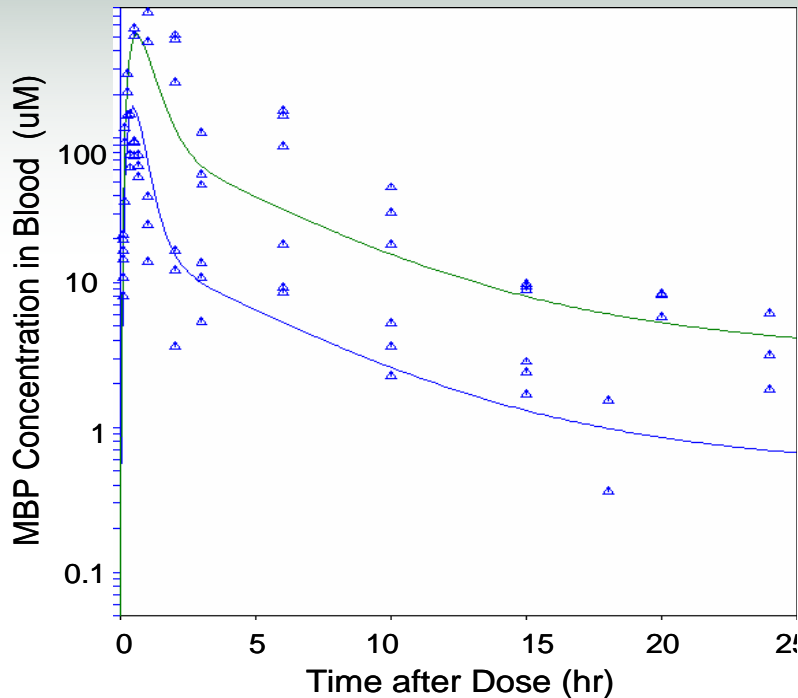


# Predicted tissue concentrations associated with effects in rats



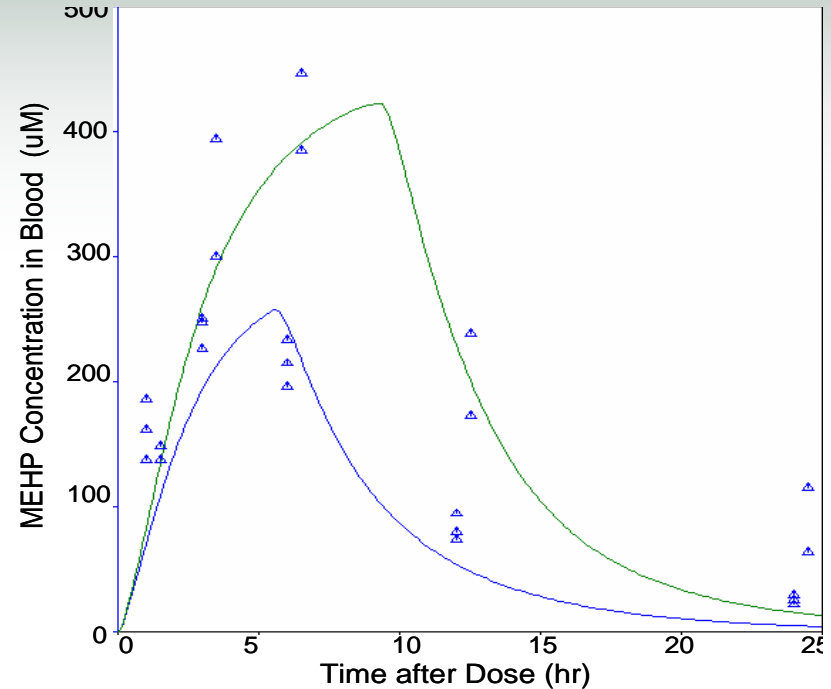
# Extending DBP model to other phthalates

## MBP in adult rat



50 and 200 mg/kg DBP  
Data of NIEHS 1994, 1995

## MEHP in adult rat



1 and 2 g/kg DEHP  
Data of Oishi *et al.*, 1989, 1990

# Comparing Internal Dose – DEHP and DBP

## DBP model extrapolated to DEHP

- Minor structural changes – oxidative metabolism
- Refitted metabolic parameters (P450, UGT)

## Validate model vs. DBP and DEHP data

## Run model at described dosing regimen for testosterone inhibition

## Calculate average fetal plasma monoester concentration

- DBP:  $ED_{50} = 35 \text{ mg/kg/day}$ ,  $IC_{50} = 1.9 \text{ } \mu\text{M}$
- DEHP:  $ED_{50} = 63 \text{ mg/kg/day}$ ,  $IC_{50} = 1.4 \text{ } \mu\text{M}$

*Active metabolites MEHP and MBP have similar potency.*

# Extending PBPK model to humans

## Preliminary model: allometrically scaled from rat

### Controlled dosing study in humans

- Exposed 6 or 7 subjects to  $^{13}\text{C}$ -DBP on buttered toast (255  $\mu\text{g}$  and 510  $\mu\text{g}$ ).
- Measured 24 hour cumulative urine, reported with total amount of MBP excreted.

Dose	Amount excreted in 24 h Urine Sample	
	Predicted	Measured
255 $\mu\text{g}$	108 $\mu\text{g}$	127 $\pm$ 36 $\mu\text{g}$ (91.6 to 148)
510 $\mu\text{g}$	217 $\mu\text{g}$	298 $\pm$ 88 $\mu\text{g}$ (191 to 438)

Data of Anderson et al. (2001) Food Addit Contam, 18(12):1068-74.

