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Cumulative effects of anti-androgenic chemical mixtures and their relevance to human health risk assessment

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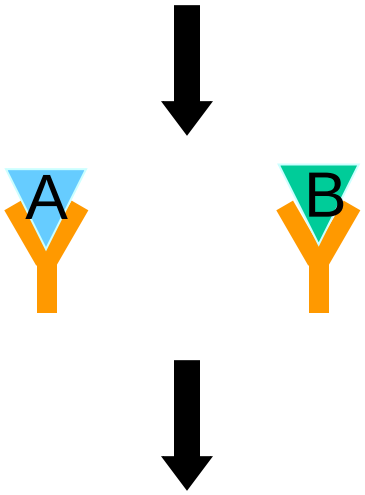
Basic study design

- Pregnant Sprague-Dawley rats are given oral doses of chemical(s) during critical window of male reproductive tract development
- Male offspring are assessed for either fetal testosterone production or reproductive parameters
- Individual chemicals are present in the mixture at or below their no observed adverse effect levels (NOAELs)
- Mixtures designed to produce significant responses

Theories of mixture toxicity

Dose Addition (DA)

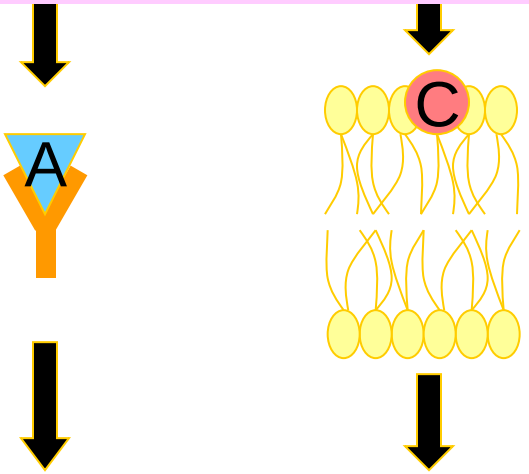
$$R_{mix} = \frac{1}{1+\left(\sum_{i=1}^{n} \frac{D_i}{ED50_i}\right)^{\rho'}}$$



Mixture Response

Response Addition (RA)