# Comparison with Other Available Human Data

## Silva et al. (2001) biomonitoring data

- Measured free MBP vs. glucuronidated MBP  
(subset of NHANES III urine samples)
- No exposure, time of collection or subject data
- Predict percent free MBP for “average man” (70 kg) with PBPK model  
(steady state exposure conditions)
- Amount excreted in urine averaged for a 24 h

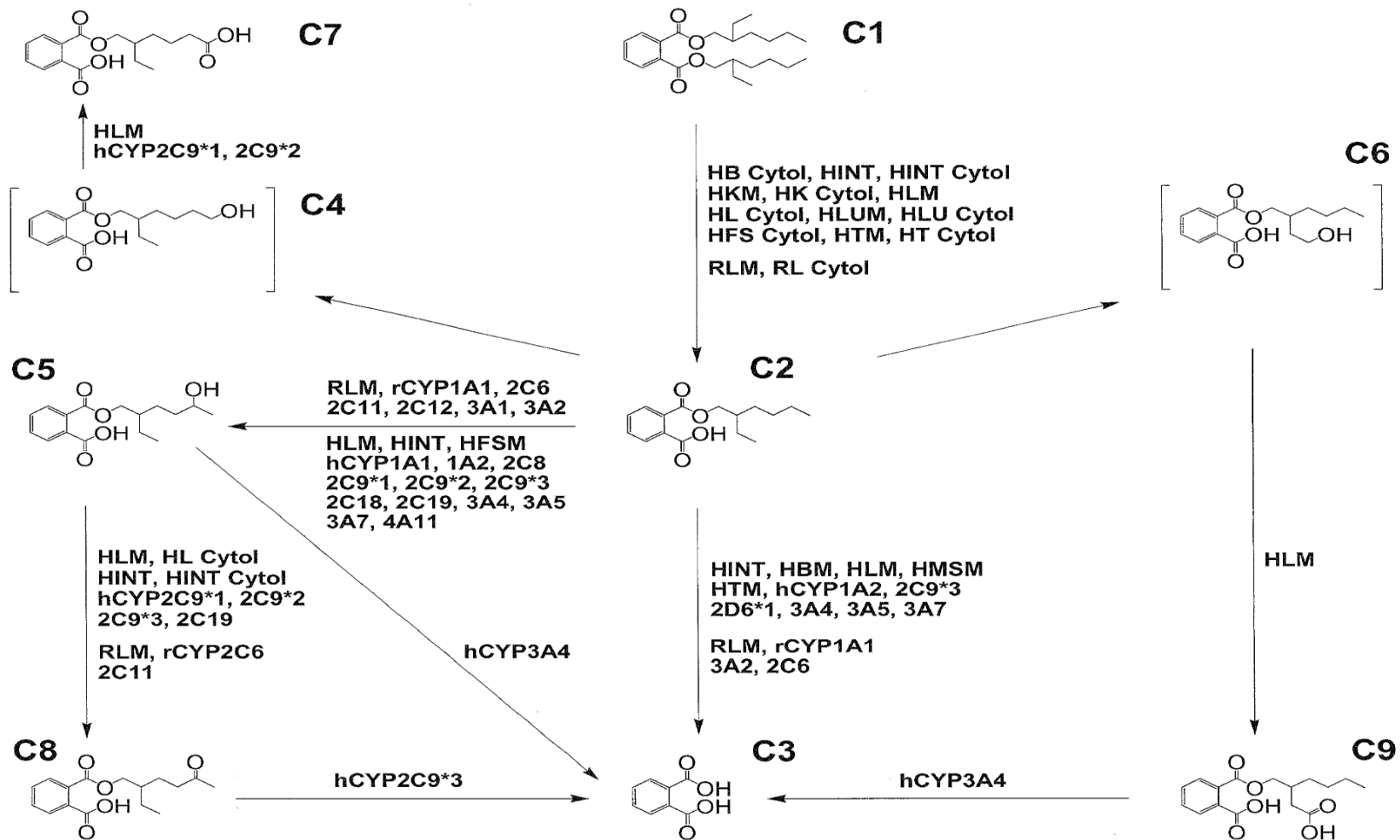
## Results

- Silva reported:
  - Mean concentration in urine - **28.95  $\mu\text{g/L}$**
  - Measured fraction of free MBP in urine - **5.6 %**
- The model predicts:  **$\approx 8.0\%$**  free MBP
  - average urine concentration of **28.9  $\mu\text{g/L}$** .