$$R_{mix} = 1 - \prod_{i=1}^{n} (1 - R_i)$$



Response A + Response B

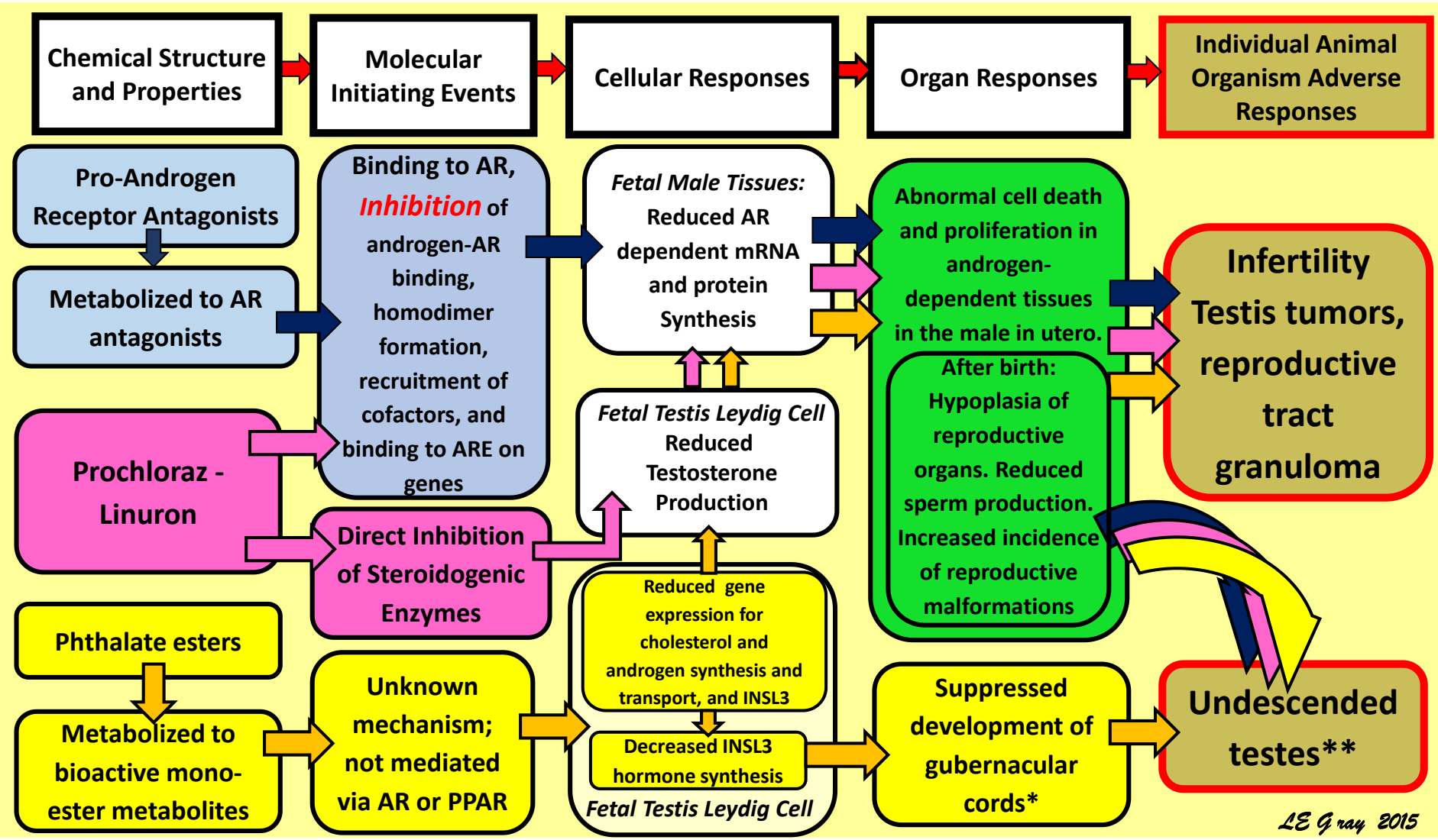
Mixture Response

Integrated Addition: Combination of DA and RA

Mixture toxicity assessment

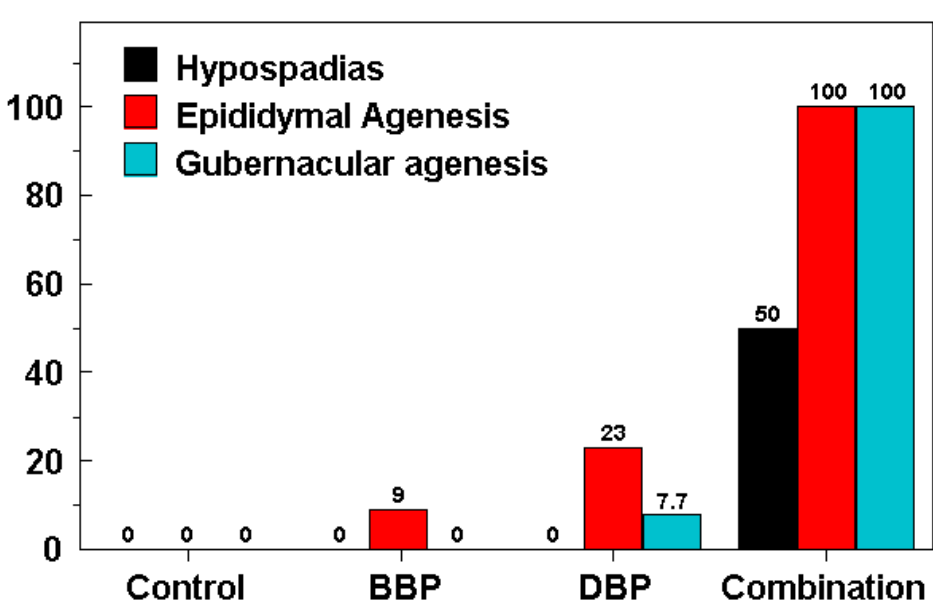
1. Characterize effects of individual chemicals
   - Fit with a four parameter variable slope logistic regression
2. Use data from single chemical exposures to predict mixture effects
   - ED50 and Hill slope
3. Compare mixture model predictions to experimental (observed) results
   - Response and dose addition models

Adverse outcome pathway for antiandrogenic chemicals

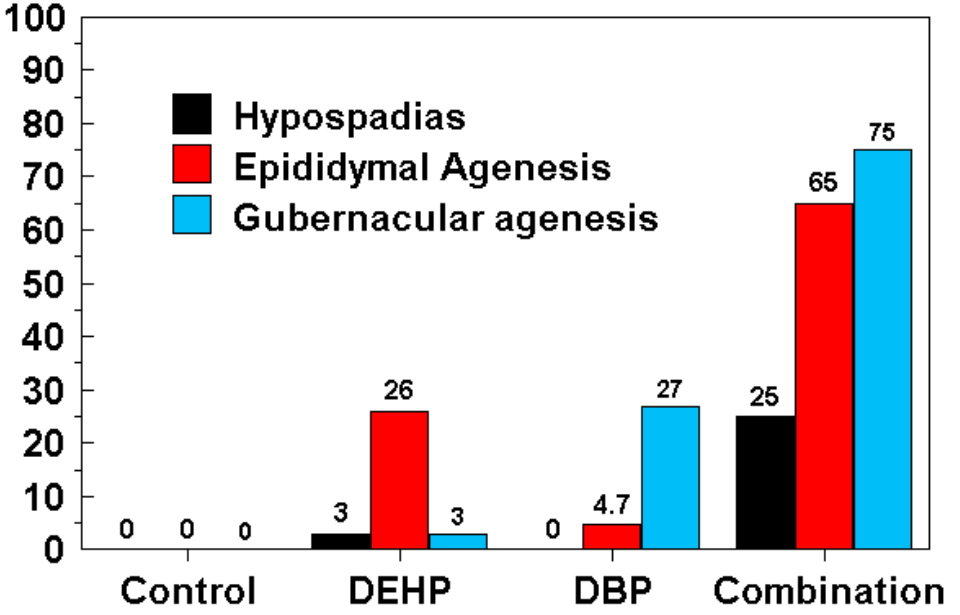


Binary phthalate mixture with same mode of action

Phthalates with a common bioactive metabolite



Phthalates with different bioactive metabolites



Hypospadias: 0+0=50%
Epididymal agenesis: 9+23=100%

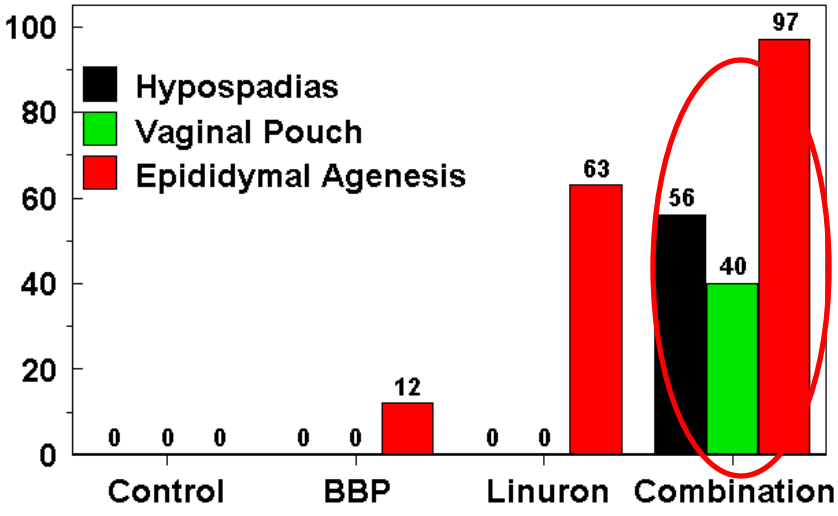
Hypospadias: 0+3=25%
Epididymal agenesis: 5.4+28.6=70%