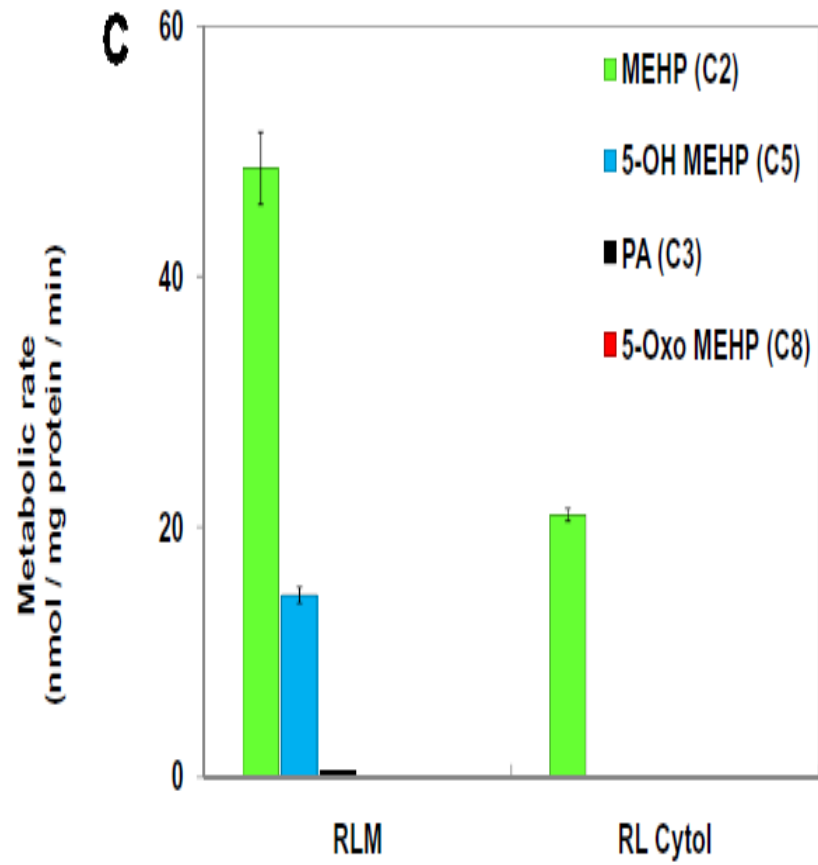
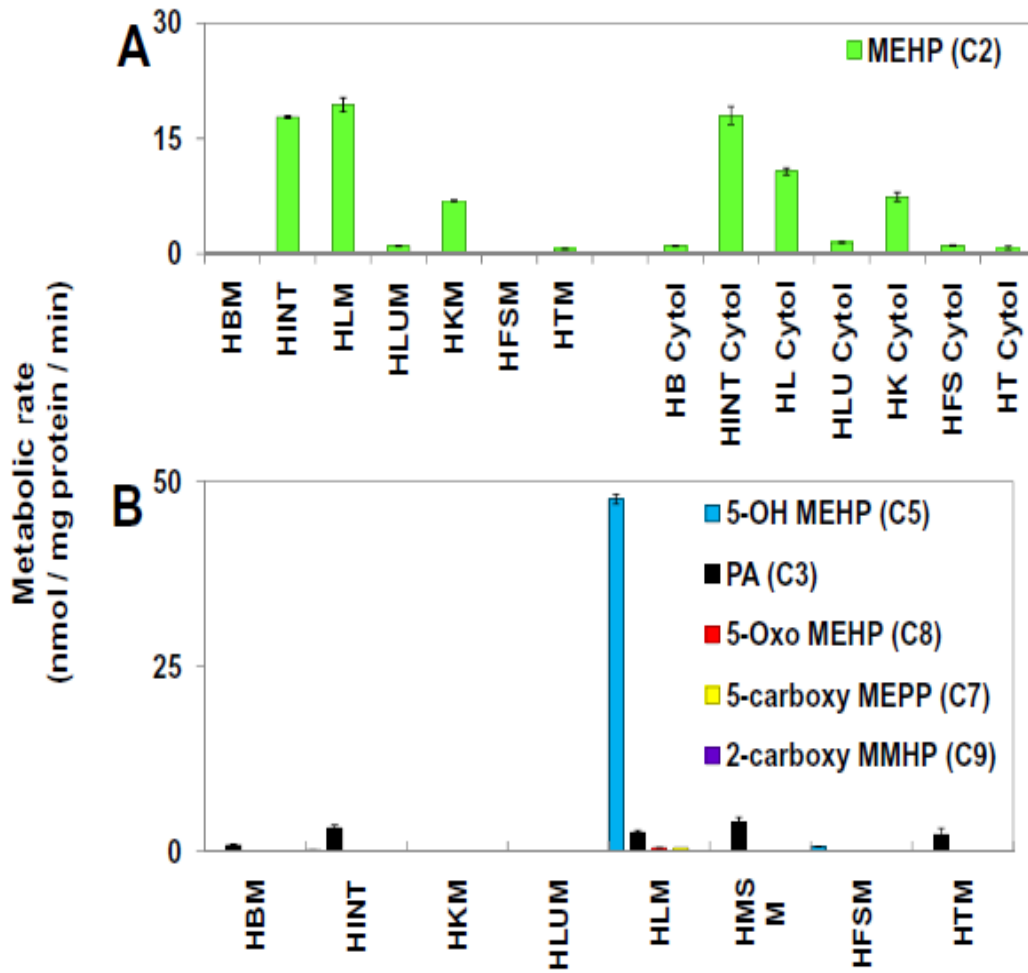
# *In Vitro* Metabolism of DEHP in humans and rats

## Part I. Phase I biotransformation of DEHP

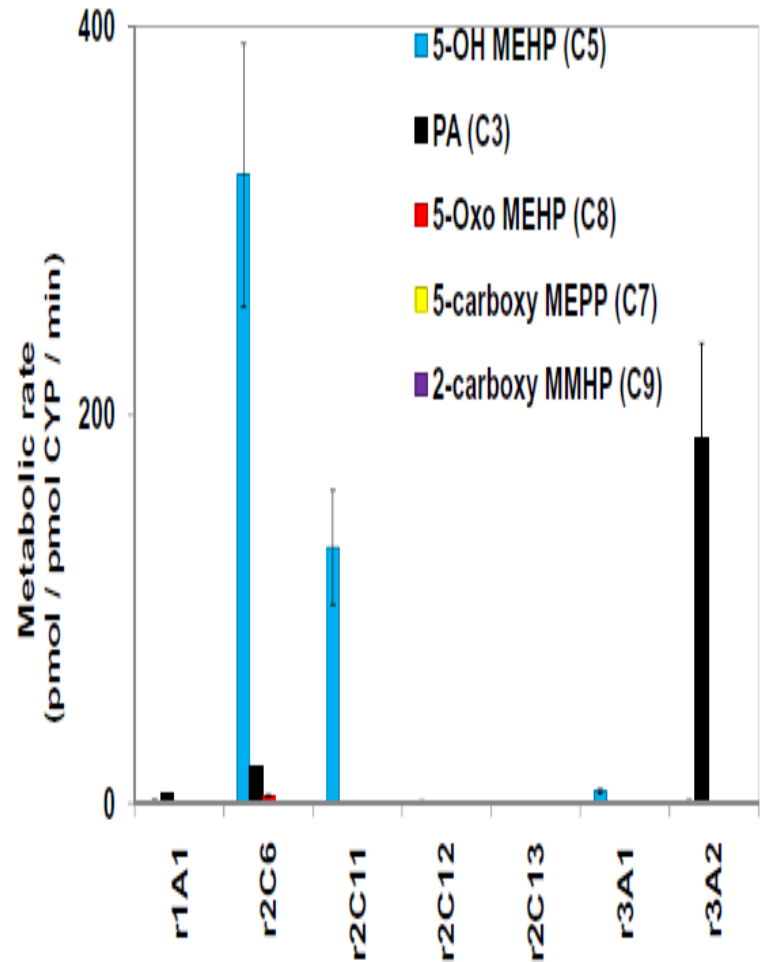
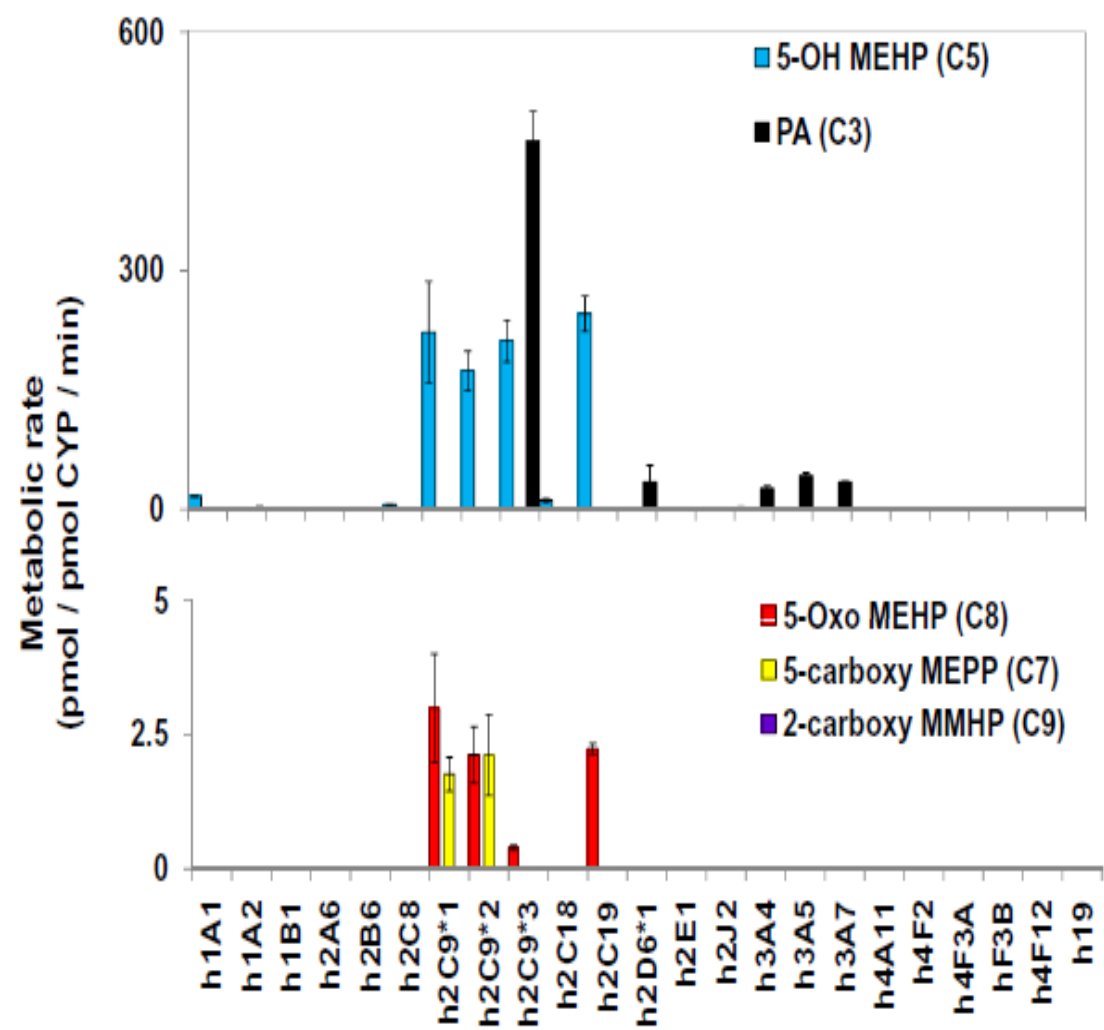
- **Organ specific metabolism of DEHP**
  - Subcellular fractions of 7 different human organs.
    - Human brain, intestine, kidney, liver, lung, skin and testis.
  - Rat hepatic subcellular fractions
- **Major CYP isoforms involved in MEHP metabolism**
  - Recombinant human CYP isoforms (n=23).
  - Recombinant rat CYP isoforms (n=7).