Hotchkiss et al. 2011 Repro Tox 30:261

Howdeshell et al. 2007. Tox Sci 99:190

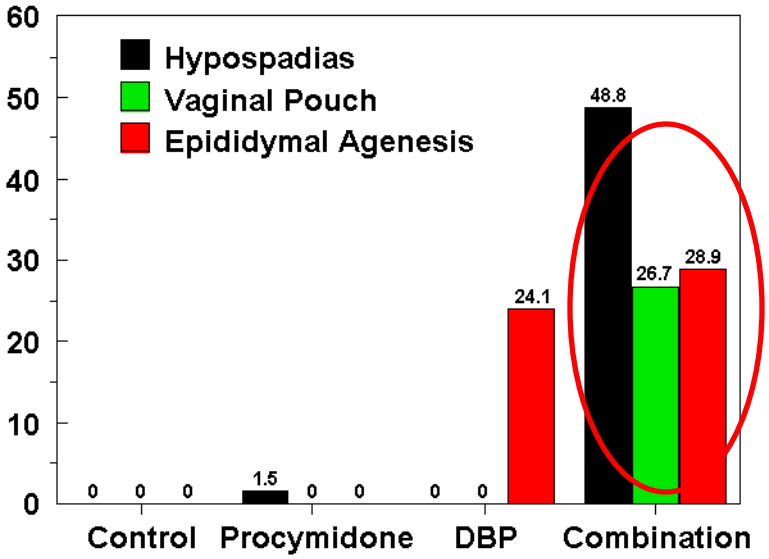
Binary mixtures with different modes of action

Inhibitor of fetal androgen synthesis and mixed mechanism antiandrogen



Hypospadias: 0+0=56%

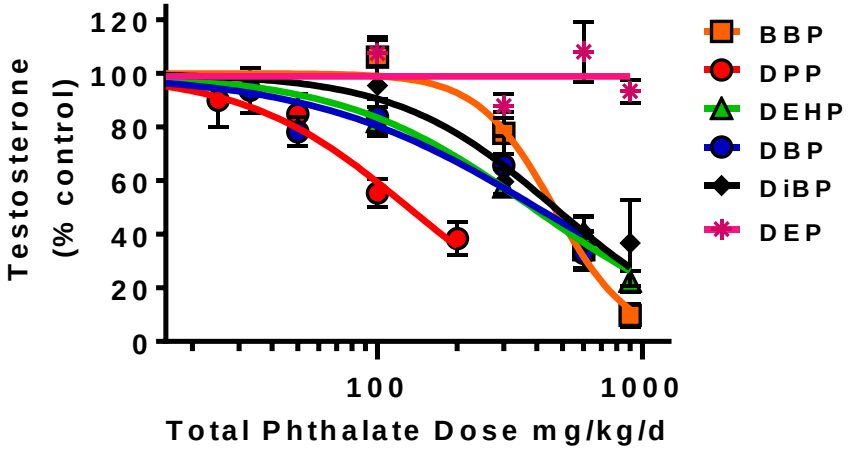
Inhibitor of fetal androgen synthesis and AR agonist

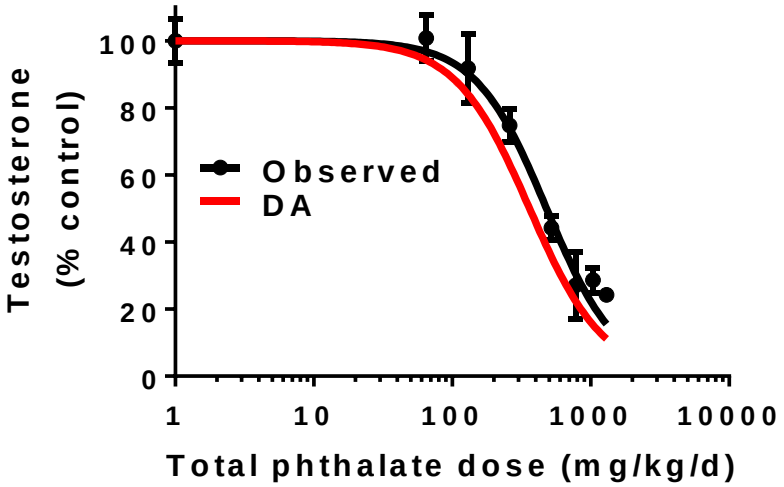


Hypospadias: 1.5+0=49%

Hotchkiss et al. 2011 Repro Tox 30:261

Mixture of 5 phthalates with common mode of action





	5% top dose	10% top dose	20% top dose	40% top dose	60% top dose	80% top dose	100% top dose
Phthalates in mixture (mg/kg/d):							
BBP	1	30	60	120	180	240	300
DBP	15	30	60	120	180	240	300
DiBP	15	30	60	120	180	240	300
DEHP	15	30	60	120	180	240	300
DPP	5	10	20	40	60	80	100
Total dose	65	130	260	520	780	1040	1300

Howdeshell et al. 2008 Toxicol Sci 105:153

Human fetuses are exposed to same bioactive metabolites as rat fetuses

Table 1. Distribution of mEP, mBP and mEHP (in ng/mL) in amniotic fluid samples from 54 pregnant women.[a]

Phthalate Metabolite	Min	Max	Percentile					
			10th	25th	50th	75th	90th	95th
mEP	<LOD	9.0	<LOD	<LOD	<LOD	4.8	7.5	8.1
mBP	<LOD	263.9	2.5	3.3	5.8	8.5	14.2	15.9
mEHP	<LOD	2.8	<LOD	<LOD	<LOD	0.8	1.7	2.6

[a] The limits of detection (LOD) for mEP, mBP and mEHP are 1.2 ng/mL, 0.97 ng/mL and 0.86 ng/mL, respectively.