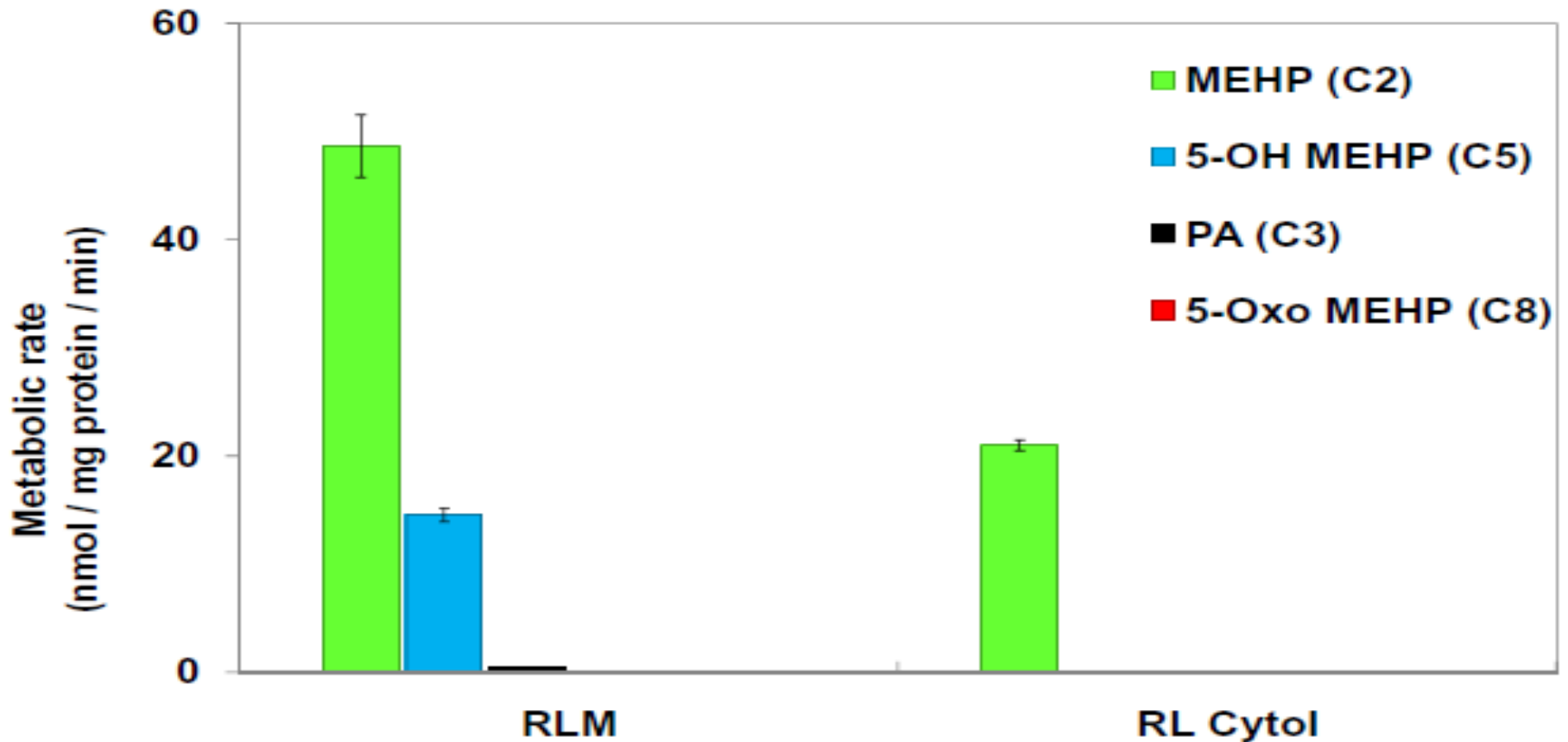
**Proposed phase I biotransformation scheme for DEHP in subcellular fractions of human organs and rat liver and varying CYP isoforms of human and rat. (Choi et al., 2011)**



**Metabolic rates for the production of MEHP (A) and MEHP-derived metabolites (B) determined in microsomal and cytosolic fractions of various human organs and of rat liver (C). (Choi et al., 2011)**



**Metabolism of MEHP by 23 selected human CYP isoforms and 7 selected rat CYP isoforms. (Choi et al., 2011)**



**Metabolic rates of the production of MEHP, 5-OH MEHP, 5-Oxo MEHP, 5-carboxy MEPP, 2-carboxy MEPP and PA in rat liver microsomes (RLM) and cytosols (RL Cytol). (Choi et al., 2011)**

## Part II. Phase II biotransformation of DEHP

### 1. Conjugation of MEHP and its oxidative metabolites

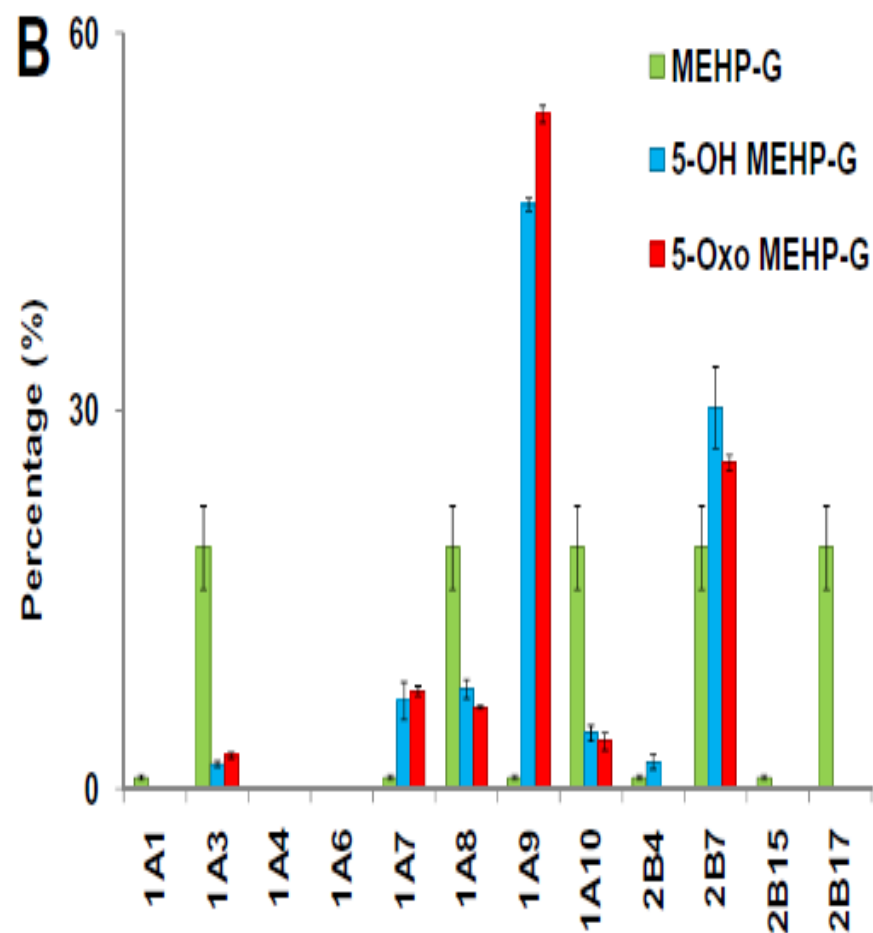
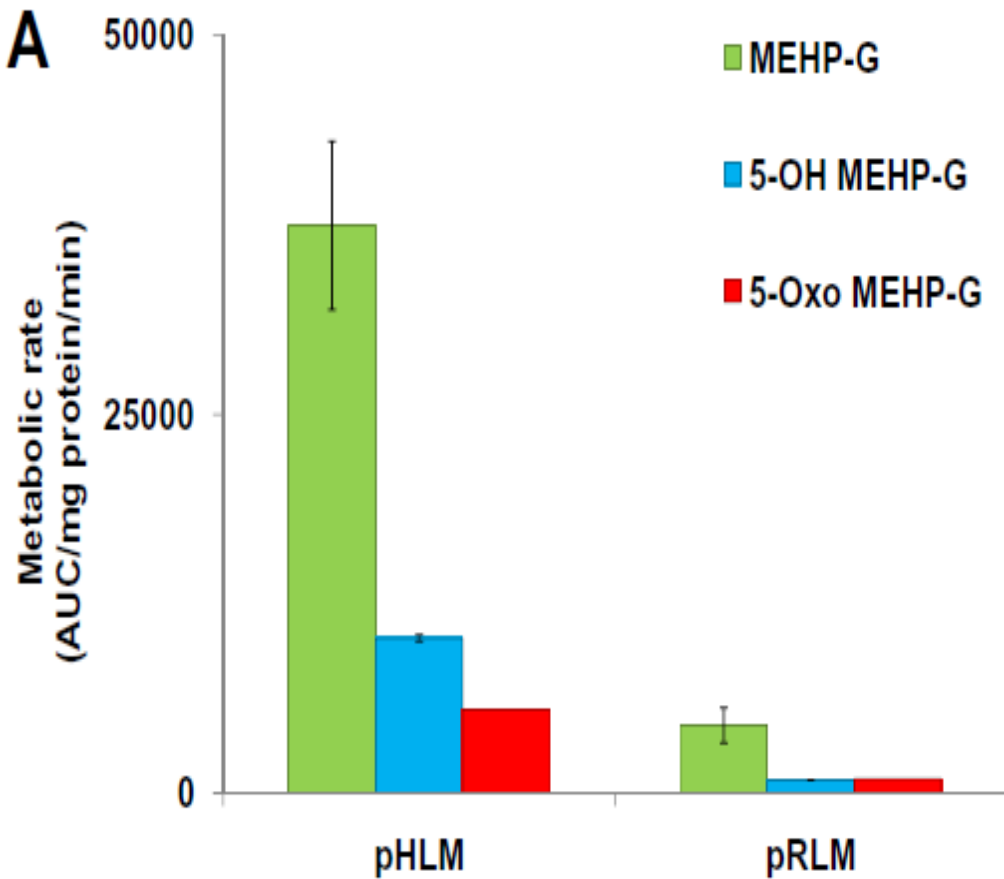
- Measurement of glucuronide and sulfate using LC-MS/MS
- Human hepatic subcellular fractions.