Silva et al. 2004 Bull Environ Contam Toxicol 72:1226

24x

5x

MEHP

Oral dose to rat dam (GD7-18)	Amniotic fluid (GD18)
11 mg/kg/dy	68.4 ng/mL
33 mg/kg/dy	166 ng/mL
100 mg/kg/dy	766 ng/mL
300 mg/kg/dy	2921 ng/mL

MBP

Oral dose to rat dam (GD13-18)	Amniotic fluid (GD18)
100 mg/kg/dy	1400 ng/ml
250 mg/kg/dy	13400 ng/ml

Calafat et al. 2004. Toxicol 217:22

Predicting dose additive effects of a phthalate mixture based on fetal T production

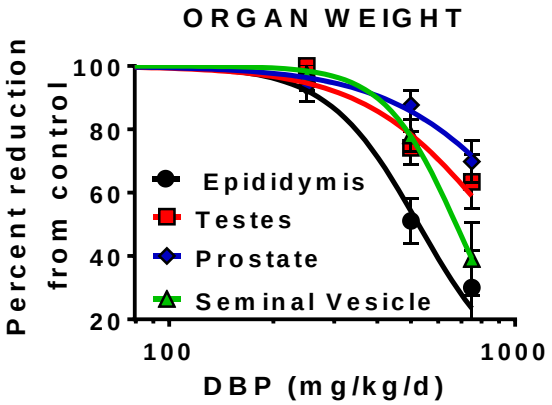
Fetal T production data for each of 5 individual phthalates

Postnatal data for the reference chemical, DBP

Use ED_{50} for fetal T production to determine potency of the individual phthalates relative to DBP

For example:

Phthalate	DBP	DEHP
ED50 (mg/kg/d)	398.5	383.4
Potency relative to DBP	1	1.04



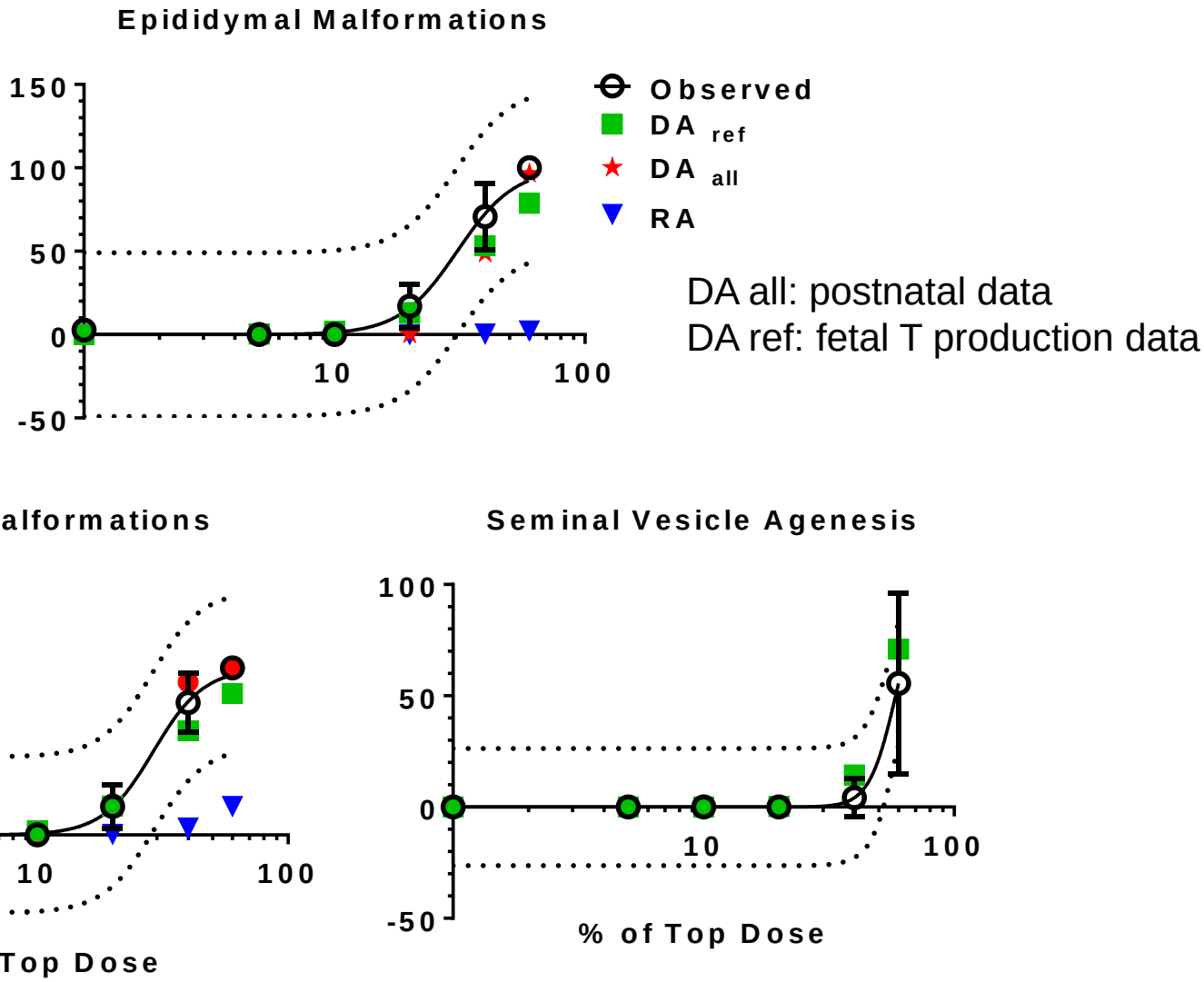
Express mixture dose as DBP equivalents

Use the log ED_{50} and Hillslope of the postnatal data for DBP to predict DA using a logistic regression equation

$$R = \frac{1}{1 + \left(\frac{ED50}{D}\right)^{\rho}}$$

Howdeshell et al. 2015 Toxicol Sci 148:488

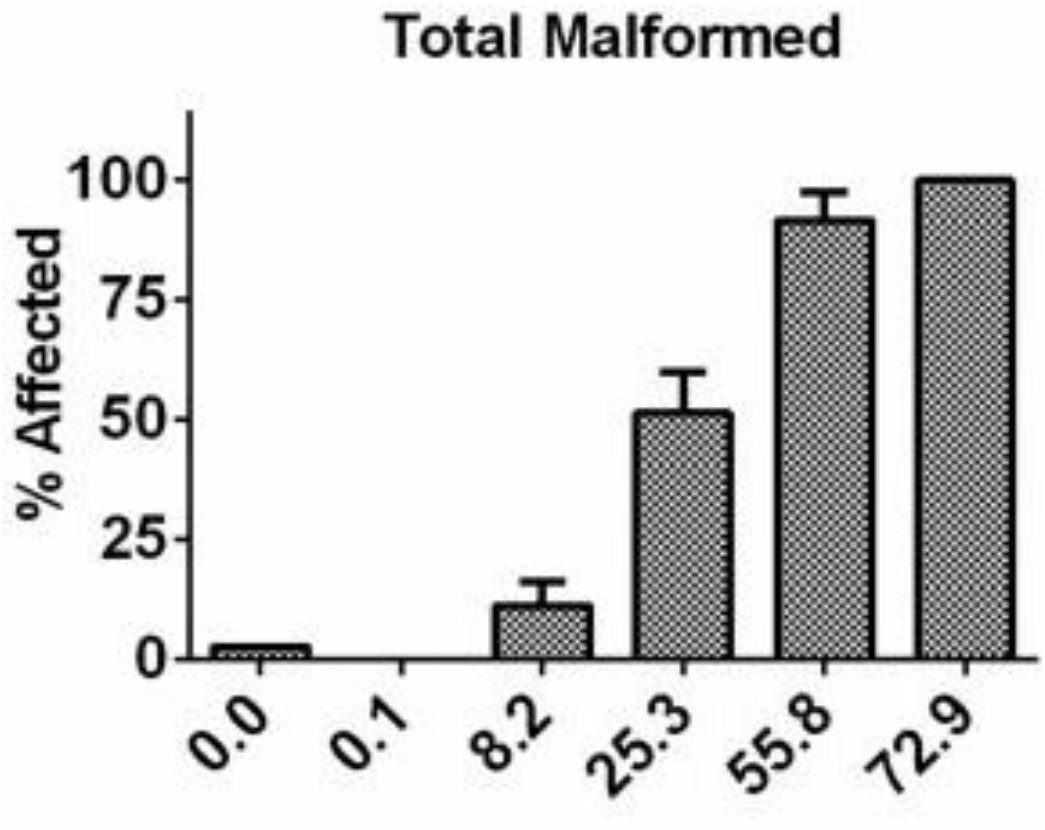
Dose addition based on fetal T predicted postnatal effects of a mixture of five phthalates



Howdeshell et al. 2015 Toxicol Sci 148:488

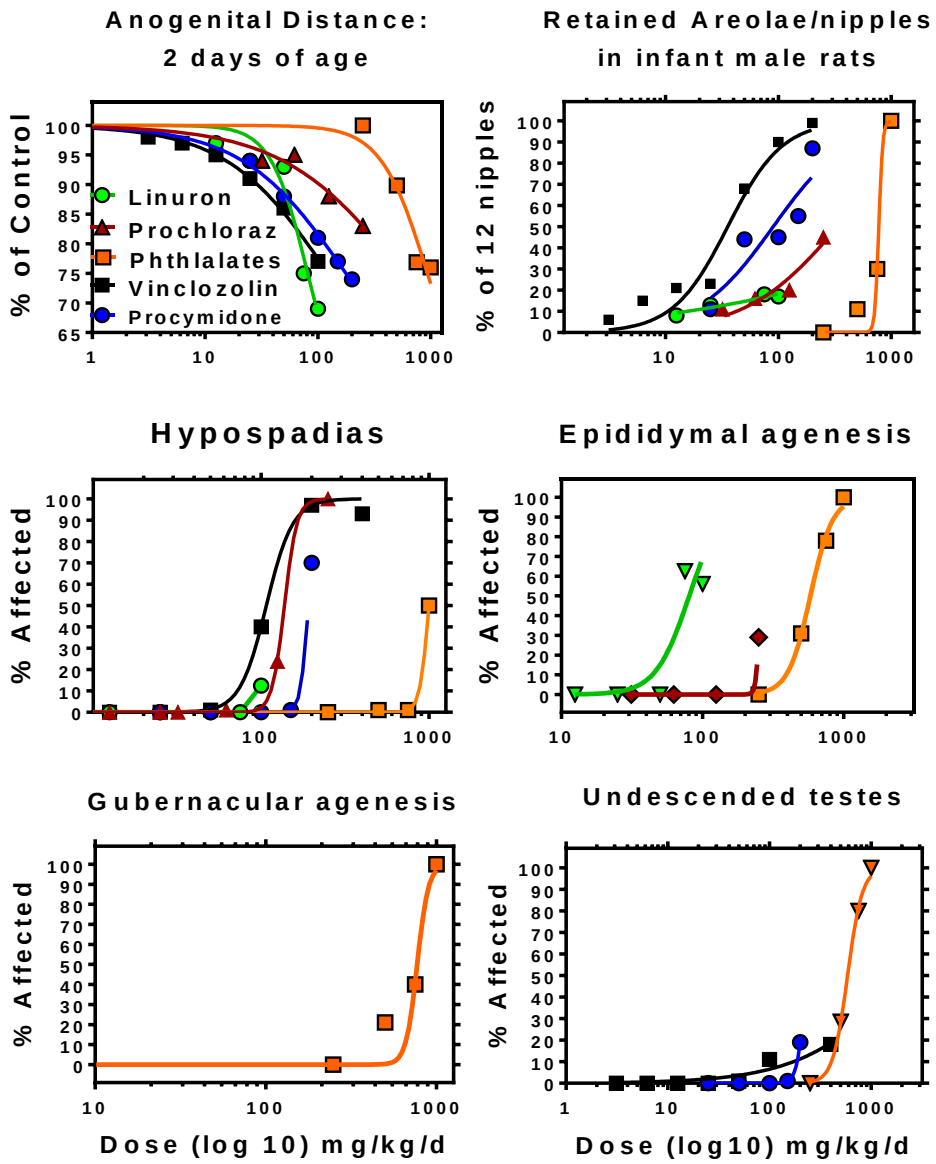
Biologically-relevant reductions in fetal T