### 2. Major UGT isoforms involved in glucuronide conjugation

- Recombinant human UGT isoforms (n=12).

### 3. Biosynthesis of MEHP glucuronide and characterization using LC-MS/MS and NMR

- To serve as standard for *in vitro* metabolism studies



**A) Metabolism of MEHP and its oxidative metabolites glucuronides in hepatic microsomes of humans and rats.**

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

# Evaluating Phthalate Potency *In Vitro*

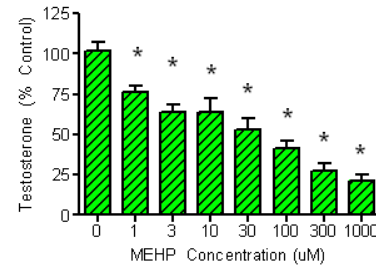
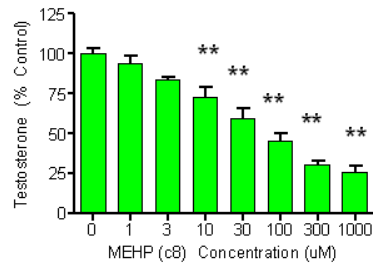
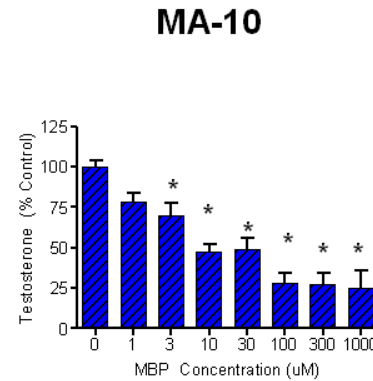
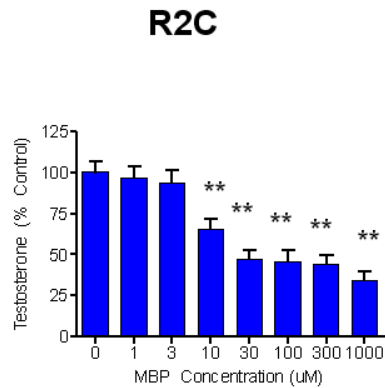
**Male developmental effects are consistently preceded by a reduction in testosterone**

**Testosterone inhibition can be evaluated in Leydig cells *in vitro***

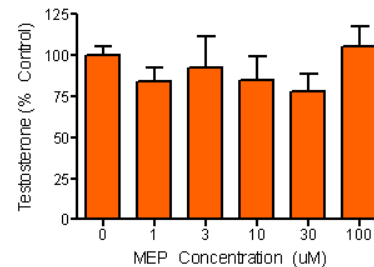
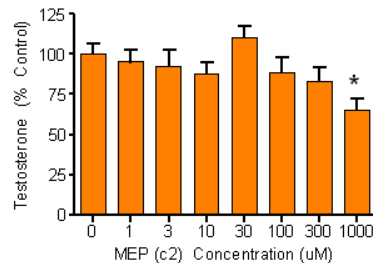
- MA-10 cells: **mouse** Leydig cancer cell
  - Produces testosterone in response to luteinizing hormone (LH)
- R2C cells: **rat** Leydig cancer cell
  - Produces testosterone without stimulation (constitutively active)
- Human?
  - No human Leydig cell line available

# Evaluating Phthalate Potency *In Vitro*

“Active”  
phthalates



“Inactive”  
phthalate

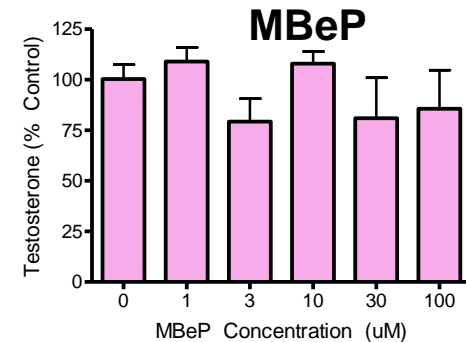
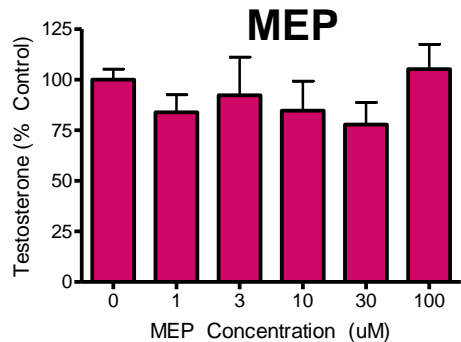
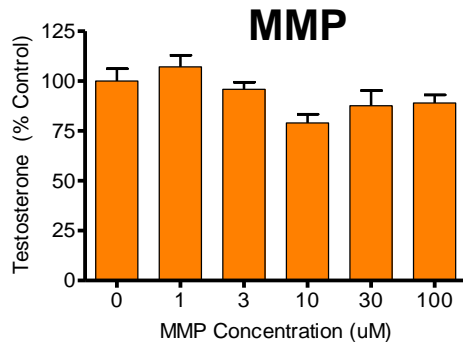
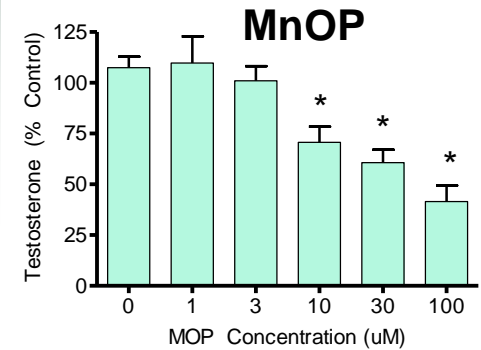
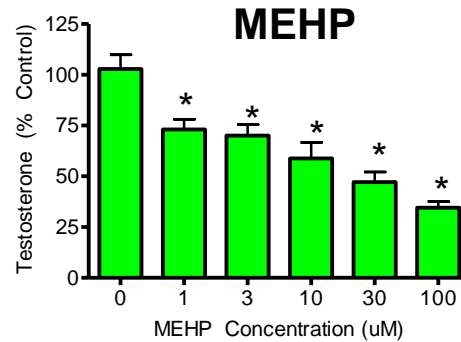
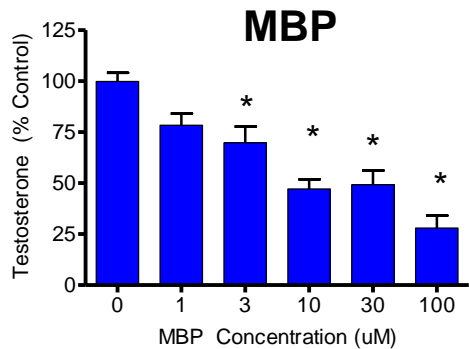