- Percent reduction in fetal T production that alters postnatal male reproductive tract development in rats prenatally exposed to a 5 phthalate mixture on GD8 to PND3



Howdeshell et al. 2015 Toxicol Sci 148:488

Mixture of 10 chemicals with different modes of action



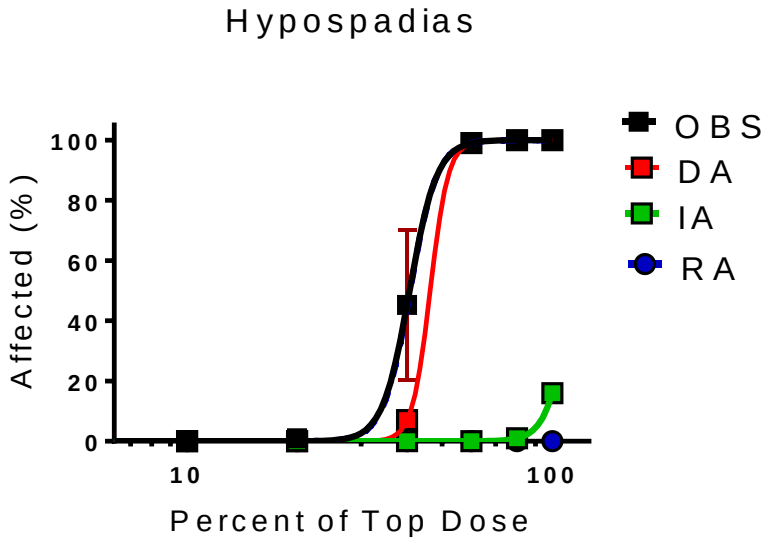
- Chemicals included:
  - 6 phthalates
  - 2 AR antagonists
  - 2 mixed mechanism antiandrogens

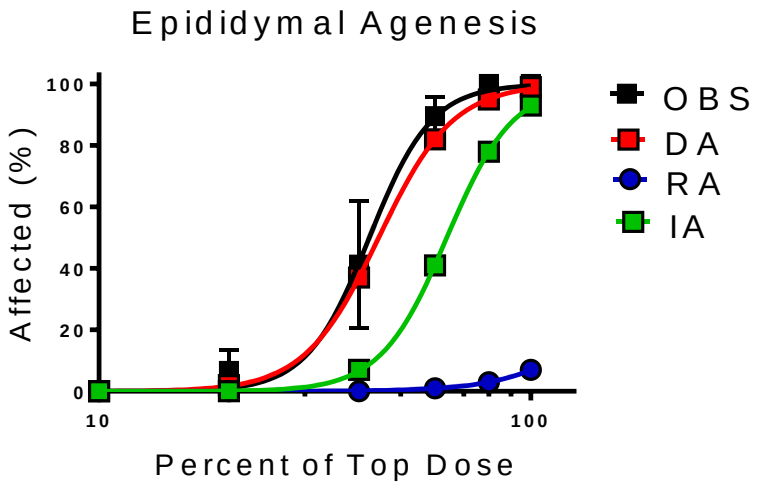
- Mixture dose based on ED50s of individual chemicals to induce hypospadias or gubernacular agenesis

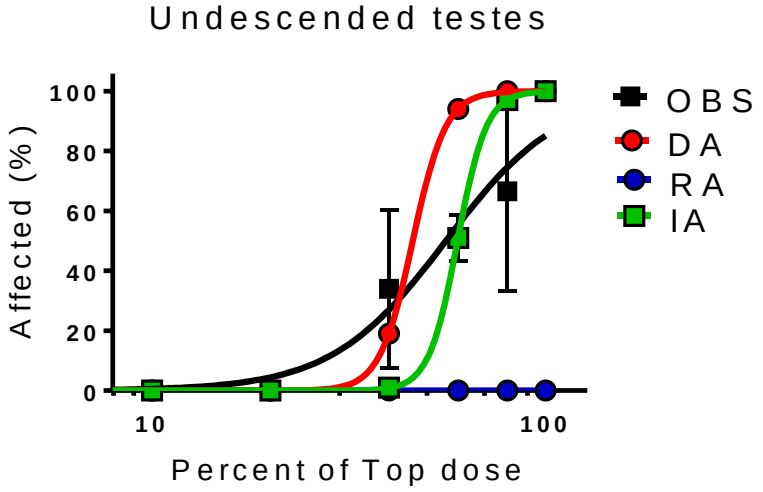
- Doses of individual chemicals were below the NOAEL

Rider et al. 2010 Int J Androl 33: 443

Dose addition predicts effects of mixture of 10 chemicals with different modes of action







Rider et al. 2010 Int J Androl 33: 443

Chronic Hazard Advisory Panel on Phthalates and Phthalate Alternatives

- Convened by the United States Consumer Product Safety Commission
- Used animal data for basis of phthalate risk assessment due to lack of human data to directly quantify risk
- A hazard index (HI) approach was applied for the antiandrogenic phthalates only: DBP, DIBP, BBP, DEHP, and DINP.

$$Hazard\ Quotient\ (HQ_j) = \frac{DI_j\ (\mu g / kg - day)}{PEAA_j\ (\mu g / kg - day)}$$

and

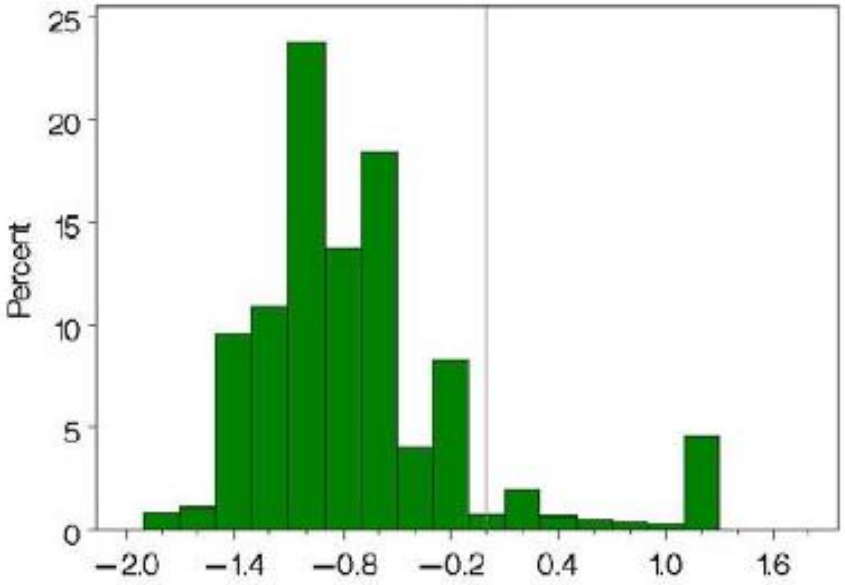
$$\textbf{Hazard Index (HI)} = \sum_{j=1}^{c} \mathbf{HQ_j}$$

PEAA = potency estimates for antiandrogenicity;
j=individual chemical; c=number of chemicals in mixture

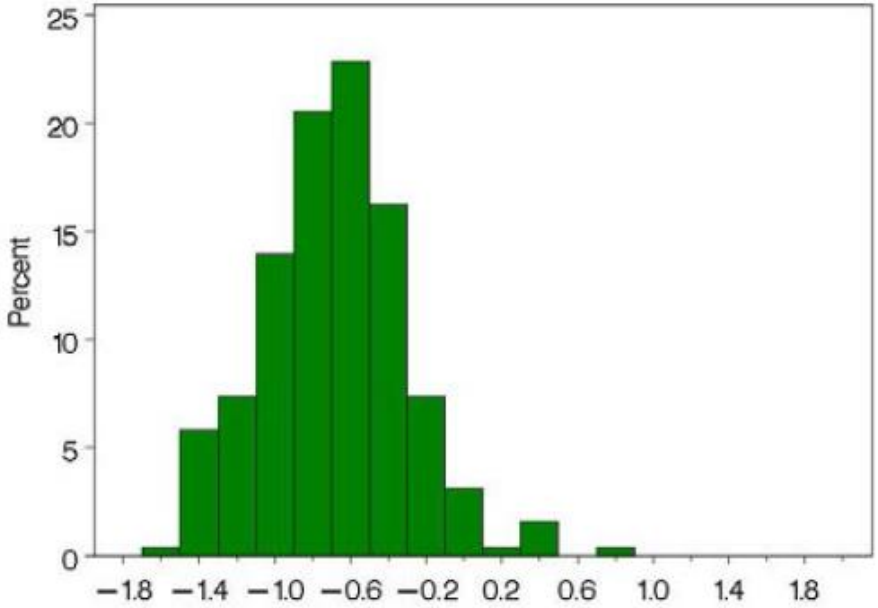
CHAP 2014

Elevated Hazard Index (HI) for Pregnant Women and Infants

Pregnant women



Infants



Log-transformed HI for 5 phthalates (DBP, DiBP, BBP, DEHP, DiNP)

- In the United States, ~10% of pregnant women and 4-5% of infants that exceed 1.0 and 4–5% of infants have HI values that exceed 1.0.