<b>Biological Response</b>	<b>Model</b>	<b>Study Type</b>	<b>IC<sub>50</sub> (μM)<sup>a</sup> MEHP</b>	<b>IC<sub>50</sub> (μM)<sup>a</sup> MBP</b>	<b>IC<sub>50</sub> (μM) MBP</b>
Testosterone Inhibition <sup>a,b</sup>	Rat Testes - Fetus	<i>In vivo</i>	1.4 (0.1-25)	1.9 (1-5)	> 350
Testosterone Inhibition	Rat Leydig Cell	<i>In vitro</i>	16 (9-29)	13 (8-23)	951
Testosterone Inhibition	Mouse Leydig Cell	<i>In vitro</i>	4 (1-19)	4 (1-20)	>100

-Can determine “MBP equivalents” from *in vitro* assay.

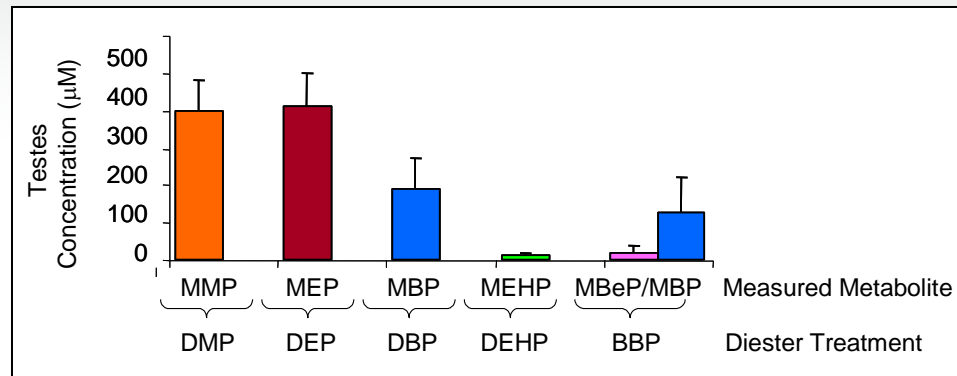
MEHP = 1 MBP equivalent

# PD differences: inhibition of testosterone production



# Conclusions about PK v. PD differences

Inactivity of short-chain phthalates not due to reduced fetal testes exposure to monoester (i.e., not kinetic)



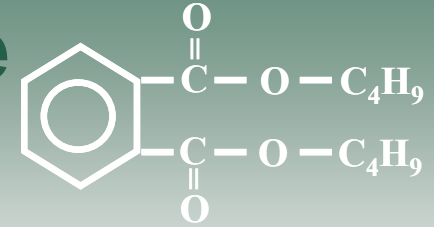
Fetal testes dose of various phthalates

– 2 hr after 500 mg/kg/day dose (GD 12-19)

Risk assessment must account for pharmacodynamic differences

- Combination of *in vitro* activity and PBPK IVIVE can be used to estimate cumulative effects of phthalate mixtures

# Research to Support a Phthalate Cumulative Risk Assessment



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

## Approach:

- Assume additivity of risk based on summation of potency-weighted target tissue dose:
  - $R_{cum} = \sum (P_i * D_i) * RPP$ 
    - where  $P_i$  = in vitro relative potency factor for phthalate  $i$  compared to reference phthalate
    - $P_i = EC50_r / EC50_i$
    - $D_i$  = target tissue dose for phthalate  $i$
    - RPP = reference phthalate potency
      - Based on target tissue dose
      - R = MBP or MEHP, depending on critical effect

# Acknowledgements

## **Rebecca Clewell**

- DINP studies
- PBPK model for dibutyl phthalate and di-ethylhexyl phthalate during gestation in rat
- In vitro MA-10 Leydig cell testosterone inhibition assay

## **Jerry Campbell**

- PBPK model for dibutyl phthalate and di-ethylhexyl phthalate in human

## **Kyoungju Choi, Hyun Joo**

- In vitro metabolism data on dibutyl phthalate and di-ethylhexyl phthalate in rat and human

## **Pergentino Balbuena**

- In vitro data on phthalate testosterone inhibition in R2C Leydig cells

## **Funding**

- ACC LRI
- EPA STAR Grant