CHAP Report 2014

Conclusions

- Dose addition accurately predicts the effects of a mixture of antiandrogenic chemicals with diverse mechanisms of action
- Prenatal endocrine changes are predictive of postnatal malformations for phthalate mixtures
  - Fetal T inhibition data for individual phthalates in a 5-day assay accurately predicted the dose additive effects on postnatal development
  - Biologically-relevant reductions in fetal T can be determined
- Biologically-based cumulative risk assessments may lead to more accurate assessment of the developmental reproductive toxicity of antiandrogenic chemical mixtures

Next Steps

- Characterization of less tested phthalates and phthalate alternatives (USEPA)
  - Fetal Phthalate Screen tests for ability to inhibit fetal T
  - Continued collaboration with CDC to understand rat metabolism of these chemicals and to inform biomarker selection (e.g., DINCH)
- Exploring the limits of dose addition with an 18-chemical mixture of antiandrogens (USEPA)
- NIEHS-EPA Collaborative Exposure Project to assess exposure to chemicals in personal care and consumer product use

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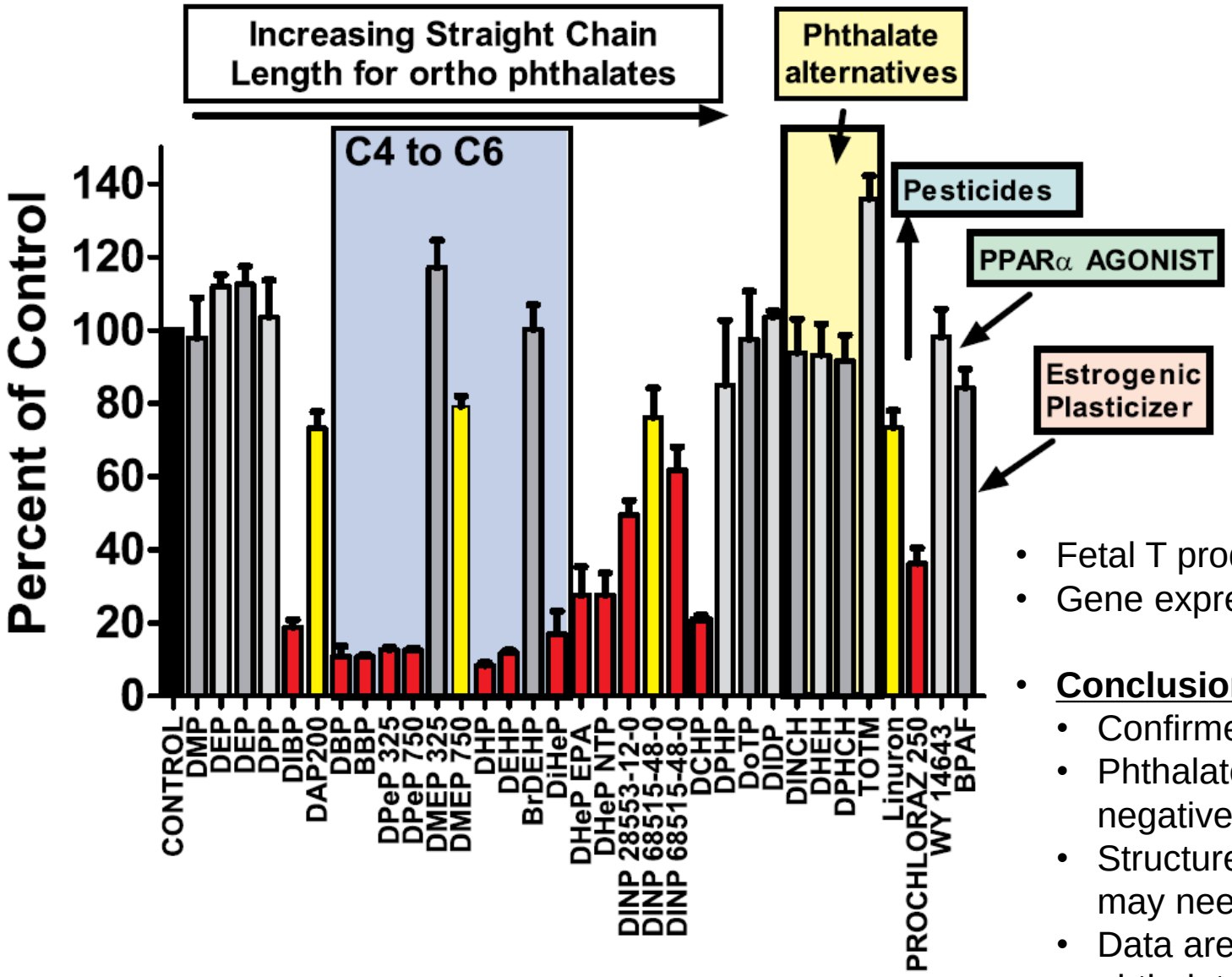
Disclaimer: This presentation does not necessarily reflect USEPA policy.

Fetal Phthalate Screen



- Fetal T production assay
- Gene expression (rtPCR arrays)

- **<u>Conclusions</u>:**
  - Confirmed published results
  - Phthalate alternatives tested negative
  - Structure-activity relationship may need to be revised
  - Data are useful in selecting phthalates for in vivo testing

(Furr et al. 2014 Toxicol Sci 140:403)

NIEHS-EPA Collaborative Exposure Project

- Objectives
  - Evaluate utility of questionnaire instruments to assess personal care and consumer product use
    - Daily diaries and biological samples (urine and serum)
    - Targeted analysis planned for NHANES chemicals via an interagency agreement with CDC
  - Evaluate USEPA's exposure models designed to predict exposures to chemicals in the environment
    - Collection of air and dust samples
    - High throughput chemical analysis of environmental samples

Kyla Taylor, NIEHS/NTP

NIEHS-EPA Project Data and Survey Tools

- Questionnaires
  - Personal care product questionnaire
  - Environmental exposures/residential history questionnaire
  - Food packaging and processing questionnaire
- Daily diaries
- Biomonitoring
  - Whole blood: two blood collections (first and last day)
  - Daily urine collection (all voids)
- Portable exposure measurement devices
- Indoor and outdoor air samplers
- House dust samples
  - Surface wipe, vacuum dust, and door frame dust)
- Consumer product inventory
- Video diaries
- Duplicate diet and photographs

Kyla Taylor, NIEHS/NTP